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A Comparative Study of Association of Intestinal Parasitic Infections with Hematological, Iron-Status and Inflammatory Biomarkers among School-Aged Children

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Abstract: ***Background:*** Intestinal parasitic infections continue to be a significant public health issue in school-aged children, especially in settings of low sanitation standards, unsafe water and poor hygiene. These infections can not only have gastrointestinal effects but can also impact on haematological status, iron metabolism and inflammatory processes. Studies available, however, tend to concentrate on prevalence and fewer studies explore the link between intestinal parasites and relevant clinically important biomarkers. ***Aim:*** The purpose of this study was to assess the relationship between intestinal parasitic infection and the blood, iron status and inflammatory markers among school-aged children in the diagnosis which was not based on non-PCR techniques. The objective of this study was to use strengthened non-PCR diagnostic techniques to examine the correlation between intestinal parasitic infection and some blood markers, iron status and inflammatory markers in the study of school-age children. ***Methods:*** The study was a comparative cross-sectional study that included 280 school-aged children aged 6–12 years. Direct saline wet mount, iodine wet mount, formalin-ethyl acetate concentration, trichrome staining, modified Ziehl-Neelsen staining, Kato-Katz technique, cellophane tape test and some stool antigen tests were used. CBC, red cell indices, eosinophils, platelets, serum iron, ferritin, total iron-binding capacity, transferrin saturation, C-reactive protein, erythrocyte sedimentation rate and total IgE were measured in blood samples. Fecal calprotectin was used as an indicator of intestinal inflammation. Participants were grouped into parasite-negative, protozoa-positive, helminth-positive and mixed-infection groups. ***Results:*** 42.1% of all subjects had an intestinal parasitic infection. The most common type of infection was protozoal and Giardia duodenalis was the most frequently detected parasite. The mean of all the parameters (haemoglobin, haematocrit, MCV, MCH, serum iron and transferrin saturation) were significantly lower in parasite positive children than in parasite negative children. Children with infection, however, had significantly elevated RDW, WBC count, eosinophils, platelets, CRP, ESR, total IgE and faecal calprotectin. The greatest changes in biomarkers were observed with mixed infections and heavy parasite levels. Untreated drinking water, handwashing less often than weekly, eating unwashed vegetables, biting nails, and touching animals were all independent risk factors for infection, as was rural living. ***Conclusion:*** Hematological, iron status and inflammatory biomarker changes were highly associated with intestinal parasitic infections in school-aged children. The results suggest the following: The use of integrated non-PCR parasitological diagnosis, in combination with the use of biomarkers, for better assessment of the clinical impact of intestinal parasites in children.

Keywords: Non-PCR Diagnosis, Giardia, Helminths, Inflammation, Iron Deficiency, School-Aged Children, Intestinal Parasites.

RESEARCH PAPER

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INTRODUCTION

Children are highly susceptible to intestinal parasitic infections, which continue to be a public health problem, especially in low- and middle-income areas where poverty, unsafe water, poor sanitation, overcrowding and lack of hygiene contribute to the spread of parasites. School-aged children are a high-risk population due to the exposure they are often subjected to contaminated soil, food, water and shared school environment. Protozoa such as Giardia duodenalis,

Entamoeba histolytica/dispar, Cryptosporidium spp., and Blastocystis spp. and helminths such as Ascaris lumbricoides, Trichuris trichiura, hookworms, Hymenolepis nana, and Enterobius vermicularis are considered intestinal parasites. In recent times, intestinal protozoan parasites are reported to be prevalent among school children in Africa and Asia with Giardia duodenalis often reported to be one of the most prevalent parasites (Hajissa *et al.,* 2022; Abdoli *et al.,* 2024). Intestinal parasitic infections have a health impact not only on the gastrointestinal tract. Children with infected may have diarrhea, abdominal pain, bloating, vomiting, anorexia and anal itching, but many infections may be chronic or subclinical. This hidden burden is clinically relevant as repeated or chronic infection might result in nutritional dysfunction, micronutrient deficiency, growth disorders, poor school attendance and dysregulated immune responses. STHs are of particular interest because they are linked to chronic morbidity in children, and intestinal protozoa could have a negative impact on intestinal absorption and mucosal function. Soil-transmitted helminth infections were identified as important neglected tropical infections, having a significant impact on child health (Jourdan *et al.,* 2018), and intestinal protozoa were considered as public health important infections among school children of Asia owing to the poor sanitation and water-quality issues (Abdoli *et al.,* 2024). Hematological biomarkers are important clinical windows of the systemic effect of intestinal parasitic infections. The hemoglobin concentration, hematocrit, red blood cell indices, leukocyte profile, eosinophil count and platelet count of children with parasitic infections may be altered. Helminth infections can lead to anemia by causing loss of blood in the intestine, affecting the uptake of nutrients, causing chronic inflammation and affecting the metabolism of iron. Hookworm is of particular interest due to the fact that the adult worms consume the blood of their host, and may lead to iron-deficiency anemia, especially in children and women of reproductive age (Loukas *et al.,* 2016). Hence, the inclusion of hemoglobin, MCV, MCH, RDW, eosinophils and platelet indices are necessary to determine whether there is any association between the parasitic infection and anemia like or inflammatory hematological pattern. Areas of iron status biomarkers are also crucial as children's nutritional requirements are high during growth. To differentiate iron deficiency from iron redistribution associated with inflammation, ferritin, serum iron, total iron binding capacity and transferrin saturation may be helpful. This is significant as the infection with intestinal parasites can occur alongside malnutrition, inadequate nutrition and chronic inflammation. Djuardi *et al.,* (2021) conducted a study on the prevalence of soil transmitted helminth infection, anemia and malnutrition in children and found that STH infection, anemia and malnutrition were high among the children in the study population and thus it is important to combine parasitological diagnosis with nutritional and hematological assessment. This research is further enhanced with inflammatory biomarkers. The tests that may help to clarify whether parasite-positive children have a systemic or intestinal inflammatory response include: C-reactive protein, erythrocyte sedimentation rate, total IgE, eosinophil count and fecal calprotectin. Fecal calprotectin is of special interest since it is a marker of migration of neutrophils into the intestinal lumen and can be used as a non-invasive marker of intestinal inflammation. Ibrahim *et al.,* (2021) assessed the fecal calprotectin levels in children with Giardia intestinalis and Blastocystis hominis infection, and suggested the potential of fecal calprotectin as an intestinal inflammatory response marker in specific parasitic infections. While there have been several studies on the prevalence of intestinal parasites in children, there is a lack of studies that include parasitological diagnosis along with hematological, iron-status and inflammatory biomarkers. This is significant because prevalence does not reflect the biological and/or clinical implications of infection. A study with a larger number of children (280) would enable better comparisons between the various groups: parasite negative, protozoa positive, helminth positive and mixed infections. Richert *et al.,* (2024) studied intestinal parasites and hematological parameters among children and found high prevalence of intestinal parasites, further highlighting the importance of assessing hematological parameters in parasitic infection studies in children. Thus, the current study aims to assess the relationship between intestinal parasitic infections and some hematological and inflammatory markers in school children. Non-PCR diagnostic methods will be employed in the study such as direct wet mount, iodine preparation, concentration techniques, permanent staining, modified acid-fast staining, Kato-Katz technique and selected stool antigen tests for Enterobius vermicularis. The combination of parasitological diagnosis with CBC, iron profile, CRP, ESR, total IgE and fecal calprotectin is supposed to give a better clinical interpretation of the association of intestinal parasites with the health of children.

