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Seasonal Haematological and Biochemical Patterns of Paediatric Entamoeba Infection in Benghazi: One Health–Sustainability Implications

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ABSTRACT:

Background: Paediatric intestinal amoebiasis remains clinically relevant in settings affected by limitations in water safety, sanitation and food hygiene. Evidence from Libya regarding its seasonal distribution and associated haematological, inflammatory and biochemical patterns remains limited. This study characterised these profiles among children with microscopy-detected *Entamoeba histolytica/dispar* complex in Benghazi and considered their One Health and sustainable development implications.

Methods: This retrospective laboratory-based analytical study included 799 microscopy-positive paediatric cases examined at Benghazi Children's Hospital from January to December 2023. Demographic, temporal, haematological, inflammatory and biochemical variables were analysed. Months were grouped into four seasons, and anaemia was classified using the 2024 World Health Organization age- and sex-specific haemoglobin thresholds. Data were summarised using frequencies, percentages, medians and interquartile ranges. Group comparisons, Spearman's correlations and robust multivariable regression were applied, with false-discovery-rate correction for multiple comparisons.

Results: Males accounted for 450 cases (56.3%), and 790 cases (98.9%) involved children aged one or two years. The highest number of positive cases occurred in summer (261/799; 32.7%), followed by autumn (251/799; 31.4%). Anaemia was identified in 371 cases (46.4%): 22.5% mild, 19.8% moderate and 4.1% severe. Two-year-olds had greater odds of anaemia than one-year-olds (odds ratio 2.65; 95% confidence interval 1.99–3.54). Serum iron correlated with haemoglobin (Spearman's ϱ=0.470; $p<0.001$), while white blood cell count correlated with C-reactive protein (ϱ=0.314; $p<0.001$). After multiple-

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testing correction, haemoglobin, white blood cell count and C-reactive protein did not differ significantly by season. Seasonal differences in serum iron, calcium and urea had small effect sizes.

Conclusion: Microscopy-positive paediatric *Entamoeba* cases showed a high frequency of anaemia and coherent iron-related and inflammatory laboratory relationships. The summer–autumn concentration represented case distribution rather than seasonal prevalence because testing denominators were unavailable. Integrated paediatric laboratory assessment and strengthened surveillance linking clinical services with water, sanitation and food-safety sectors may support One Health-informed prevention and progress towards Sustainable Development Goals 2, 3, 6 and 17.

1. INTRODUCTION

Amoebiasis is an intestinal and extra-intestinal parasitic disease caused by *Entamoeba histolytica* and acquired predominantly through ingestion of mature cysts in faecally contaminated food, water or material transferred by inadequately cleaned hands. Most infections remain asymptomatic, but invasive disease may produce colitis, mucosal ulceration, bloody diarrhoea and, less commonly, extra-intestinal disease, particularly amoebic liver abscess. Clinical expression reflects a complex interaction among parasite virulence, host immunity, nutritional status, intestinal microbiota and environmental exposure (Kashwani et al., 2023; Carrero et al., 2020; Nasrallah et al., 2022; Guillén, 2023; Morán et al., 2023).

Diagnosis remains difficult in routine practice. Direct saline and iodine microscopy is accessible and inexpensive, yet it cannot reliably distinguish pathogenic *E. histolytica* from the morphologically similar *E. dispar* and *E. moshkovskii*. Species-level antigen assays and molecular methods improve specificity, while microscopy-only studies should adopt conservative terminology such as the *E. histolytica/dispar* complex (Ravi A et al., 2025; Calle-Pacheco et al., 2022; Cooney et al., 2025). This limitation is particularly important in Libya, where historical microscopy-based investigations documented the *E. histolytica/dispar* complex in Benghazi and Al-Khoms and may have combined morphologically indistinguishable pathogenic and non-pathogenic *Entamoeba* species (El-Ammari and Nair, 2003; El-Ammari et al., 2004; El-Ammari and Nair, 2015; Ghenghesh et al., 2016).

Young children are vulnerable to the combined consequences of enteric infection, reduced dietary intake, inflammation, blood loss and underlying micronutrient deficiency. Haemoglobin, haematocrit, red blood cell count, erythrocyte volume and serum iron may reveal clinically relevant iron-related haematological patterns, whereas white blood cell count, platelet count and C-reactive protein (CRP) provide complementary information about inflammatory activity. Invasive amoebic colitis can induce mucosal inflammation through both parasite and host mediators, and recent clinical work has renewed interest in complete blood count-derived inflammatory indices in adhesin-confirmed amoebiasis (Ngobeni et al., 2017; Uğraklı et al., 2026). Nevertheless, these laboratory abnormalities are non-specific and cannot be attributed causally to *Entamoeba* without an appropriate comparison group and stronger diagnostic confirmation. Paediatric studies from African settings have likewise documented substantial burdens of intestinal protozoa and other diarrhoeal pathogens, underscoring the need for integrated parasitological and clinical assessment (Oyegue-Liabagui et al., 2020; Agyekum et al., 2021; Mero et al., 2021).

Seasonality may influence enteric pathogen detection through changes in temperature, precipitation, water availability, household water storage, wastewater integrity, food handling and healthcare attendance. Studies of childhood diarrhoeal pathogens show that seasonal patterns differ by organism and environmental setting, while shifts in drinking-water sources and interrupted supplies may modify exposure pathways (Nguyen et al., 2021; Mero et al., 2023). The distinction between seasonal case counts and seasonal prevalence is essential: a larger number of positive cases in a given season does not establish greater infection risk unless the total number tested during the same period is known. Epidemiological patterns and associated risk factors also vary across populations and exposure settings (Lin et al., 2022).

