



Prevalence and Epidemiological Patterns of *Entamoeba histolytica* Infection among Children in Western Libya: A Comparative Study of 2023 and 2024

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تاريخ الاستلام: 2025/10/10 تاريخ المراجعة: 2025/10/13 - تاريخ القبول: 2025/10/17 - تاريخ النشر: 2025 /10/27

ABSTRACT:

Background: Amebiasis is an intestinal infection caused by the protozoan *Entamoeba histolytica*. The infection mainly spread through the fecal-oral route, infected food and water, and bad hygiene. **Aim:** The study aimed to assess the prevalence of *Entamoeba histolytica* among children in Western Libya during 2023 and 2024, using data collected from some laboratories. **Methods:** A total of 250 cases with ages ranging from one month to 14 years were included. Statistical analysis was performed using the chi-square (χ^2) test in SPSS software (version 26.0, IBM Corp., Armonk, NY). A significant level of < 0.05 was used to assess the associations between the number of *Entamoeba histolytica* infections and variables such as number of cases, month, and gender. **Results:** In 2023, the overall cases were 104. The highest infection rates occurred during the warmer months, particularly in May (18 cases, 17%) and June (18 cases, 17%). A significant monthly variation was observed based on the Chi-squar test ($\chi^2 = 32.86$, p-value = 0.0007). In contrast, in 2024, the total number of cases increased to 146, which represents to a 40% increase compared to the previous year. The highest number of infections was recorded during the cold months, with November (24 cases, 16%) and December (30 cases, 20%). The Chi-squar test ($\chi^2 = 28.77$) p-value showed statistically significant variation across months ($P < 0.05$). Males were slightly more affected than females, accounting for about 58% of cases, however, there was no significant association between gender and infection rates in both years (2023: $\chi^2 = 2.31$, p = 0.128; 2024: $\chi^2 = 3.42$, p = 0.064). Most of the affected children were under 14 years of age, with a few newborn children who were 1 month old becoming infected. In 2024, there was a noticeable change, with the majority of infections occurring during the colder months, whereas in 2023, the majority of cases occurred during the warmer months. **Conclusion:** The results indicate that infections are on the rise and no longer solely occur

on the warmer months as previously believed. In order to protect children during is a critical requirement for improved awareness, hygiene practices, and early prevention initiatives.

KEYWORDS: Amebiasis, *Entamoeba histolytica* (*E. histolytica*), Children, Western Libya

المستخلص

الخلفية: داء الأميبا (Amebiasis) هو مرض معوي يُسببه طفيلي المتحولة النسيجية (*Entamoeba histolytica*))، ويُعد من الأمراض المنتشرة على نطاق واسع في مختلف أنحاء العالم. ينتقل هذا المرض بشكل رئيسي عن طريق البراز-الفم، من خلال المياه الملوثة، والطعام، وسوء ممارسات النظافة. الأهداف: هدفت هذه الدراسة إلى تقييم مدى انتشار داء المتحولة النسيجية بين الأطفال في المنطقة الغربية من ليبيا خلال عامي 2023 و2024، استنادًا إلى بيانات جُمعت من عدد من المختبرات في تلك المنطقة. المواد والطرق: شملت الدراسة 250 حالة من الأطفال، تراوحت أعمارهم بين شهر و14 سنة وتم استخدام اختبار كاي تربيع χ^2 للتحليل الإحصائي من أجل دراسة العلاقة بين عدد الحالات والمتغيرات مثل الشهر والسنة مع استخدام قيمة P (P value) لتحديد ما إذا كانت هذه الفروق ذات دلالة إحصائية أم لا. النتائج: أظهرت النتائج أنه في عام 2023، تم تسجيل 104 حالات، وبلغت ذروة الإصابات خلال الأشهر الدافئة، خاصة في شهري مايو (18 حالة، 17%) ويونيو (18 حالة، 17%). وقد أظهر اختبار كاي-تربيع وجود فروق شهرية ذات دلالة إحصائية ($\chi^2 = 32.86$ ، $p = 0.0007$). بينما ارتفع عدد الحالات في عام 2024 إلى 146 حالة، أي بزيادة قدرها 40% مقارنة بالعام السابق. وقد سُجّلت معظم الحالات في عام 2024 خلال الأشهر الباردة، حيث سُجّلت 24 حالة في نوفمبر (16%) و30 حالة في ديسمبر (20%). كما أظهر اختبار كاي-تربيع مرة أخرى وجود فروق شهرية ذات دلالة إحصائية ($\chi^2 = 28.77$ ، $p < 0.05$). وقد لوحظ أن معظم الأطفال المصابين كانوا دون سن الرابعة عشر، كما لوحظ أن الذكور كانوا أكثر تأثرًا من الإناث بنسبة بلغت حوالي 58% من إجمالي الحالات. كما تم تسجيل عدد قليل من الحالات بين حديثي الولادة بعمر شهر واحد. الملخص: تشير هذه النتائج إلى أن انتشار العدوى الطفيلية المعوية لم يعد مقصورًا على الأشهر الدافئة فقط كما كان يُعتقد سابقًا يسلط الانتشار الملحوظ للمرض بين الأطفال دون سن الخامسة، وخصوصًا الذكور، الضوء على الحاجة الماسة إلى تعزيز التوعية، وتحسين ممارسات النظافة، وتكثيف جهود الوقاية المبكرة لحمايتهم خلال سنواتهم الأولى الحرجة. بل أصبح انتشارها يمتد عبر فصول السنة المختلفة.