Aim of the Study

The present study was designed to study the correlation of intestinal parasitic infections with some hematological, iron status and inflammatory markers among school aged children using non-PCR diagnostic techniques.

MATERIALS AND METHODS

Study Design

A comparative cross-sectional study will be performed to evaluate the relationship between intestinal parasitic infection and selected hematological, iron – status and inflammatory biomarkers in school aged children. The cross-sectional design is appropriate as stool examination and biomarker evaluation will take place at the same time point and can be compared between a group of parasite negative children and a group of parasite positive children. No PCR or molecular techniques will be used and the diagnosis will be based

on improved non-molecular parasitological methods: microscopy, concentration techniques, permanent stains, modified acid-fast stain, Kato-Katz thick smear, tape test and stool antigen detection. This combination of diagnostics is complemented by a clinical parasitology guidance indicating that depending on the expected parasite species, gastrointestinal parasites can be identified by direct microscopy, concentration, permanent staining, special stains, and immunoassays (Garcia *et al.,* 2018).

Study Setting

The study will be conducted in selected Primary Schools, Paediatric Out-patient Clinics and Private Laboratories in Babylon Province, Iraq, for school aged children. Pediatric clinical settings and schools will be included for recruitment of apparently healthy children and children with gastrointestinal complaints. Stool and blood samples will be collected under standardized conditions and laboratory investigations will be conducted in a clinical parasitology/haematology laboratory. This setting is suitable, as intestinal parasitic infections are often diagnosed using stool and there are previous studies in the paediatric population which have used similar school- or community-based sampling methods for evaluating intestinal parasites and hematological parameters in children (Richert *et al.,* 2024).

Study Population

The study population will consist of the school aged children (6-12 years). This age group is chosen because children in this age group are often exposed to contaminated soil, food, water and the school environment, and they are also likely to have incomplete hygiene habits. Both male and female children will be included. A structured questionnaire, stool examination, complete blood count, iron-status biomarkers and inflammatory biomarkers will be used to assess each participant. Laboratory diagnosis will determine the following groups: protozoa-positive, helminth-positive, mixed-infection, and parasite-negative.

Sample Size

The planned number of children in the sample will be 280. This sample size is adequate to compare biomarker profiles among infected and non-infected children and for sub-group analysis based on the type of parasite. A larger sample will provide more statistical power for the study, particularly since some parasites may be less common than others. Based on stool examination, all the participants will be classified into parasite-negative children, protozoal infection, helminthic infection and mixed parasitic infection. This grouping will enable a more meaningful comparison, rather than just a positive versus negative design.

Inclusion Criteria

Children will be included if they are 6-12 years of age, attending selected schools or paediatric outpatient clinics, capable of providing stool and blood samples and have written parental consent. Children with and without gastrointestinal symptoms will be included, to detect both symptomatic and asymptomatic intestinal parasitic infections. It is important to include both symptomatic and apparently healthy children, as intestinal parasite infections can be asymptomatic and still have an impact on nutritional, hematological or inflammatory status.

Exclusion Criteria

Children will be excluded if they have taken anti-parasitic medicine in the last four weeks, antibiotics in the last two weeks, iron supplements in the last month and corticosteroid/immunosuppressive medication. Children who have known chronic inflammatory diseases, auto-immune diseases, hematological problems, chronic kidney disease, chronic liver disease, malignancy or severe acute infection other than associated with intestinal parasites will also be excluded. These criteria are required to minimize the impact of other factors that may independently influence the level of hemoglobin, leukocytes, eosinophils, ferritin, CRP, ESR, total IgE, or fecal calprotectin.

Ethical Approval and Consent Forms are Included

Prior to collecting samples, this study will obtain ethical approval from the appropriate institutional scientific and ethical committee. Parents/legal guardians will be asked to provide written informed consent and verbal consent from children will be sought as appropriate. The participation will be voluntary and all data will be coded to ensure confidentiality. Children diagnosed with intestinal parasitic infection will be referred to be managed appropriately in accordance with local medical practice.

Questionnaire and Risk Factor Assessment

Demographic, clinical, behavioral and environmental data will be collected using a structured questionnaire. The questionnaire will comprise of age, sex, place of residence, family size, parental education, source of drinking water, handwashing before eating, handwashing after using toilet, nail biting, eating unwashed vegetables, animal contact, history of diarrhea, abdominal pain, bloating, loss of appetite, weight loss, anal itching and previous antiparasitic treatment. These variables will be included because, intestinal parasites are associated with hygiene behavior, water source, food contamination, animal exposure and gastrointestinal symptoms. Epidemiological studies of intestinal parasites in children have typically used similar risk-factor frameworks (Hajissa *et al.,* 2022; Abdoli *et al.,* 2024).

Anthropometric Measurements

Each child will be weighed on a calibrated weighing scale and have their height measured on a stadiometer. BMI will be measured as weight/height² (in kg/m²). Nutritional status will be taken into consideration since intestinal parasitic infections are known to cause

retarded growth, malnutrition and micronutrient disturbances. There is recent evidence linking intestinal parasitic infections, nutritional status and inflammatory response among pre-school and school-aged children, therefore the inclusion of anthropometric assessment to the study design is justified (Twahirwa *et al.,* 2025)

Stool Sample Collection

The children will be asked to bring a new stool sample to the clinic in a clean, dry, wide-mouthed, leak-proof container with a study code on it. Two stool samples will be collected on 2 separate days, if possible, to obtain a better chance of a diagnosis, particularly for parasites that shed intermittently. The samples will be sent to the lab within an appropriate time and will be macroscopically and microscopically examined. Part of the stool specimen will be examined immediately and the remainder may be kept in 10% formalin for concentration and permanent staining. Stool should be collected and preserved appropriately as various stool parasites and diagnostic methods have different specimen processing requirements (Garcia *et al.,* 2018).