Earlier Libyan investigations conducted in Benghazi and Al-Khoms documented the persistent occurrence of intestinal protozoa, including the microscopy-detected *E. histolytica/dispar* complex, across Libyan and other resident populations (El-Ammari and Nair, 2003; El-Ammari et al., 2004; El-Ammari and Nair, 2015). The present analysis extends this local evidence base by integrating seasonal, haematological, inflammatory and biochemical measurements among paediatric cases confirmed

as microscopy-positive. The study also applies a One Health-informed interpretation. In this context, One Health does not imply that domestic animals are a proven principal reservoir of human *E. histolytica*; rather, it connects child health with laboratory surveillance, water and wastewater management, food hygiene, environmental contamination and multisectoral governance (One Health High-Level Expert Panel, 2022).

The primary objective was to characterise the seasonal, haematological, inflammatory and biochemical profiles of microscopy-positive paediatric *Entamoeba* cases at Benghazi Children's Hospital. Secondary objectives were to quantify anaemia using contemporary WHO thresholds, compare laboratory profiles by age, sex and season, examine relationships among iron-related and inflammatory variables, and translate the findings into One Health surveillance and sustainable development priorities.

2. MATERIALS AND METHODS

2.1 Study design and setting

This retrospective laboratory-based analytical study used laboratory records from Benghazi Children's Hospital, Benghazi, Libya, covering 1 January to 31 December 2023. The analytical question concerned seasonal and laboratory variation within microscopy-positive cases; the study was not designed to estimate prevalence or to compare infected and uninfected children. Reporting was structured in accordance with the STROBE recommendations for observational studies (von Elm et al., 2008).

2.2 Study population and case definition

The study included 799 paediatric cases examined at the Parasitology Laboratory of Benghazi Children's Hospital between January and December 2023. All included cases had a positive stool microscopy result for *Entamoeba*. The study included male and female children aged 1, 2 or 15 years who were referred from the gastroenterology department (GASTRO), medical unit A (MUA), medical unit B (MUB), medical unit C (MUC), newborn ward (NNW), isolation unit (ISO), nephrology department (NEPHRO), intensive care unit (ICU) and haematology department.

All 799 microscopy-positive cases with the required principal demographic, haematological and biochemical measurements were included in the main analysis.

Demographic variables included age, sex and referring hospital department. Age was recorded in completed years, and sex was classified as male or female. The laboratory assessment included white and red blood cell counts, haemoglobin, haematocrit, mean corpuscular volume, platelet count, C-reactive protein, serum iron, calcium, urea, aspartate aminotransferase and alanine aminotransferase. Ferritin and magnesium were available for a limited subset of cases and were therefore included only in descriptive supplementary analyses.

To investigate temporal patterns, the month of examination was recorded for each case and subsequently classified into four seasons: winter comprised December, January and February; spring comprised March, April and May; summer comprised June, July and August; and autumn comprised September, October and November. As all included cases were microscopy-positive, monthly and seasonal findings were interpreted as distributions of recorded positive cases rather than prevalence or positivity rates.

2.3 Parasitological examination

Stool specimens were examined in the hospital parasitology laboratory using routine direct wet-mount microscopy with physiological saline and Lugol's iodine. Microscopic examination was performed for the detection of *Entamoeba* cysts and/or trophozoites. Because conventional light microscopy cannot reliably differentiate pathogenic *E. histolytica* from the morphologically similar species *E. dispar* and *E. moshkovskii*, the parasitological finding was reported throughout the study as microscopy-detected *E. histolytica/dispar* complex. The term *E. histolytica* was reserved for discussion of the pathogenic species in its established biological and clinical context.

2.4 Haematological and biochemical variables

The haematological variables included white blood cell count (WBC), red blood cell count (RBC), haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV) and platelet count. The inflammatory and biochemical variables included C-reactive protein (CRP), serum iron, calcium, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), ferritin

and magnesium. Ferritin and magnesium were summarised descriptively because they were available for only a limited subset of cases.

2.5 Anaemia definition

Anaemia was classified using the 2024 WHO age- and sex-specific haemoglobin thresholds. For children aged 6–23 months, no anaemia was defined as Hb ≥10.5 g/dL, mild anaemia as 9.5–10.4 g/dL, moderate anaemia as 7.0–9.4 g/dL and severe anaemia as <7.0 g/dL. For children aged 24–59 months, the corresponding categories were ≥11.0, 10.0–10.9, 7.0–9.9 and <7.0 g/dL. For cases aged 15 years, sex-specific WHO thresholds for adolescents aged 15–19 years were applied (World Health Organization, 2024).

2.6 Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics, version 28. Categorical variables were presented as frequencies and percentages, whereas continuous variables were summarised as medians and interquartile ranges. Group differences were assessed using appropriate non-parametric tests, and associations were examined using Spearman's correlation and robust multivariable regression. Multiple comparisons were adjusted using the Benjamini–Hochberg procedure, and sensitivity analyses were conducted for flagged observations, with $p<0.05$ considered statistically significant. Approximate 95% confidence intervals were calculated for selected correlation coefficients.

2.7 One Health and sustainable development framework

Findings were interpreted across four linked domains: paediatric clinical care; parasitology and laboratory surveillance; water, sanitation, food and environmental health; and multisectoral governance. The framework prioritised SDG 2 (nutrition), SDG 3 (health), SDG 6 (water and sanitation) and SDG 17 (partnerships), while avoiding claims that environmental exposures were directly measured.

2.8 Ethical considerations

The study used retrospective hospital laboratory records and involved no direct patient contact. Data were analysed without names or direct identifiers. Administrative permission was taken from Benghazi Children's Hospital and the relevant ethics committee.

3. RESULTS

3.1 Demographic and temporal characteristics

A total of 799 microscopy-positive paediatric cases were analysed. Male cases accounted for 450 (56.3%) and female cases for 349 (43.7%). The age distribution was strongly concentrated in early childhood: 409 cases (51.2%) involved children aged one year and 381 (47.7%) involved children aged two years; only nine cases (1.1%) involved 15-year-olds. Gastro contributed 651 cases (81.5%). The remaining cases originated from MUA (27; 3.4%), NNW (26; 3.3%), MUB (24; 3.0%), ICU (21; 2.6%), MUC (18; 2.3%), Haematology (12; 1.5%), Nephro (11; 1.4%) and ISO (9; 1.1%).