الكلمات المفتاحية: المتحولة النسيجية (*Entamoeba histolytica*) ، داء الأميبا (Amebiasis) ، الأطفال، غرب ليبيا.

INTRODUCTION

Amebiasis is an intestinal parasitic disease caused by an anaerobic protozoan *Entamoeba histolytica* belonging to the family Entamoebidae within the phylum Amoebozoa of Eukaryota [1]. Although the parasite occurs worldwide, its prevalence is difficult to estimate due to latent infections and limited diagnostic and surveillance capacities in many endemic regions [2]. Globally, it is estimated that up to 90% of infections are asymptomatic [3]. However, the infection remains common in populations with poor hygiene and low socioeconomic conditions. Key risk factors include fecal-oral transmission, inadequate hand hygiene, and contamination of water sources, sometimes associated with close contact with animals [4].

Studies have also reported higher infection rates among homosexual males, suggesting the possibility of sexual transmission [5].

The life cycle of *E. histolytica* is relatively simple. The infective stage is the cyst, a spherical structure measuring 10–16 μm . Immature cysts contain a single nucleus, while mature cysts have four nuclei, each with a nuclear membrane, fine chromatin granules, and a central genetic reservoir. Upon excystation in the gastrointestinal tract, each cyst releases eight trophozoites, the motile vegetative form of the parasite, ranging from 20 to 40 μm in diameter [6]. Cysts are resistant to desiccation and can survive in humid environments or water for several weeks. When ingested by a susceptible host, excystation occurs, and trophozoites move in response to stimuli to initiate infection [7].

The parasite has a very simple life cycle in which the infective form is the cyst, spherical structures roughly 10–16 micrometers in size that exhibit a unique maturation process. Immature cysts contain a solitary nucleus, while their mature counterparts boast four distinct nuclei. Each nucleus features a rounded membrane adorned with fine chromatin particles and a central reservoir of genetic material, when in excystation, each cyst produces eight vegetative forms, or trophozoites, which are the motile form of the parasite; they are 20–40 μm in diameter. The cysts are resistant to dehydration in soil and can survive in humid environments and in water for several weeks. Susceptible hosts exposed to the aforementioned infection sources ingest the cysts, which then undergo excystation during their passage through the gastrointestinal tract [6]. In response to unknown stimuli, the trophozoite moves and initiates pathogenesis [7].

Both *E. histolytica* and *E. dispar* infect about one in ten people worldwide, although most infections are asymptomatic. Despite this, amebiasis generally causes 110,000 deaths each year [8]. Persistent *E. histolytica* trophozoites can penetrate the intestinal mucosa, causing tissue destruction, ulcers, and symptoms including diarrhea, abdominal pain, weight loss, fatigue, and flatulence (intestinal amebiasis). Liver involvement may lead to abscess formation [8]. The species' name, "histolytica," reflects its tissue-destructive properties. Diagnosis usually depends on microscopic stool examination, but recent methods include detection of specific antigens and DNA through molecular tests, such as PCR (conventional or real-time), ELISA, and other antibody-based assays, which are easily available for use in diagnostic settings [8].