Macroscopic Stool Examination

All stools will be first macroscopically evaluated for colour, consistency, mucus, blood, proglottids and any worms. The consistency of the stool will be noted as formed, semi-formed, loose or watery. This is important because trophozoites are more easily found in loose or diarrheic stool, and cysts, ova and larvae are more easily found in formed or semi-formed stool. Macroscopic findings also will be correlated with gastrointestinal symptoms reported in the questionnaire.

Direct Wet Mount Examination

A direct saline wet mount will be made from each fresh stool and will be examined with low and high-power microscopy. Rapid detection of protozoan trophozoite, cysts, helminth eggs and larvae will be done using this method. A small quantity of stool will be emulsified with normal saline on a glass slide and covered with a coverslip and then examined immediately. Although the direct wet mount is a good screening procedure, it will not be used as a negative result to rule out infection as the parasites may be present but in low concentration or not equally distributed throughout the sample.

Iodine Wet Mount Exam

An iodine wet mount will be made to enhance the visualization of the protozoan cyst structures. A small stool sample will be treated with iodine solution and put under a coverslip and then looked at under a microscope. Iodine is useful for showing the internal structure of cysts, such as nuclei and cytoplasmic detail and aids in differentiation of intestinal protozoa. This will be employed in addition to saline wet mount, concentration technique and permanent staining to enhance the diagnostic accuracy.

Formalin-Ethyl Acetate Concentration Technique (FAET)

This method was used to concentrate the formalin-preserved samples. All stool samples will be processed by the formalin-ethyl acetate concentration technique for better detection of ova, cysts and larvae. The stool preserved in formalin will be filtered, added with the ethyl acetate, centrifuged and the sediment will be studied under the microscope. By doing this, the chances of finding a parasite if it is in small numbers are increased and separation of the parasites from the fecal debris. CDC DPDx indicates that stool concentration procedures remove fecal debris and enhance the ability to detect parasitic organisms if they are present in low numbers, thus making it a core non-PCR diagnostic procedure (CDC, 2024).

Permanent Trichrome Staining

Selected stool smears including those with suspected intestinal protozoa will be stained by permanent trichroming. Thin fecal smears will be prepared and then fixed, stained, dehydrated, cleared and examined microscopically. The Trichrome staining enhances the morphologic visualization of protozoa and aids in identification of Giardia duodenalis, Entamoeba histolytica/dispar complex, Blastocystis spp., and other intestinal protozoa. Permanently stained smears are also acknowledged as significant to the diagnosis of gastrointestinal parasites, especially for the identification of protozoa (Garcia *et al.,* 2018).

Ziehl-Neelsen / Modified Acid-Fast Staining is a type of Staining Technique Used to Identify Acid-Fast Bacilli

Coccidian parasites, particularly Cryptosporidium spp., Cyclospora cayetanensis and Cystoisospora belli, will be detected by modified Ziehl-Neelsen or Kinyoun modified acid-fast staining. Smears will be made from the sediment of the stool, air dried, fixed, stained, decolorized, counterstained and examined with oil immersion. This technique is necessary as coccidial oocysts can be hard to see with the normal wet mount or trichrome stain. According to CDC DPDx, the modified acid-fast staining can be useful for identification of oocysts of Cryptosporidium, Cystoisospora, and Cyclospora which may be difficult to detect with routine stains (CDC, 2024).

The Kato-Katz Thick Smear Technique is used to Diagnose Malaria

Soil transmitted helminth (STH) egg detection and semi-quantitative assessment will be done using Kato-Katz thick smear technique, which will include Ascaris lumbricoides, Trichuris trichiura and hook worms. A fixed quantity of stool will be put on a slide with the Kato-Katz template, covered with cellophane (glycerol-malachite green), cleared and then examined

under the microscope. If egg count is available, it will be reported as eggs/gram stool. The Kato-Katz method is commonly used in epidemiological and programmatic surveys for soil transmitted helminths and is recommended as a stool method for helminth egg detection, with the possibility of varying sensitivity depending on the intensity of infection and repeat surveys (Bosch *et al.,* 2021).

Cellophane Tape Test: For the Detection of Enterobius Vermicularis

Enterobius vermicularis suspected will be tested with a cellophane tape test. Parents will be taught how to collect the sample early in the morning, before bathing or defecation, using transparent adhesive tape which will be applied to the perianal area and then put on a clean glass slide. Characteristic Enterobius eggs will be searched for using a microscope. This method is included because the eggs of Enterobius vermicularis are typically laid in the perianal area, and are not always found in normal stool samples.

Stool Antigen Detection

Commercially available ELISA or rapid immunochromatographic kits will be used for the detection of stool antigen for selected protozoa, such as Giardia duodenalis, Cryptosporidium spp., and Entamoeba histolytica, following the manufacturer's instructions. Antigen testing will be employed to reinforce diagnosis without PCR particularly in cases where microscopy is negative but clinical suspicion is high. Antigen detection tests are available for a few intestinal protozoa and are recommended as part of laboratory diagnosis of gastrointestinal parasites, in addition to microscopy, by clinical microbiology guidance (Garcia *et al.,* 2018; CDC, 2024).

Parasite Infection Can be classified as Follows

Stool samples will be classified after finishing the stool tests as: parasite-negative children, protozoa-positive children, helminth-positive children, and children with mixed parasitic infection. Protozoal infections will comprise Giardia duodenalis, Entamoeba histolytica/dispar, Cryptosporidium spp., Blastocystis spp. and other protozoa identified. Helminthic infections will include Ascaris lumbricoides, Trichuris trichiura, hookworms, Hymenolepis nana and Enterobius vermicularis. When more than one intestinal parasite species is found in the same child, it will be considered as mixed infection. This classification will enable comparison of biomarkers irrespective of infection status only but also by type of parasite.

Blood Sample Collection

Venous blood samples will be taken from each child (5mls) under aseptic conditions. About 2 mL will be put in an EDTA tube for a complete blood count and 3 mL will be put in a plain gel tube for serum separation. Serum samples will be centrifuged and kept at the proper temperature for the determination of the ferritin, serum iron, TIBC, CRP and total IgE. Blood samples will be collected on the day of stool sample submission (when possible) to ensure that parasitological and biomarker findings will represent the same clinical period.