Monthly case counts were lowest in February (n=34), increased progressively from March onwards, reached 99 in August and peaked at 108 in September. Summer contributed 261 cases (32.7%), autumn 251 (31.4%), spring 154 (19.3%) and winter 133 (16.6%). These proportions describe the distribution of positive cases and do not represent seasonal positivity rates. (Table 1; Figures 1 and 2).

Table 1. Demographic, departmental and seasonal characteristics of the analysed cases.

Characteristic	Category	n	%
Sex	Male	450	56.3
	Female	349	43.7
Age (years)	1	409	51.2
	2	381	47.7

	15	9	1.1
Department	Gastro	651	81.5
	MUA	27	3.4
	MUB	24	3.0
	MUC	18	2.3
	NNW	26	3.3
	ISO	9	1.1
	Nephro	11	1.4
	ICU	21	2.6
	Haematology	12	1.5
Season	Winter	133	16.6
	Spring	154	19.3
	Summer	261	32.7
	Autumn	251	31.4

Note: Percentages are calculated from 799 microscopy-positive cases. Department abbreviations are presented as used in the hospital register.

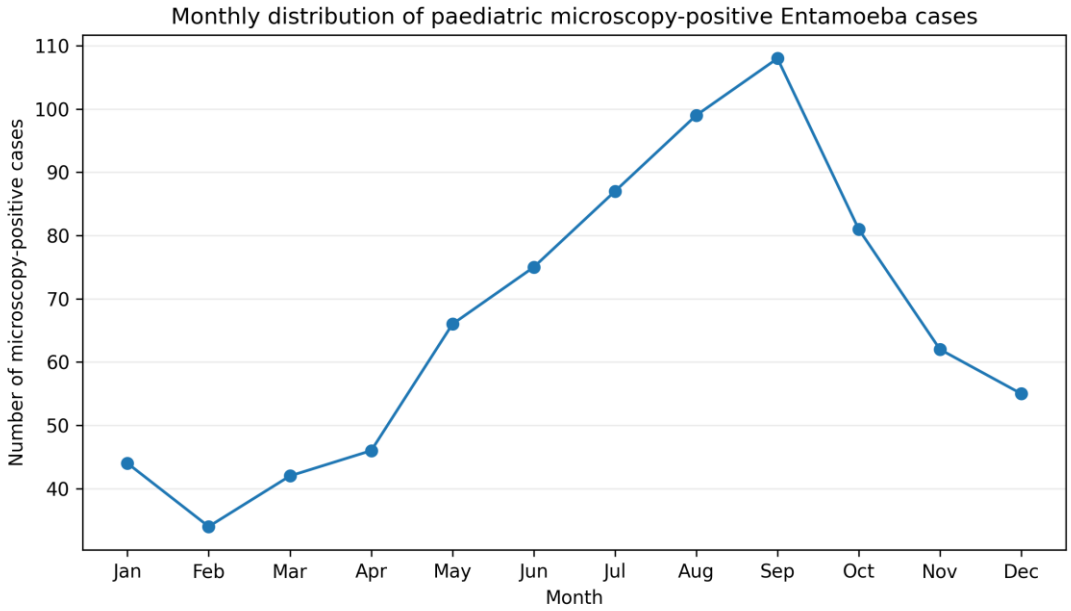

Figure 1. Monthly distribution of the 799 paediatric microscopy-positive Entamoeba cases. The figure presents counts rather than positivity rates because monthly testing denominators were unavailable.

Natural Resources for Human Health
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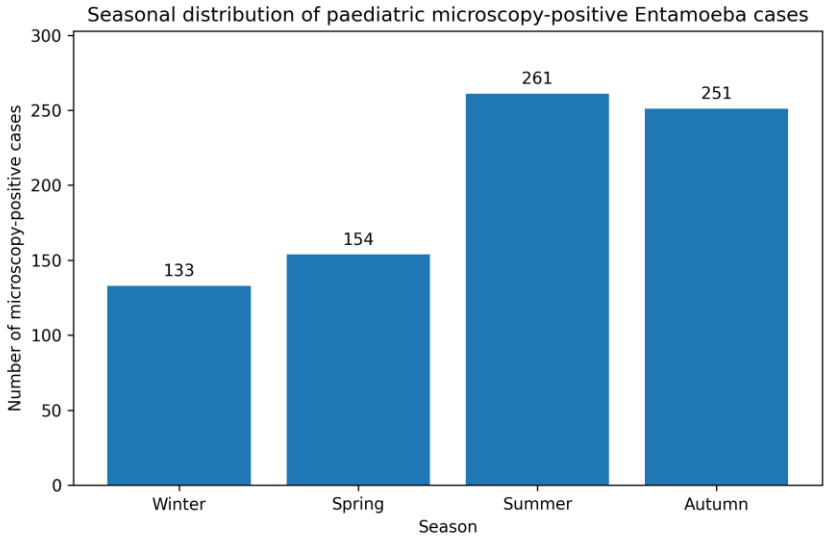

Figure 2. Seasonal distribution of paediatric microscopy-positive Entamoeba cases. Summer accounted for the largest number of positive cases, followed closely by autumn.

3.2 Overall haematological and biochemical profile

Median WBC was 11.70×10^9/L (IQR 8.60–15.66), median haemoglobin was 10.90 g/dL (IQR 9.85–12.00), median HCT was 32.30% (IQR 29.10–35.10), and median MCV was 75.30 fL (IQR 70.70–81.05). Median platelet count was 321×10^9/L. The inflammatory marker CRP was right-skewed, with a median of 6.62 mg/L (IQR 2.65–21.23) and a maximum of 246.86 mg/L. Median serum iron was 44.00 µg/dL (IQR 37.00–47.00). AST and ALT medians were 32 and 18 U/L, respectively. Ferritin and magnesium were summarised descriptively for the limited number of cases in which these measurements were available. (Table 2).