Recent data suggest that *Entamoeba histolytica* may play a minimal role in Libya [9]. The prevalence rates observed were 0.8%–36.6% for *E. histolytica* in children with gastroenteritis and 0.8%–16.3% in those without gastroenteritis. The review also indicated that the prevalence of *E. histolytica* was significantly higher in children than adults in the Libyan

population; high prevalence rates for *E. histolytica* were reported mainly from the city of Sirt [10]. A recent study in Al-khoms reported a significantly higher prevalence rate of *E. histolytica* among foreign workers, mainly from Egypt and other African countries, compared to Libyan individuals [11]. Children less than 15 years old, particularly those aged 5–9 years, are most affected [12]. Recent research indicates that the illness may have severe health implications for children in children in susceptible populations, including anemia [13].

The study objective was to investigate the prevalence of *Entamoeba histolytica* infection among children in Western Libya and to assess the influence of socio-demographic factors, including age, gender, month, and year, on infection rate.

Methods

Study design and population: A cross-sectional study analyzed the prevalence of *Entamoeba histolytica* infection among children in Western Libya over two years (2023 and 2024). The study population comprised two hundred and fifty (250) cases, ranging in age from one month to 14 years. Data were collected from records of laboratory-confirmed *E. histolytica* infection reported by several laboratories (Al-Riyada, Al-Wiqayah, Al Yusr, Masirat Al-Hayat, Al-Kubra Surman Clinic, and Zawia Medical Center) within the specified region.

Data collection and analysis: Data extracted from laboratory records included monthly case numbers for both 2023 and 2024 and gender and age at the time of diagnosis.

Statistical analysis: Statistical analysis was performed using the Chi-square (χ^2) test in SPSS software (version 26.0, IBM Corp., Armonk, NY); a significant level at < 0.05 was used to assess the associations between the number of *Entamoeba histolytica* cases and variables such as month and gender.

RESULTS:

Results of 2023: During 2023, 104 children were diagnosed with *Entamoeba histolytica* infection. Among the patients who were investigated, the infection ranged from moderate to high.

The number of reported annual cases varies clearly by month (Table 1). May and June had the highest number of cases (18 cases each, 17%), followed by September and October (14 cases, 13%, and 13 cases, 12.5%, respectively). August and December had the lowest infection rates (one case each). A strong seasonal trend was indicated by the chi-square test, which revealed a statistically significant variance between months ($\chi^2 = 32.86$, p -value = 0.0007).

Table (1): Distributed sample based on number of case

Month	1	2	3	4	5	6	7	8	9	10	11	12
Number of cases	7	3	9	4	18	18	8	1	14	13	8	1
Chi-squar test (χ^2)	32.86											
P-value	0.0007											
Total	104											

From the total number of cases, there were male 60 cases (58%), while there were 44 female cases (42%) (Table 2). Figure 1 shows that throughout 2023, males had slightly higher infection rates than females. Gender and infection rate did not statistically significantly correlate, according to the Chi-square test ($\chi^2 = 2.31$, p-value = 0.128). This suggests that gender could not be a significant independent risk factor for *E. histolytica* infection in this population.

Table (2): Distributed sample based on gender:

Gender	Number	Percent
Male	60	58%
Female	44	42%
Chi -squar test (χ^2)	2.31	
P-value	0.128	
Total	104	

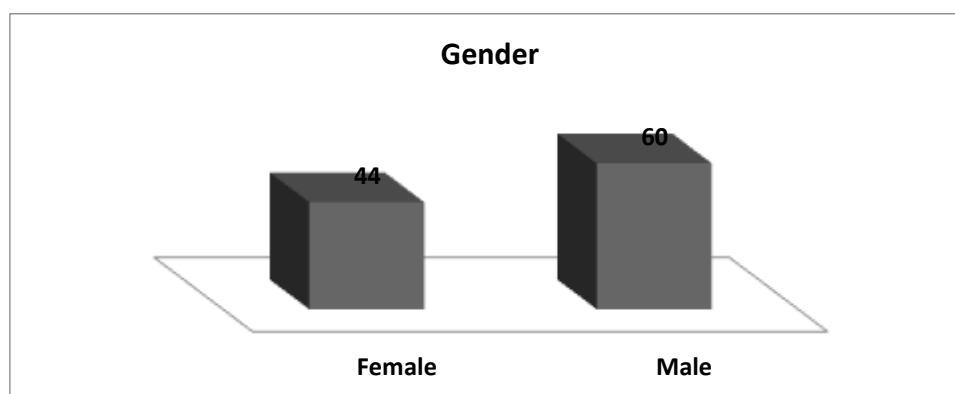


Figure (1): Frequency of gender

The age of infected children ranged from 3 months to 14 years. The broad age range indicates that all pediatric age groups are susceptible. However, the highest number of cases occurred in the 6–10 –year –old age group (45 cases), followed by 1–5 years (30 cases) and 11–15 years (25 cases). Based on available data, the estimated distribution by age groups is shown in the chart below.