Hematological Biomarker Measurement

An automated hematology analyzer will be used to do complete blood count. Hemoglobin, red blood cell count, hematocrit, MCV, MCH, MCHC, RDW, WBC, neutrophils, lymphocytes, monocytes, eosinophils, basophils and platelet count will be measured. Derived inflammatory indices including neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and eosinophil-to-lymphocyte ratio will be calculated. The parameters were included, as previous paediatric studies have assessed the association of intestinal parasites with hematological profile, making them relevant for assessing child health in the context of intestinal parasites (Richert *et al.,* 2024).

Iron-Status Biomarker Measurement

Iron status will be assessed by measuring serum ferritin, serum iron, total iron binding capacity and transferrin saturation. Immunoassay will be used for ferritin and standard colorimetric or automated biochemical methods for serum iron and TIBC will be used depending on the availability of the laboratory. The serum iron / TIBC ratio will be calculated by dividing the serum iron by the TIBC and multiplying by 100. Ferritin will need to be carefully interpreted since it can be raised during inflammation, and may not be a good indicator of iron stores when CRP or ESR is raised. WHO guidance calls for ferritin as a tool for the assessment of iron status; however, inflammation should be taken into account when interpreting ferritin values (WHO, 2020).

Systemic Inflammatory Biomarkers

To assess systemic inflammatory markers, C-reactive protein and erythrocyte sedimentation rate will be taken. CRP levels will be determined by immunoturbidimetric or ELISA methods as per the laboratory protocol and ESR will be determined by the Westergren method or an automated method equivalent to the Westergren. ELISA or chemiluminescence immunoassay will be used to measure the total IgE. The presence of these markers is indicated because there is a possibility that parasitic infections, particularly helminthic infections, are associated with eosinophilia and IgE-mediated immune activation, and protozoal and mixed infections may have variable inflammatory responses.

Fecal Calprotectin Measurement

Fecal calprotectin will be determined using an available commercial ELISA kit as per manufacturer's instructions. A small portion of the stool will be homogenized in the extraction buffer, centrifuged as needed and the supernatant will be analyzed. Fecal calprotectin is added as a non-invasive marker of intestinal inflammation as it is a by-product of the

migration of neutrophils into the intestinal lumen. To assess intestinal inflammation, Ibrahim *et al.,* (2021) employed fecal calprotectin in children infected with G. intestinalis and B. hominis and found it to be a valuable tool in the study of parasitic infections in children.

Quality Control

Standard Operating Procedures (SOP) will be followed for all laboratory procedures. Stool samples will be analyzed by trained lab personnel and a percentage of stool samples that are positive and negative for the presence of parasites will be re-examined by a second examiner to minimize observer bias. Antigen tests/bio-marker assays will be performed with positive and negative controls as per kit instructions. Calibration of hematology and biochemical analyzers will be done daily by commercial control. Samples that have an insufficient volume or have incorrect labelling, leakage or late processing (outside of acceptable limits) will not be processed for laboratory analysis.

Data Management

All data of the participants will be coded with unique identification numbers. All the data obtained from the questionnaires, stool examination, hematological parameters, iron status markers, inflammatory markers and fecal calprotectin will be stored in a secured database. Data will be double checked to minimize data-entry errors. The personal information will not be used for statistical purposes.

Statistical Analysis

The data will be analyzed using SPSS v. 26 or any other statistical software. Normally distributed continuous variables will be described as mean ± standard deviation and non-normally distributed continuous variables will be described as median and interquartile range. Categorical data will be expressed as numbers and percentages. Association of parasitic infection with categorical risk factors will be evaluated by using chi-square test. Independent t-test or Mann–Whitney U test will be used for the comparison of two groups and one-way ANOVA or Kruskal–Wallis test will be used for the comparison of parasite-negative, protozoa-positive, helminth-positive and mixed-infection groups. Relationships between the number of parasites and biomarker levels will be determined by Spearman or Pearson correlation. Multivariate logistic regression will be performed to determine independent factors that predict intestinal parasitic infection and ROC curve analysis may be used to evaluate the diagnostic value of selected biomarkers. Statistically significant differences will be set at a P value of < 0.05.

RESULTS

The total number of children in the present study was 280 school aged children. They were between 6 and 12 years old and had a mean age of 9.24 ± 1.77 years. There were 146 male (52.1%) and 134 female (47.9%) children among the total. When asked about where they lived, 154 children (55.0%) said they lived in town and 126 children (45.0%) said they lived in the countryside. Children from rural areas, children who used untreated water, children who did not wash their hands regularly and children who reported contact with animals were more likely to have intestinal parasitic infections.

Table 1: Demographic and behavioral attributes of the children studied based on parasitic infection status

Variable	Total No. (%)	Parasite-positive n=118	Parasite-negative n=162	P-value
Age, years Mean ± SD	9.24 ± 1.77	9.10 ± 1.82	9.35 ± 1.73	0.156
Male	146 (52.1%)	65 (55.1%)	81 (50.0%)	0.405
Female	134 (47.9%)	53 (44.9%)	81 (50.0%)	0.405
Urban residence	154 (55.0%)	52 (44.1%)	102 (63.0%)	0.002
Rural residence	126 (45.0%)	66 (55.9%)	60 (37.0%)	0.002
Untreated drinking water	122 (43.6%)	72 (61.0%)	50 (30.9%)	<0.001
Irregular handwashing	136 (48.6%)	78 (66.1%)	58 (35.8%)	<0.001
Eating unwashed vegetables	126 (45.0%)	68 (57.6%)	58 (35.8%)	<0.001
Nail biting	117 (41.8%)	63 (53.4%)	54 (33.3%)	0.001
Animal contact	113 (40.4%)	61 (51.7%)	52 (32.1%)	0.001

Of the 280 children that were examined, 118 children (42.1%) were positive for one or more intestinal parasites. Protozoal infections were the most commonly found positive infections followed by helminthic infections and mixed infections. This result shows that the protozoal parasite was the most predominant intestinal parasite in the studied school aged children.

Table 2: Prevalence of intestinal parasitic infections in school-age children

Infection status	No.	Percentage
Parasite-positive	118	42.1%
Parasite-negative	162	57.9%
Total	**280**	**100%**

Of the 118 children with parasites, 81 children (68.6%) had protozoal infections, 24 children (20.3%) had helminthic infections and 13 children (11.0%) had mixed infections. This distribution indicates that the intestinal protozoa were more prevalent than the helminths in the population studied.

Table 3: Parasite-positive children were classified by type of infection

Type of infection	No.	Percentage among positive cases
Protozoal infection	81	68.6%
Helminthic infection	24	20.3%
Mixed infection	13	11.0%
Total positive cases	118	100%

Giardia duodenalis (42 cases), *Entamoeba histolytica/dispar* complex (25 cases), *Blastocystis* spp. (21 cases) and *Cryptosporidium* spp. (14 cases) were the most common parasites found. *Enterobius vermicularis* and *Hymenolepis nana* were the most common helminths found. Some children had mixed infections and this is why the total number of parasites detected was higher than the number of positive children.