Table 2. Overall haematological, inflammatory and biochemical profile.

Variable (unit)	Available n (%)	Median (IQR)	Range
WBC ($\times10^9$/L)	799 (100.0)	11.70 (8.60–15.66)	4.00–44.30
RBC ($\times10^{12}$/L)	799 (100.0)	4.29 (3.85–4.68)	1.82–10.70
Haemoglobin (g/dL)	799 (100.0)	10.90 (9.85–12.00)	4.90–17.80
Haematocrit (%)	799 (100.0)	32.30 (29.10–35.10)	12.70–78.40
MCV (fL)	799 (100.0)	75.30 (70.70–81.05)	6.89–105.00
Platelet count ($\times10^9$/L)	799 (100.0)	321.00 (239.00–431.50)	67.40–1132.00
Calcium (mg/dL)	799 (100.0)	8.20 (7.23–9.07)	1.75–13.00
Serum iron (µg/dL)	799 (100.0)	44.00 (37.00–47.00)	16.70–121.80
Urea (mg/dL)	799 (100.0)	16.00 (12.00–22.00)	3.00–128.00
CRP (mg/L)	799 (100.0)	6.62 (2.65–21.23)	0.06–246.86
AST (U/L)	799 (100.0)	32.00 (22.00–42.50)	7.00–88.00
ALT (U/L)	799 (100.0)	18.00 (11.00–26.00)	2.00–48.00

Ferritin (ng/mL)	18 (2.3)	39.09 (5.50–55.47)	3.00–164.00
Magnesium (mg/dL)	33 (4.1)	1.36 (1.10–2.12)	0.27–2.80

Note: Ferritin and magnesium are reported as available-case summaries only and were excluded from primary inferential models because more than 95% of values were unavailable.

3.3 Anaemia profile

Using WHO 2024 thresholds, 371 cases met criteria for anaemia, corresponding to 46.4% (95% CI 43.0–49.9). Mild anaemia was present in 180 cases (22.5%), moderate anaemia in 158 (19.8%) and severe anaemia in 33 (4.1%). Anaemia was more frequent at age two years than at age one year (58.8% versus 35.0%). The odds of anaemia among two-year-olds were 2.65 times those among one-year-olds (95% CI 1.99–3.54). Anaemia frequency did not differ significantly by sex or season. (Table 3; Figure 3).

Table 3. Anaemia severity overall and in the principal age and sex subgroups.

Group	N	No anaemia n (%)	Mild n (%)	Moderate n (%)	Severe n (%)	Any anaemia n (%)
Overall	799	428 (53.6)	180 (22.5)	158 (19.8)	33 (4.1)	371 (46.4)
Age 1 year	409	266 (65.0)	66 (16.1)	61 (14.9)	16 (3.9)	143 (35.0)
Age 2 years	381	157 (41.2)	110 (28.9)	97 (25.5)	17 (4.5)	224 (58.8)
Male	450	238 (52.9)	111 (24.7)	86 (19.1)	15 (3.3)	212 (47.1)
Female	349	190 (54.4)	69 (19.8)	72 (20.6)	18 (5.2)	159 (45.6)

Note: Anaemia severity was assigned using age- and sex-specific WHO 2024 haemoglobin thresholds. The nine 15-year-old cases were described separately and were not used as an independent inferential subgroup.

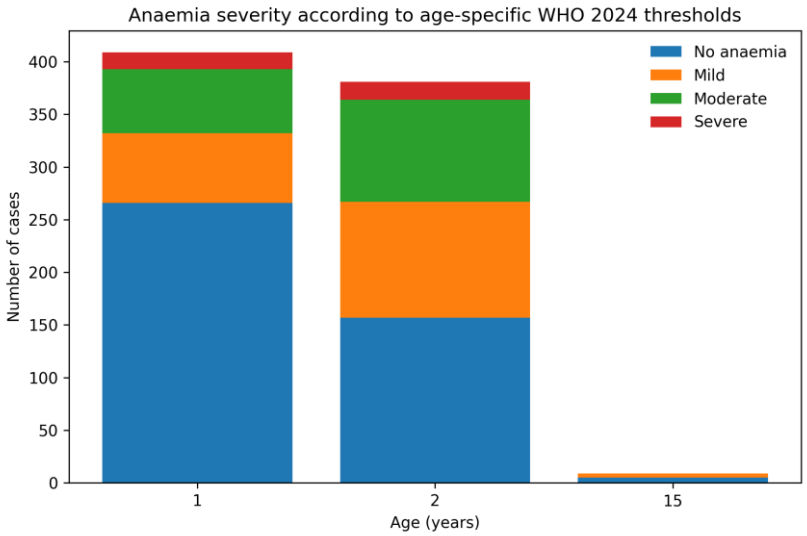

Figure 3. Anaemia severity according to age-specific WHO 2024 haemoglobin thresholds. The nine cases aged 15 years are shown descriptively because of their small number.

3.4 Seasonal laboratory variation

After false-discovery-rate correction, seasonal differences remained for calcium ($q<0.001$; epsilon-squared=0.028), urea ($q=0.0046$; epsilon-squared=0.017) and serum iron ($q=0.0168$; epsilon-squared=0.013). Serum iron was highest in summer and lowest in winter, with a small effect size. Calcium was lower in autumn than winter and summer, while urea was lower in autumn

than spring and summer. No FDR-significant seasonal differences were detected for haemoglobin, WBC, CRP, RBC, HCT, platelets, AST or ALT. Thus, the larger summer–autumn case counts were not accompanied by a corresponding seasonal increase in the principal inflammatory markers. (Table 4).

Table 4. Seasonal variation in selected laboratory markers.