Table (3): Distributed sample based on age group:

Comparative Study of 2023 and 2024

Month	1	2	3	4	5	6
Average age	3m –9y	5m– 8y	4y– 12y	4y –10y	9m– 14y	3y– 11y
Month	7	8	9	10	11	12
Average age	5y– 13y	12y	6m–13y	3m– 14y	8m–11y	1y
Total	104					

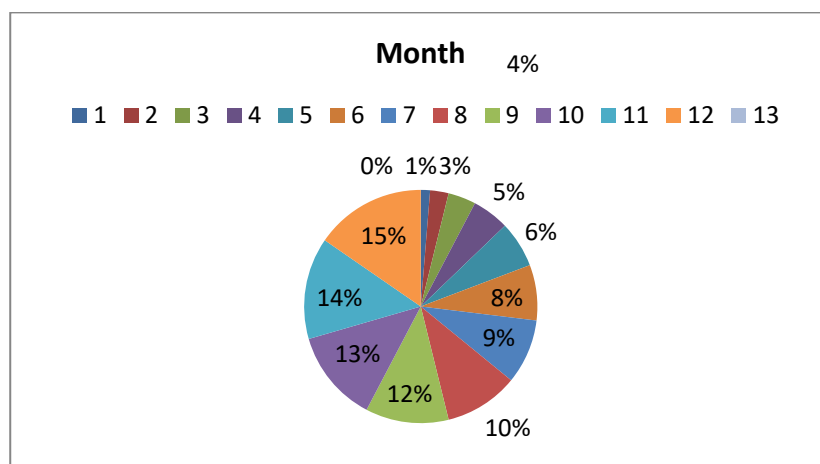


Figure (2): Frequency of age group

Results of 2024:

Entamoeba histolytica infection increased to 146 total cases overall in 2024. The frequency of infection changed by season. There were 24 cases (16%) in November and 30 cases (20%) in December, both of which represented a significant increase. There were higher numbers of cases in June, 16 cases (10%), July, 16 cases (10%); and September, 14 cases (9%). Only 3 cases (2%) were reported during April. Chi-square test (χ^2) = 28.77, p = 0.001. P-value showed statistically significant variation across months ($P < 0.05$). Table 4 suggests that infections seem to rise significantly in months of the year.

Table (4): Distributed sample based on number of cases:

Month	1	2	3	4	5	6	7	8	9	10	11	12
Number of cases	7	6	8	3	7	16	16	5	14	10	24	30
Chi squar test (χ^2)	28.77											
P-value	0.001											
Total	146											

85 male cases (58%) and 61 female cases (42%) represented the total infection of 146. Chi-square test (χ^2) = 3.42, p = 0.064. Gender and infection rate did not significantly correlate ($p > 0.05$). Table 5 shows that the observed male frequency of infection is reliable with the pattern discovered in 2023.

Table (5): Distributed sample based on gender:

Gender	Number	Percent
Male	85	58%
Female	61	42%
Chi-squar test (χ^2)	3.42	
P-value	0.064	
Total	146	

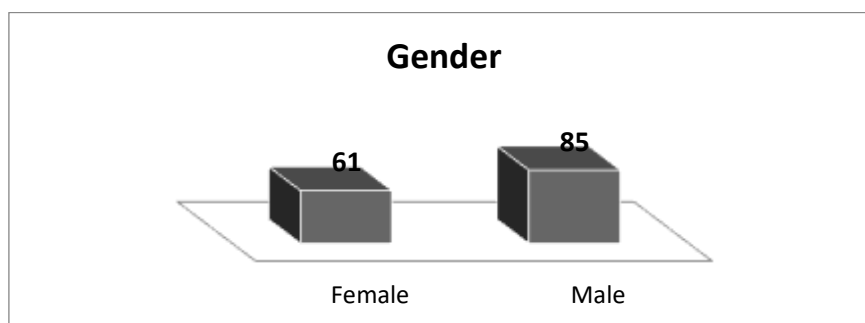


Figure (3): Frequency of gender

The age range of those infected varied continuously from one month to 14 years. The majority of illnesses affected children older than a year. Wider age ranges were noted in June, November, and December, among other months, according to Table 6.