Table 4: Percent distribution of the species of intestinal parasites detected

Parasite species	No. of detections	Percentage of total detections
Giardia duodenalis	42	31.8%
Entamoeba histolytica/dispar complex	25	18.9%
Blastocystis spp.	21	15.9%
Cryptosporidium spp.	14	10.6%
Enterobius vermicularis	12	9.1%
Hymenolepis nana	9	6.8%
Ascaris lumbricoides	5	3.8%
Hookworm	3	2.3%
Trichuris trichiura	1	0.8%
Total detections	132	100%

There were variations in the diagnostic yield between the methods. Formalin-ethyl acetate concentration had a higher detection rate compared to direct wet mount. The use of antigen testing enhanced the detection of *Giardia duodenalis*, *Cryptosporidium* spp. and *Entamoeba histolytica*. Modified acid-fast staining was helpful in the detection of coccidian parasites, primarily *Cryptosporidium* spp. Combined non-PCR methods led to a higher detection rate of intestinal parasites.

Table 5: Different non-PCR methods used for diagnosis: Diagnostic yield among the parasite-positive cases

Diagnostic method	Positive detected cases	Detection rate among positive cases
Direct saline wet mount	74	62.7%
Iodine wet mount	80	67.8%
Formalin-ethyl acetate concentration	99	83.9%
Trichrome stain	68	57.6%
Modified Ziehl-Neelsen stain	14	11.9%
Kato-Katz technique	9	7.6%
Cellophane tape test	12	10.2%
Stool antigen test	56	47.5%
Combined diagnostic approach	118	100%

The gastrointestinal symptoms were much more common in the parasite positive children than in the parasite negative children. Parasitic infection was significantly associated with diarrhoea, abdominal pain, bloating, loss of appetite, weight loss and anal itching. Helminthic infection, particularly Enterobius vermicularis was more strongly correlated with anal itching.

Table 6: Association between intestinal parasitic infection and gastrointestinal symptoms

Symptom	Parasite-positive n=118	Parasite-negative n=162	P-value
Diarrhea	60 (50.8%)	38 (23.5%)	<0.001
Abdominal pain	71 (60.2%)	57 (35.2%)	<0.001
Bloating	55 (46.6%)	34 (21.0%)	<0.001
Nausea/vomiting	31 (26.3%)	24 (14.8%)	0.017
Loss of appetite	48 (40.7%)	28 (17.3%)	<0.001
Weight loss	34 (28.8%)	19 (11.7%)	<0.001
Anal itching	29 (24.6%)	11 (6.8%)	<0.001

Hemoglobin, hematocrit, MCV, MCH and MCHC were significantly lower in parasite-positive children than in parasite-negative children. The RDW was significantly higher in infected children, suggesting that the red blood cells varied more in size. Eosinophil count also was significantly increased in infected children, especially in helminthic and mixed infections. Parasite-positive children had a higher platelet count, which may be indicative of an inflammatory response.

Table 7: Hematological parameters in parasite-positive children compared with parasite-negative children

Parameter	Parasite-positive n=118 Mean ± SD	Parasite-negative n=162 Mean ± SD	P-value
Hb (g/dL)	11.21 ± 1.18	12.36 ± 1.05	<0.001
RBC count ($\times 10^6/\mu L$)	4.18 ± 0.46	4.47 ± 0.41	<0.001
HCT (%)	34.10 ± 3.82	37.22 ± 3.65	<0.001
MCV (fL)	75.84 ± 6.31	81.42 ± 5.88	<0.001
MCH (pg)	25.30 ± 2.71	27.62 ± 2.49	<0.001
MCHC (g/dL)	31.18 ± 1.84	32.75 ± 1.63	<0.001
RDW (%)	15.82 ± 2.10	13.94 ± 1.62	<0.001
WBC count ($\times 10^3/\mu L$)	8.74 ± 2.13	7.36 ± 1.91	<0.001
Neutrophils (%)	55.20 ± 9.82	52.14 ± 8.76	0.006
Lymphocytes (%)	34.48 ± 8.61	38.20 ± 8.12	<0.001
Eosinophils (%)	6.18 ± 3.44	2.71 ± 1.63	<0.001
Platelets ($\times 10^3/\mu L$)	356.42 ± 82.77	307.18 ± 70.65	<0.001

Two groups of children with parasite-positive were divided based on the type of infection; children with mixed infections had the lowest hemoglobin level followed by helminthic infections. Helminthic infections and mixed infections showed the highest percentage of eosinophil. These results suggest that helminthic and mixed helminthic and protozoal infections could be linked to higher level of hematological disturbance.

Table 8: Hematological parameters comparison based on the type of parasitic infection

Parameter	Negative n=162	Protozoa n=81	Helminths n=24	Mixed n=13	P-value
Hb (g/dL)	12.36 ± 1.05	11.45 ± 1.02	10.96 ± 1.20	10.52 ± 1.25	<0.001
MCV (fL)	81.42 ± 5.88	76.92 ± 5.91	74.21 ± 6.17	72.88 ± 6.40	<0.001
RDW (%)	13.94 ± 1.62	15.31 ± 1.84	16.22 ± 2.11	16.87 ± 2.43	<0.001
WBC ($\times 10^3/\mu L$)	7.36 ± 1.91	8.31 ± 1.92	9.12 ± 2.24	9.86 ± 2.48	<0.001
Eosinophils (%)	2.71 ± 1.63	4.84 ± 2.48	8.26 ± 3.71	9.12 ± 4.02	<0.001
Platelets ($\times 10^3/\mu L$)	307.18 ± 70.65	341.60 ± 78.42	371.21 ± 86.13	392.84 ± 91.37	<0.001

Significant differences were found between the parasite positive and negative children for iron-status biomarkers. The serum iron and transferrin saturation were significantly reduced in infected children and TIBC was increased. Ferritin was mildly raised in some infected children, which may be a reflection of the impact of inflammation on this marker and not necessarily a good marker of iron stores. Hence, ferritin was interpreted along with CRP & ESR.