Variable	Winter	Spring	Summer	Autumn	Adjusted q
Panel A. Haematological and inflammatory markers					
WBC	**12.00 (8.60–15.90)**	**10.87 (8.27–15.97)**	**11.90 (9.00–16.18)**	**11.40 (8.37–14.90)**	**0.4640**
Haemoglobin	**10.90 (9.70–11.90)**	**11.10 (10.00–12.00)**	**10.80 (9.80–11.90)**	**10.80 (9.80–12.15)**	**0.6556**
MCV	**75.90 (71.70–82.50)**	**75.85 (71.93–81.22)**	**73.70 (68.80–79.10)**	**76.00 (69.50–81.10)**	**0.0762**
Platelet count	**340 (230–432)**	**328 (247–433)**	**326 (243–433)**	**297 (228–410)**	**0.1904**
CRP	**8.12 (3.35–24.46)**	**9.25 (2.52–22.77)**	**6.23 (3.08–15.06)**	**6.43 (2.52–21.11)**	**0.4200**
Panel B. Biochemical markers					
Serum iron	**42.45 (34.25–46.23)**	**43.86 (37.88–47.63)**	**45.00 (40.00–48.00)**	**43.17 (35.50–46.00)**	**0.0168**
Calcium	**8.59 (7.68–9.40)**	**7.89 (7.11–8.79)**	**8.40 (7.40–9.40)**	**7.87 (6.56–8.96)**	**0.0002**
Urea	**15.00 (11.00–22.00)**	**17.00 (12.00–22.00)**	**18.00 (12.00–23.00)**	**14.00 (12.00–19.00)**	**0.0046**
AST	**25.00 (17.00–42.00)**	**29.50 (19.75–44.00)**	**33.00 (22.00–43.00)**	**34.00 (23.50–38.00)**	**0.0757**
ALT	**16.00 (9.00–28.00)**	**14.50 (9.00–27.75)**	**21.00 (12.00–24.00)**	**21.00 (11.00–26.00)**	**0.4640**

Note: Values are median (IQR). q values were obtained after Benjamini–Hochberg correction. Statistically detectable seasonal differences in serum iron, calcium and urea had small effect sizes.

3.5 Age- and sex-related laboratory differences

Compared with one-year-olds, two-year-olds had lower median haemoglobin (10.70 versus 11.20 g/dL), RBC (4.22 versus 4.39×10^{12}/L) and HCT (31.61% versus 32.80%); these differences remained significant after false-discovery-rate correction. Serum iron was also lower at age two in the unadjusted comparison, although the adjusted q value was 0.060. No laboratory variable differed significantly between males and females after correction for multiple testing. (Table 5).

Table 5. Age- and sex-related differences in selected laboratory markers.

Variable	Group 1 median (IQR)	Group 2 median (IQR)	Adjusted q
Panel A. Age comparison: 1 year versus 2 years			
WBC	11.60 (8.60–16.10)	11.80 (8.60–15.50)	0.9196
RBC	4.39 (3.94–4.72)	4.22 (3.75–4.62)	0.0054

Haemoglobin	11.20 (9.90–12.20)	10.70 (9.80–11.60)	0.0041
Haematocrit	32.80 (29.30–35.60)	31.61 (28.90–34.40)	0.0054
MCV	75.10 (70.90–79.70)	75.40 (70.00–81.00)	0.6949
Serum iron	44.22 (38.00–47.45)	43.00 (36.45–46.65)	0.0601
CRP	7.54 (2.77–23.81)	6.26 (2.63–18.46)	0.2207
Panel B. Sex comparison: male versus female			
WBC	11.70 (8.70–15.28)	11.70 (8.60–16.61)	0.8362
RBC	4.26 (3.87–4.68)	4.35 (3.83–4.68)	0.8889
Haemoglobin	10.80 (9.90–11.90)	11.00 (9.70–12.00)	0.8362
Haematocrit	32.00 (29.20–34.70)	32.60 (29.00–35.50)	0.7071
MCV	74.65 (70.12–79.67)	76.10 (71.00–82.10)	0.2516
Serum iron	43.99 (37.45–46.74)	44.00 (36.63–48.00)	0.8362
CRP	6.23 (2.44–20.23)	8.02 (3.23–22.07)	0.2516

Note: In Panel A, Group 1 denotes age one year and Group 2 denotes age two years. In Panel B, Group 1 denotes male and Group 2 denotes female. No sex-related difference remained significant after multiple-testing correction.

3.6 Correlation analysis

The strongest correlations were between haemoglobin and HCT (rho=0.786), and between AST and ALT (rho=0.785). Serum iron correlated moderately with haemoglobin (rho=0.470) and HCT (rho=0.386). WBC correlated with CRP (rho=0.314) and more weakly with platelet count (rho=0.145). MCV correlated with haemoglobin (rho=0.266) and serum iron (rho=0.149). All of these associations remained significant after false-discovery-rate correction, except where otherwise specified in figure 6. (Figures 4–6).