Table (6): Distributed sample based on age group:

Month	1	2	3	4	5	6
Average age	2y -7y	1m- 10y	8m- 10y	2y -12y	1y- 9y	3m- 14y
Month	7	8	9	10	11	12
Average age	3m- 13y	1y-14y	2m-14y	4m- 14y	4m-14y	1y-14y
Total	146					

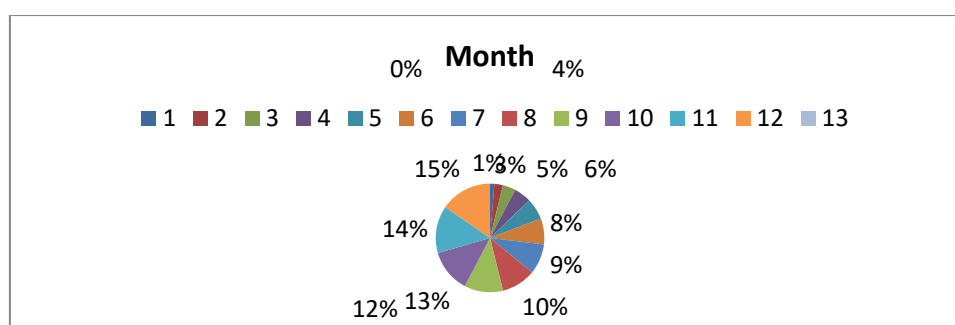


Figure (4): Frequency of age group

Comparison between 2023 and 2024:

Table 7 shows that there were 104 cases in 2023 and 146 in 2024. Between 2023 and 2024, the number of *Entamoeba histolytica* cases recorded increased by almost 40%. The chi-square test (χ^2) = 7.07, p = 0.008 indicated statistically significant differences in the case distribution between the two years. Males continued to be slightly more affected by the infection than females during the study period, but there was no marked shift in the gender distribution during that time. The age range continued to vary throughout the early years and puberty. The 2024 sample includes infants as young as one month. Seasonal patterns were observed in the highest infection numbers in the warmer months of 2023 (May 18 cases, June 18 cases, September 14 cases, and October 13 cases), while the lowest months were August (1 case) and December (1 case).

However, the coldest months of November (24 cases) and December (30 cases) had the largest number of cases in 2024, while April (3 cases) had the lowest. This could be a sign of changes in environmental conditions or seasonal variations in transmission.

Table (7): Comparison between 2023 and 2024 cases:

Year	Total cases	Percent %	Chi-squar test (χ^2)	p-value
2023	104	42%	7.07	0.008
2024	146	58%		

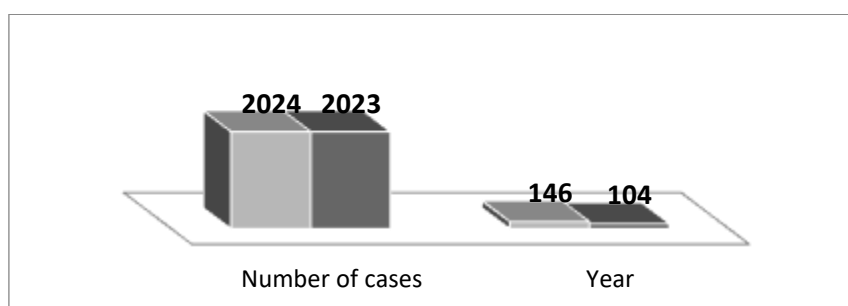


Figure (5): Comparison between 2023 and 2024 cases

In the years 2023 and 2024, a total of 104 and 146 cases were documented, respectively. Among these, male cases represented 60 (58%) in 2023 and 85 (58%) in 2024, while female cases were composed of 44 (42%) in 2023 and 61 (42%) in 2024. These results show a stable, slight majority of male children compared to female children in both years. Despite this minor male dominance, the gender distribution stayed fairly constant, displaying no significant change in the male-to-female ratio over the two years, Chi-square test (χ^2) = 0.0069, p = 0.933. This

stability indicates that there was no noticeable gender-related risk factor affecting the infection prevalence during the study period, as presented in Table 8.

Table (8): Comparison between 2023 and 2024 based on gender:

Year	2023	2024
Male	60 (58%)	85(58%)
Female	44(42%)	61(42%)
Chi-squar test (χ^2)	0.933	
P-value	0.0069	

The children included in the study represented a broad age range over both years; in 2023, their ages varied from 3 months to 14 years, whereas in 2024, the range extended slightly to include younger infants. The addition of younger participants in 2024 obtained a more complete understanding of early childhood and assisted in identifying possible variations in vulnerability among different age groups.