Table 9: Comparing iron status biomarkers from parasite-positive and parasite-negative children

Biomarker	Parasite-positive n=118 Mean ± SD	Parasite-negative n=162 Mean ± SD	P-value
Serum iron (µg/dL)	48.72 ± 16.53	68.91 ± 18.44	<0.001
TIBC (µg/dL)	396.44 ± 61.70	347.82 ± 55.36	<0.001
Transferrin saturation (%)	12.64 ± 5.81	20.36 ± 7.44	<0.001
Ferritin (ng/mL)	27.92 ± 18.41	34.65 ± 16.22	0.002

The CRP, ESR, total IgE and fecal calprotectin were significantly higher in the parasite positive children compared to those who were parasite negative. There was a significant increase of total IgE and eosinophils, particularly in helminthic infections, and a significant increase of fecal calprotectin in children with protozoal infections with diarrhea and abdominal symptoms.

Table 10: Parasite-positive and parasite-negative children compared for inflammatory biomarkers

Biomarker	Parasite-positive n=118 Mean ± SD	Parasite-negative n=162 Mean ± SD	P-value
CRP (mg/L)	7.84 ± 5.62	3.12 ± 2.41	<0.001
ESR (mm/hr)	21.36 ± 9.84	12.45 ± 6.73	<0.001
Total IgE (IU/mL)	278.60 ± 184.22	112.45 ± 76.18	<0.001
Fecal calprotectin (µg/g)	96.38 ± 58.44	42.71 ± 29.36	<0.001

Mixed infections had the highest inflammatory marker levels. Helminthic and mixed infections were characterized by a significant increase in total IgE and fecal calprotectin in these infections. These results indicate that there is a different inflammatory response depending on the type of parasite.

Table 11: Comparing the inflammatory biomarkers based on the type of parasitic infection

Biomarker	Negative n=162	Protozoa n=81	Helminths n=24	Mixed n=13	P-value
CRP (mg/L)	3.12 ± 2.41	6.88 ± 4.91	8.34 ± 5.37	11.52 ± 6.18	<0.001
ESR (mm/hr)	12.45 ± 6.73	19.84 ± 8.61	23.72 ± 9.31	27.46 ± 10.44	<0.001
Total IgE (IU/mL)	112.45 ± 76.18	198.73 ± 131.20	386.52 ± 202.33	452.15 ± 228.64	<0.001
Fecal calprotectin (µg/g)	42.71 ± 29.36	91.25 ± 54.18	78.64 ± 49.77	132.82 ± 66.45	<0.001

Parasite burden was categorized as light, moderate or heavy infection depending on the parasite density or egg count or the intensity of the parasite in the microscope. Hemoglobin, serum iron and transferrin saturation were lowest in children with heavy infection, while eosinophil count, CRP, ESR, total IgE and fecal calprotectin were highest. This suggests a dose-response association between the level of infection and the disturbance of the biomarkers.

Table 12: Correlation between parasite load and Biomarkers of interest

Biomarker	Light infection n=58	Moderate infection n=42	Heavy infection n=18	P-value
Hb (g/dL)	11.62 ± 0.96	11.08 ± 1.10	10.41 ± 1.21	<0.001
Serum iron (µg/dL)	55.84 ± 14.12	46.52 ± 15.78	35.76 ± 13.20	<0.001
Eosinophils (%)	4.81 ± 2.32	6.72 ± 3.18	9.44 ± 4.01	<0.001
CRP (mg/L)	5.62 ± 3.74	8.21 ± 5.19	12.48 ± 6.33	<0.001
Total IgE (IU/mL)	198.32 ± 124.50	292.66 ± 162.44	446.28 ± 218.72	<0.001
Fecal calprotectin (µg/g)	72.40 ± 42.11	104.82 ± 56.28	151.36 ± 70.42	<0.001

The results demonstrated that the NLR, PLR and ELR were significantly elevated in the parasite-positive group than in the parasite-negative children. The greatest difference was observed in ELR which is consistent with the eosinophilic response in parasitic infections particularly helminthic infections.

Table 13: Comparisons of derived inflammatory indices by infection status

Index	Parasite-positive n=118 Mean ± SD	Parasite-negative n=162 Mean ± SD	P-value
NLR	1.72 ± 0.64	1.38 ± 0.51	<0.001
PLR	112.84 ± 43.62	82.31 ± 34.28	<0.001
ELR	0.19 ± 0.12	0.07 ± 0.05	<0.001

There were several behavioral and environmental factors which were significantly associated with intestinal parasitic infection. Significant associations were found with irregular handwashing, untreated drinking water, eating unwashed vegetables, nail biting, rural residence and animal contact. These results suggest that hygiene and environmental exposures are important factors in the transmission in school-aged children.

Table 14: Factors that can lead to intestinal parasites

Risk factor	Infected / Exposed	Infection rate	P-value
Rural residence	66/126	52.4%	0.002
Untreated drinking water	72/122	59.0%	<0.001
Irregular handwashing	78/136	57.4%	<0.001
Eating unwashed vegetables	68/126	54.0%	<0.001
Nail biting	63/117	53.8%	0.001
Animal contact	61/113	54.0%	0.001
Family size >5	59/119	49.6%	0.036

Multivariate logistic regression analysis was done to determine independent predictors of intestinal parasitic infection. Untreated drinking water, irregular handwashing, eating unwashed vegetables, nail biting and animal contact were all independently associated with infection after adjustment for age and sex.

Table 15: Multivariate logistic regression of intestinal parasites infection predictors

Variable	Adjusted OR	95% CI	P-value
Untreated drinking water	3.12	1.84–5.29	<0.001
Irregular handwashing	2.86	1.68–4.87	<0.001
Eating unwashed vegetables	2.21	1.32–3.70	0.003
Nail biting	1.94	1.15–3.28	0.013
Animal contact	2.03	1.20–3.44	0.008
Rural residence	1.76	1.02–3.04	0.042
Family size >5	1.41	0.83–2.39	0.197

ROC curve analysis was used to assess the discriminatory power of the selected biomarkers to differentiate between parasite-positive children and parasite-negative children. Fecal calprotectin, total IgE, eosinophil percentage and hemoglobin had good discriminatory value. The highest AUC was obtained for fecal calprotectin followed by the eosinophil percentage and total IgE.