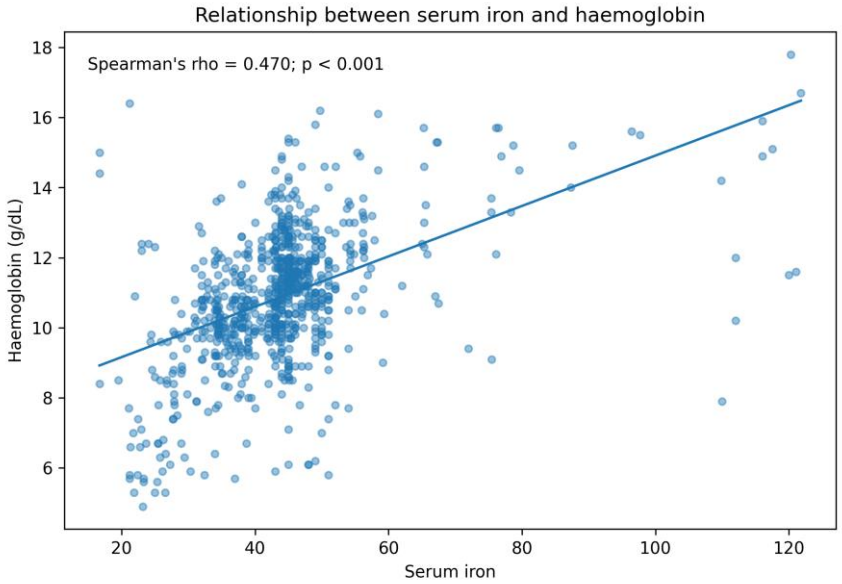

Figure 4. Positive relationship between serum iron and haemoglobin (Spearman's rho=0.470; p<0.001). The fitted line is illustrative; inference is based on rank correlation and multivariable analysis.

Natural Resources for Human Health
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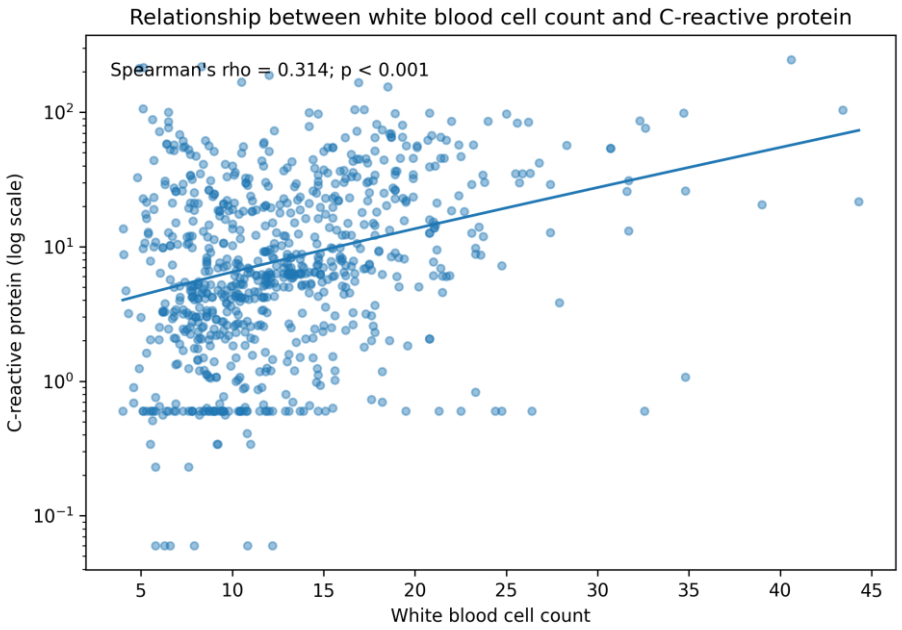

Figure 5. Positive relationship between white blood cell count and C-reactive protein (Spearman's rho=0.314; p<0.001). CRP is displayed on a logarithmic scale because of marked right-skewness.

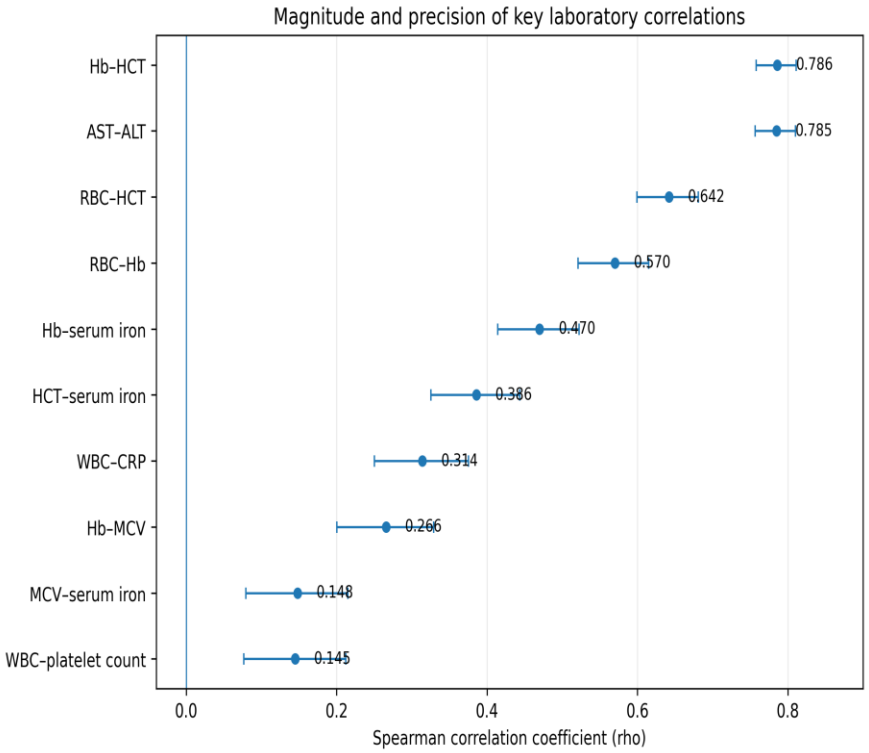

Figure 6. Ranked correlations among key haematological, inflammatory and biochemical variables. Points represent Spearman's rho and horizontal lines show approximate 95% confidence intervals.

3.7 Multivariable analysis

The haemoglobin model explained 30.0% of observed variance. After adjustment, age two years was associated with haemoglobin 0.374 g/dL lower than age one year (95% CI −0.608 to −0.139; p=0.002). Each one-unit increase in serum iron was associated with a 0.065 g/dL increase in haemoglobin (95% CI 0.049–0.081; p<0.001), while each 1 fL increase in MCV was associated with a 0.041 g/dL increase (95% CI 0.023–0.060; p<0.001). Higher WBC showed a small inverse association with haemoglobin (β=−0.028; 95% CI −0.053 to −0.003; p=0.029). Sex, season and log-CRP were not independently associated with haemoglobin.