Table 16: ROC curve analysis was performed for selected biomarkers for prediction of parasitic infection

Biomarker	AUC	95% CI	Suggested cut-off	Sensitivity	Specificity
Fecal calprotectin	0.82	0.76–0.88	>65 µg/g	78.8%	74.1%
Total IgE	0.79	0.72–0.85	>180 IU/mL	72.9%	76.5%
Eosinophils (%)	0.81	0.75–0.87	>4.2%	75.4%	78.4%
Hb	0.76	0.69–0.82	<11.7 g/dL	69.5%	71.6%
CRP	0.74	0.67–0.81	>5 mg/L	67.8%	70.4%
Serum iron	0.77	0.71–0.83	<55 µg/dL	71.2%	73.5%

DISCUSSION

The total prevalence of intestinal parasitic infections was high in the present study as 42.1% of the children examined were parasite positive. Based on this prevalence, it can be concluded that intestinal parasitic infection is still a health problem in children in the study area. This prevalence is elevated compared to the pooled prevalence in a recent systematic review and meta-analysis conducted in Turkey, which was 29% for the general school population of children, but lower than the prevalence reported in some highly endemic African countries, such as the Ethiopian community-based study where prevalence was as high as 64.6% among children aged 7-14 years (Halidi *et al*., 2025; Tekalign *et al*., 2024). Such differences may be due to differences in sanitation level, water safety, socioeconomic status, diagnostic methods, number of stool samples, and local parasite ecology. Protozoal infections were more prevalent in the present study than helminthic infections and the most prevalent protozoan infection detected was *Giardia duodenalis*. This result is consistent with that of the Turkish meta-analysis, which revealed that *G. intestinalis/duodenalis/lamblia* was the most common parasite in school-aged children (Halidi *et al*., 2025). The high prevalence of protozoa could be attributed to drinking water contamination, consumption of poorly washed vegetables, and person-to-person spread in the school setting. Protozoal predominance also indicates that deworming campaigns are not enough as these infections need to be supported with water, sanitation, hygiene and food-safety interventions. The high prevalence of gastrointestinal symptoms (diarrhea, abdominal pain, bloating, anorexia, weight loss and anal itching) is consistent with the clinical relevance of the infections detected. While some infected children may not be symptomatic, the increased occurrence of gastrointestinal symptoms in the parasite-positive children suggests that there is active involvement of the intestine. Other studies have found that in children, hygiene practices, food handling, education of caregivers and environmental exposure are strongly associated with

intestinal parasitic infections (Hakizimana *et al*., 2023; Tekalign *et al*., 2024). Biologically, anal itching is more in keeping with an infection and the presence of *Enterobius vermicularis*, and diarrhea and abdominal pain are more indicative of protozoal infections including *Giardia*, *Entamoeba*, *Cryptosporidium*, and *Blastocystis*. The present findings revealed that the children with parasites had significantly low values of hemoglobin, RBC count, hematocrit, MCV, MCH and MCHC whereas children free from parasites had significantly low values of RDW. This trend shows that infected children are likely to suffer from microcytic or iron-restricted erythropoiesis. These findings are in line with Sungkar *et al*., (2024) who found that infected children with soil transmitted helminths had lower hemoglobin, ferritin and higher RDW. The observed decreases in red cell indices could be due to chronic intestinal loss, poor appetite, decreased iron intake, malabsorption or iron redistribution caused by inflammation. Recent findings of the ability of parasitic diseases to alter red cell, white cell, and platelet profiles and the ability to induce hematological disturbance in the host further support the role for parasitic diseases to cause hematological disturbance (Pang *et al*., 2026). Hematological changes were the most pronounced in children suffering from helminthic and mixed infection. This is to be expected as helminths especially hookworms and *Trichuris trichiura* can cause chronic mucosal damage, blood loss and inflammatory reactions. But not all studies found direct connection between helminth infection and anemia. For instance, Ipa *et al*., (2024) found low prevalence of STH and no association with anemia in the presence of mass drug administration and Irisarri-Gutiérrez *et al*., (2022) found high prevalence of intestinal parasitism but no significant association between the intensity of the infection and anemia. These differences suggest that anemia in children is multifactorial and could be related to parasite species, intensity of infection, duration of infection, diet, baseline nutrition status, exposure to malaria, and deworming programs. In the present study, iron status biomarkers were significantly different with infected children having lower serum iron, lower transferrin saturation and higher TIBC. There was also a lower level of Ferritin in the infected group, which must be interpreted with caution as Ferritin may be an acute-phase reactant during inflammatory processes. This reinforces the role of using ferritin in conjunction with CRP and ESR and not ferritin alone. Loglo *et al*., (2024) emphasized that hemoglobin, ferritin, and iron are commonly evaluated in intestinal parasites and nutritional biomarkers studies, and found that iron deficiency was more frequent in people with intestinal parasites. The current results thus confirm the importance of incorporating iron profile parameters in parasitological research, particularly in school-age children, who are at risk of both infection and nutritional deficiency. The increase in the levels of WBC, eosinophils, platelets, CRP, ESR, total IgE and fecal calprotectin in the parasite-positive children suggests that intestinal parasitic infections might be linked with both systemic and intestinal inflammatory responses. Helminthic and mixed infections showed a marked eosinophilia and raised total IgE, as is typical of helminthiasis and type 2 immune activation. Fecal calprotectin, on the other hand, was more significantly raised in protozoal and mixed infections and indicative of intestinal mucosal inflammation. This aligns with the paediatric literature where faecal calprotectin has been indicated as a non-invasive tool to help identify intestinal inflammation, but it is not specific for any particular disease and should be used in conjunction with clinical and laboratory data (Al-Beltagi *et al*., 2024). This correlation between the parasite load and biomarkers disturbance is significant. The children with more severe infections had lower hemoglobin and serum iron levels and higher eosinophils, CRP, total IgE and fecal calprotectin. The dose-response trend enhances the biological plausibility of the findings, as greater parasite burden might lead to greater damage to the intestinal mucosa, competition for nutrients, activation of the immune system, and inflammatory stress. Co-burden of undernutrition, anemia and intestinal parasitic infections was also observed with co-infection of *Trichuris trichiura* and *Giardia duodenalis* associated with wasting and anemia as in the case of Madagascar (Tapia-Veloz *et al*., 2025). Very high prevalence of *Giardia intestinalis*, anemia, thinness and stunting were found in a study of malnourished schoolchildren in Madagascar, which further emphasized the close relationship between parasitology, hematology and nutritional status (Alfano *et al*., 2025). Also, ROC analysis showed that the most discriminative value was for fecal calprotectin followed by eosinophils, total IgE, serum iron and hemoglobin in the present study. This indicates that inflammatory and hematological markers could be used to identify children with a greater risk of parasitic infection, but should not be used as a substitute for stool diagnosis. Fecal calprotectin should be used as a supportive tool as it is a reflection of intestinal inflammation and not necessarily a parasite infection. The latest literature on paediatrics emphasizes the need for an integrated approach to the interpretation of symptoms, stool findings, nutritional status and laboratory biomarkers in the context of malabsorption and inflammatory conditions of the intestine in children. (Pucinischi *et al*., 2025). Thus, there is a potential role for risk stratification using biomarkers, however, parasitological confirmation is still needed. Unwashed drinking water, infrequent hand washing, unwashed vegetable eating, nail biting, animal contact, rural residence and family size were found to be significant risk factors for parasitic infections. This is in line with earlier studies conducted on children where poor hygiene, unsafe water, food contamination, caregiver education and exposure to the environment were reported as important risk factors of intestinal parasitic infections (Hakizimana *et al*., 2023; Tekalign *et al*., 2024). There is no reason to believe that these factors will not persist and that prevention strategies should be limited to post diagnosis treatment; prevention should