The log-CRP model explained 12.1% of variance. WBC was independently associated with higher log-CRP (β=0.067; 95% CI 0.051–0.083; p<0.001). Summer showed lower adjusted log-CRP than winter in the full dataset, but this estimate became borderline in the sensitivity analysis after exclusion of flagged cases. Other predictors were not independently associated with CRP. (Table 6).

Table 6. Statistically significant independent predictors in the robust multivariable models.

Outcome	Independent predictor	β coefficient	95% CI	p value
Haemoglobin (g/dL)	Age 2 versus 1 year	−0.374	−0.608 to −0.139	0.002
	Serum iron	0.065	0.049 to 0.081	<0.001
	MCV	0.041	0.023 to 0.060	<0.001
	WBC	−0.028	−0.053 to −0.003	0.029
log(CRP+1)	Summer versus winter	−0.250	−0.480 to −0.019	0.034
	WBC	0.067	0.051 to 0.083	<0.001

Note: The table displays predictors with p<0.05 from the fully adjusted models. The haemoglobin model included age, sex, season, serum iron, MCV, log-CRP and WBC. The CRP model included age, sex, season, WBC, platelet count, haemoglobin and serum iron.

4. DISCUSSION

This study provides an integrated seasonal, haematological, inflammatory and biochemical characterisation of 799 paediatric paediatric cases with microscopy-positive findings for Entamoeba in Benghazi. Three findings are particularly important. First, almost half of the cases met WHO criteria for anaemia, with a disproportionate burden among two-year-olds. Second, serum iron was moderately associated with haemoglobin and remained an independent correlate after adjustment, supporting an iron-related haematological phenotype without establishing iron-deficiency anaemia. Third, WBC was associated with CRP, identifying a coherent inflammatory pattern, although neither marker varied materially across seasons.

The concentration of cases in summer and autumn is compatible with the broader observation that enteric pathogen detection can vary over the year according to organism, setting and exposure pathway (Nguyen et al., 2021; Mero et al., 2023). Potential mechanisms include warmer conditions, greater water consumption, intermittent supply, domestic storage, wastewater intrusion, raw-food exposure and seasonal healthcare attendance. These mechanisms remain hypotheses because the present dataset contained no temperature, rainfall, water-quality, household, dietary or geospatial measurements. Furthermore, summer and autumn could have had higher testing volumes. The result should therefore be expressed as seasonal case concentration rather than increased seasonal prevalence.

The observed anaemia frequency is clinically important, particularly because 98.9% of cases involved children aged one or two years, a period of rapid growth, transition to complementary foods and high iron demand. The lower haemoglobin, RBC and HCT among two-year-olds persisted despite the slightly higher anaemia threshold applied at 24–59 months. The adjusted association between serum iron and haemoglobin adds biological coherence, while the correlation between MCV and both haemoglobin and iron is compatible with an iron-related erythrocyte pattern. Nevertheless, serum iron is affected by recent intake, diurnal variation and inflammation, and cannot establish iron deficiency in isolation. Ferritin would normally strengthen interpretation but was measured in only 18 cases and is itself an acute-phase reactant; WHO guidance therefore recommends

interpreting ferritin alongside inflammatory status (World Health Organization, 2020). Accordingly, the manuscript avoids the term confirmed iron-deficiency anaemia.

Amoebic colitis can cause mucosal inflammation and blood loss, but the present case-only design cannot determine whether *Entamoeba* caused the anaemia. Nutritional iron deficiency, bacterial or viral gastroenteritis, haemoglobinopathies, other parasitic infections and chronic inflammatory conditions were not captured. Species misclassification further limits causal interpretation because microscopy may combine pathogenic *E. histolytica* with non-pathogenic or less clearly pathogenic look-alike species, a limitation also relevant to earlier Libyan microscopy-based surveys (El-Ammari et al., 2004; El-Ammari and Nair, 2015; Ghenghesh et al., 2016). Targeted *E. histolytica* antigen or PCR confirmation, coupled with stool bacterial and viral testing where clinically indicated, would markedly improve future studies (Ravi A et al., 2025; Calle-Pacheco et al., 2022; Cooney et al., 2025).

The positive WBC–CRP relationship indicates that the two inflammatory measures moved together, while the weak WBC–platelet association may reflect reactive haematological responses. Experimental and clinical literature supports a role for both parasite-derived and host inflammatory mediators in invasive disease (Ngobeni et al., 2017; Guillén, 2023). A recent adhesin-positive case-control analysis also evaluated systemic immune-inflammatory indices and complete blood parameters in amoebiasis, highlighting the value of moving beyond parasite detection to integrated laboratory phenotyping (Uğraklı et al., 2026). The current study extends that direction to a large paediatric Libyan dataset but lacks a negative gastroenteritis comparison group.

Biochemical seasonal differences were statistically detectable for calcium, urea and serum iron, but all effect sizes were small. These findings should not be overinterpreted as direct seasonal organ dysfunction. Calcium values included unexpectedly low observations and lacked albumin correction; urea can vary with hydration, intake and renal perfusion; and serum iron is sensitive to inflammatory and pre-analytical conditions. AST and ALT were strongly correlated, as expected for related hepatic enzymes, but median values did not show a seasonal pattern. Without clinical examination, bilirubin, albumin or imaging, these data do not support claims of amoebic liver disease.

The One Health relevance of these findings lies primarily in shared environmental and institutional interfaces. Human *E. histolytica* transmission is predominantly faecal–oral, and the animal sector should not be framed as a proven major reservoir in the absence of molecular evidence. The practical system includes paediatric services, parasitology laboratories, household and municipal water, wastewater, food handling, irrigation and environmental monitoring. A sentinel programme could combine monthly *Entamoeba* counts and testing denominators with water interruptions, bacteriological indicators, wastewater events, produce-safety inspections and meteorological data. Such integration reflects the contemporary One Health definition linking human health to food, water and ecosystems (One Health High-Level Expert Panel, 2022).