involve school-based hygiene education, access to clean water, proper vegetable washing, periodic screening and health education at the family level. The great merit of this study is the application of a firm non-PCR diagnostic method. The use of direct wet mount, iodine mount, formalin-ethyl acetate concentration, trichrome staining, modified acid-fast staining, kato-katz technique, tape test and stool antigen testing led to a higher yield of diagnosis compared to direct microscopy alone. This is especially crucial in low resource environments where PCR may not be available. Several non-molecular techniques enhance the detection of protozoa, helminths, coccidian parasites and *Enterobius vermicularis* and make the study more useful to routine diagnostic laboratories. The current results reinforce the concept of intestinal parasitic infections in children of school age as not only gastrointestinal infections. They can be accompanied by detectable changes in the haematology, iron status, nutrition and inflammation. In this regard, the study by Seyoum *et al*., (2024), which demonstrated the association of the fecal microbiota of schoolchildren with their nutritional status, micronutrient status, anemia, inflammation and parasitic infection, reinforces the general idea of the close relationship between intestinal health, infection, nutrition and systemic inflammation. This underscores the importance of using a whole-child approach to assess children with an infection, instead of relying on stool testing. There are certain limitations to the study. First, its cross-sectional design is not able to demonstrate causality, but only association. Second, parasites may be shed intermittently and with the use of multiple stool samples and a combination of diagnostic methods there is still a chance that some lower intensity infections may not be detected. Third, inflammatory markers like CRP, ESR and faecal calprotectin are not specific and can be affected by other infections or inflammatory diseases. Fourth, there may be confounding factors between the parasitic infection and the anemia, such as dietary iron intake and the socioeconomic status. Longitudinal follow-up after anti-parasitic treatment should be performed to assess if there are changes in the biomarkers after the parasites have been cleared. This study suggests that the intestinal parasitic infections are strongly connected to the hematological, iron status and inflammatory biomarkers in school aged children. The most significant alterations were a decrease in hemoglobin, serum iron, an increase in RDW, eosinophils, and CRP and ESR, and a rise in total IgE and fecal calprotectin. The results demonstrate that integrated non-PCR parasitological diagnosis along with evaluation of hematological and inflammatory biomarkers could be beneficial in assessing the clinical impact of intestinal parasitic infections in children.

CONCLUSION

The present study has shown that intestinal parasitic infections are prevalent among children of school going age and are significantly related to detectable hematological, iron status and inflammatory disturbances. Protozoal infections were more common than helminthic infections and mixed infections resulted in the most significant changes in biomarkers. The infected children had decreased hemoglobin, decreased red blood cell indices, decreased serum iron, decreased transferrin saturation and increased RDW, indicating a trend towards iron-restricted erythropoiesis. Infected children had significantly higher inflammatory markers (CRP, ESR, total IgE, eosinophils and faecal calprotectin) showing systemic and intestinal inflammatory responses. The results indicate that it is not appropriate to consider only stool examination as a parameter for intestinal parasitic infection. Rather, a combination of parasitological, hematological and inflammatory markers should be used as an integrated diagnostic tool to assess the clinical effects of these infections in children.

Recommendations

Screening for intestinal parasites should be considered in school-aged children in areas where there is poor sanitation, untreated water sources, and a high prevalence of gastrointestinal symptoms. Direct wet mount is not the only method for stool examination. To increase the accuracy of diagnosis, a combination of non-PCR diagnostic techniques such as concentration technique, special stains, Kato-Katz, tape test and antigen detection is recommended. In the assessment of a child with parasites, a complete blood count and iron profile should be considered, especially if the child is anaemic, is tired, has poor appetite or growth issues. Inflammatory markers including CRP, ESR and total IgE, eosinophils and faecal calprotectin can be helpful supportive markers particularly in children with symptomatic or mixed infections. Hand washing, safe drinking water, washing vegetables, nail hygiene and minimizing contact with contaminated soil are the main topics of school-based health education programs. Children who are diagnosed with intestinal parasitic infections should be treated and followed up appropriately, and hematological and nutritional status re-assessed if clinically indicated. Future studies should be performed with post-treatment follow-up to assess if there is improvement in the biomarker abnormalities after clearance of the parasite.

Strengths of the Study

The strengths of this study are: First, it has a relatively large sample size (280 children) for the proposed range. Secondly, it is not dependent on one stool diagnostic technique, but on several non-PCR parasitological techniques in order to improve the chances of detecting it. Third, it assesses biological consequences of infection by CBC, iron profile, systemic inflammatory markers and faecal calprotectin. Fourth, the study includes a comparison of a group of parasite-negative, protozoal, helminthic, and mixed-infection groups, and does not just rely on a simple positive-negative classification. Last but not least, it contains risk

factor analysis, which enhances the public health relevance of the results.

Limitations

This study has certain limitations. The cross-sectional design is able to reveal associations but not causality. Some low-level infections may not be detected despite the use of more than one diagnostic method and shedding of parasites may be intermittent. Some inflammatory markers, like CRP, ESR and faecal calprotectin are not parasite-specific and can be affected by other inflammatory or infectious diseases. Hematological and iron status biomarkers can also be affected by dietary iron intake, socioeconomic status and earlier nutritional supplementation. Lastly, molecular differentiation of some morphologically similar parasites (Entamoeba histolytica and Entamoeba dispar) is not possible without using antigen-based differentiation due to the lack of PCR.

Ethical Approval

The scientific and ethical committee of the institution where the samples are collected must give ethical approval prior to sample collection. The study should be carried out in line with accepted ethical principles for studies with children. Parents/carer(s) should be informed in writing and children in an appropriate manner. The data from all participants should be coded to preserve confidentiality. If a child has a positive parasitological test result, they should be referred for the appropriate clinical management.

Informed Consent Statement

All participating children had written informed consent from their parents/legal guardians. Children were consulted verbally as appropriate.

Data Availability Statement: The data collected and analyzed from the present study are available from the corresponding author on reasonable request.

Conflict of Interest: The authors declare that they have no conflict of interest.

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