The sustainable development implications are direct but should remain proportionate to the evidence. The anaemia findings relate to SDG 2 and child nutrition; diagnosis and morbidity relate to SDG 3; faecal–oral prevention relates to SDG 6; and the required multisectoral surveillance architecture relates to SDG 17. The data do not measure educational performance or climate effects, so SDG 4 and SDG 13 are best treated as future research extensions rather than demonstrated outcomes. These linkages are consistent with the integrated framework of the 2030 Agenda for Sustainable Development (United Nations, 2015).

The study has several strengths: year-round coverage, a large positive-case dataset, integrated haematological and biochemical measurements, use of current WHO anaemia thresholds, false-discovery-rate correction and sensitivity analysis. Limitations include the retrospective design, absence of a microscopy-negative or diarrhoeal control group, lack of testing denominators, inability to confirm unique patients, absence of symptoms and treatment data, lack of species-specific confirmation, dominance of cases from the Gastro unit, incomplete ferritin and magnesium testing, uncertain laboratory reference intervals and several internally inconsistent values. These limitations restrict causal and prevalence inference but do not invalidate the descriptive and within-case associations when interpreted conservatively.

5. ONE HEALTH-INFORMED SURVEILLANCE PRIORITIES

- Create a hospital sentinel dataset that links stool microscopy or antigen/PCR results to unique patient identifiers, symptoms, treatment, CBC, CRP and iron-related tests.
- Report both positive counts and monthly testing denominators to distinguish seasonal workload from seasonal positivity.

- Introduce routine parasitology quality assurance and species-confirmatory testing for a representative subset of microscopy-positive samples.
- Coordinate pre-summer and summer WASH risk communication for families, childcare facilities and food handlers, with attention to household water storage and hand hygiene.
- Link hospital trends with municipal water-quality, wastewater, produce-safety and environmental incident data through a multidisciplinary surveillance group.
- Include nutritional and growth measurements in prospective paediatric studies to separate infection-associated laboratory changes from underlying malnutrition.

The proposed surveillance priorities and their links to One Health and sustainable development are summarised in Figure 7.

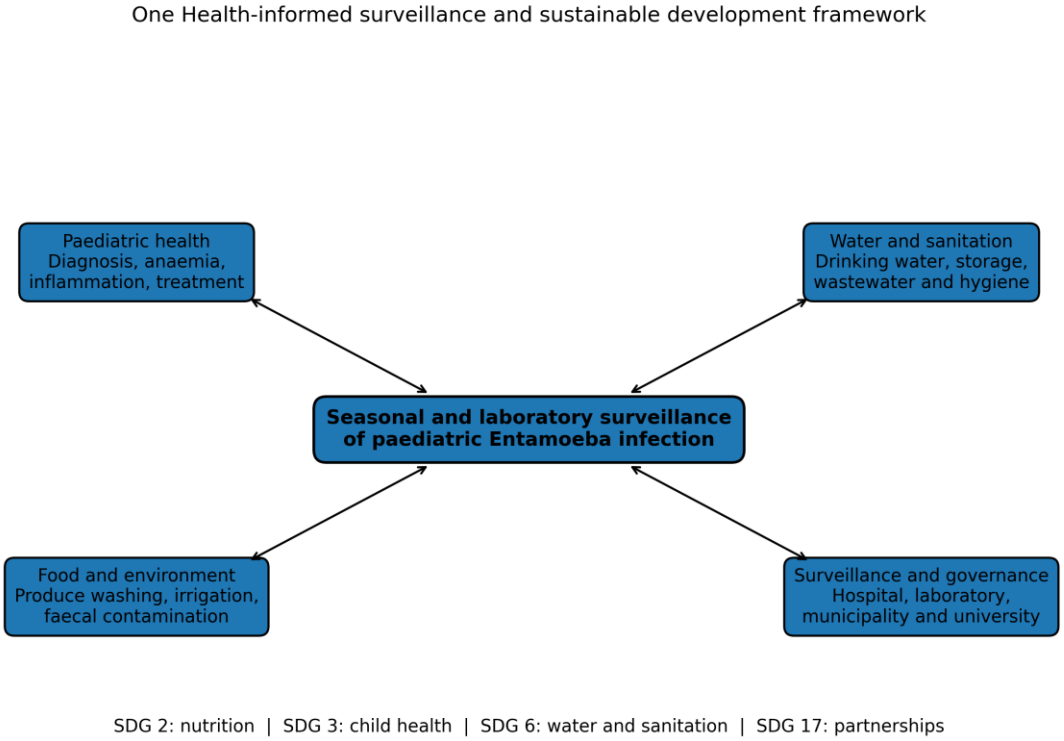

Figure 7. One Health-informed surveillance and sustainable development framework for paediatric intestinal amoebiasis in Benghazi.

6. CONCLUSION

Among 799 paediatric cases with microscopy-positive Entamoeba findings, anaemia affected nearly half of the cohort, and the data showed coherent iron-haematological and inflammatory relationships. Positive cases were concentrated in summer and autumn; however, the absence of monthly testing denominators precluded interpretation as seasonal prevalence. Lower haemoglobin was independently associated with age two years, lower serum iron, lower MCV and higher WBC, whereas WBC was the principal independent correlate of CRP. These findings support integrated laboratory assessment in symptomatic children but do not establish species-specific or causal effects of amoebic infection.

Prospective surveillance should combine unique patient identifiers, species-confirmatory testing, appropriate control groups, nutritional assessment and environmental exposure data. A One Health-informed sentinel platform linking paediatric care, parasitology, water and sanitation, food safety and municipal surveillance could translate hospital laboratory signals into targeted action relevant to SDGs 2, 3, 6 and 17.

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