Table (9): The following arrangement presents a brief overview of the key findings for each year and a direct comparison between them:

Metric	Year 2023	Year 2024
Total number of cases	104	146
Male cases	60	85
Female cases	44	61
Male percentage	58%	58.2%
Female percentage	42%	42%
Peak months (number)	May (18), Jun (18), Sep (14), Oct (13)	Nov (24), Dec (30), Jun (16), Jul (16), Sep (14)
Lowest months (number)	Aug (1), Dec (1)	Apr (3)
Age range	3m to 14y	1m to 14y

Table (10): Comparison highlighting the key differences and similarities between the two years:

Metric	Comparison (2024 vs. 2023)
Total number of cases	Increased by 40.4%
Male cases	Increased by 25 cases
Female cases	Increased by 17 cases
Peak months	Shifted towards the end of the year (Nov, Dec) in 2024, though June and September remained high
Lowest months	Different months with the lowest numbers
Overall age range	Similar (1m to 14y in 2024 vs. 3m to 14y in 2023)

Results of the Combined Analysis of 2023 and 2024:

When combining the two years (a total of 250 cases), various trends concerning *Entamoeba histolytica* infection in children in Western Libya may be easily observed. Most notably, the number of confirmed cases increased significantly between 2023 and 2024, indicating that the condition with this parasite infection in the area is either severe or may get worse. This occurred for a variety of reasons, including maybe lowered standards of sanitation, water quality, and hygiene, or rather the actual figures showed a greater awareness of events as a result of improved reporting.

The rise in case numbers is seasonally stable, although the peak months varied between the two years; however, there were a consistently high number of infections in both years, and a comparable infection burden was observed during those months. In other words, June and September had numerous reported cases, and the total number of cases encountered during those months was quite comparable across the two years. It is important to note that the total contributions from cases in November and December will be considered for possible case analysis if obtained from subsequent months in 2024.

DISCUSSION:

The current study aimed to investigate the prevalence and distribution of *Entamoeba histolytica* infections among children in Western Libya. The analysis of 250 cases raises important considerations for regional public health.

The data presented in this study is particularly important for understanding the epidemiology of *Entamoeba histolytica* infection in children between 1 month and 14 years in Western Libya during 2023 and 2024.

The results show a rising trend in infection prevalence, increasing from 104 in 2023 to 146 in 2024, which represents approximately a 40% increase in infection rates. This increase could be attributed to several factors, such as changes in environmental conditions, improvements in water sanitation and hygiene, and increasing population density.

The case distribution indicates a change in the peak infection months, moving from the warmer months in 2023 (May, June, September, and October) to the colder months in 2024 (November and December). This contradicted the expectation that amoebiasis would reach its highest point in warmer and more humid conditions [14]; however, the change in local climate and indoor sedentary population during the colder months possibly contributed to an increase in transmission by late 2024. Other developing regions are showing a similar change in temporal patterns during climate and infrastructure transition [15].

The study showed a stable higher incidence of infection in male children across both years, with an estimated 58% of the infected population being male. This is important because male children, as end-user hosts, are more likely to encounter human-made environmental contaminants due to increased environmental exposure, leading to an increased infection. These results supported by prior studies, showing that male children are frequently more exposed to environmental contaminants due to their active behaviors, spend more time outdoors, and have less supervised hygiene [16]. The age range of the participants was 1 month to 14 years, with children 5 years of age and younger showing the highest concentration of infection. This is consistent with several studies showing that children aged five years and under face a higher risk of parasitic intestinal infection, partly because of their immature immune systems, hand-to-mouth activities, and increased contact with contaminated food or water sources [17–18]. The presence of infants as young as one month old in 2024 may simply indicate vertical transmission within households, poor weaning practices, or contamination from feeding equipment.

In conclusion, this two-year study provides important data about *Entamoeba histolytica* prevalence in western Libya. Deeper investigation of the environmental, behavioral, and public health causes explaining these trends is required in light of the significant increase in cases in 2024 as well as the change to seasonal patterns. Developing effective methods to reduce the effect of this parasitic infection on local children requires an understanding of these processes.

CONCLUSION:

In comparison to 2023, there was a noticeable rise in *E. histolytica* infection cases in 2024. A change in the peak infection months between the two years is one of the monthly data trends that suggest potential seasonal or environmental factors. Males have been found to

have higher rates of infection than females, which require for additional research to find possible biological, social, or behavioral factors. The age distribution of cases, on the other hand, stayed comparatively constant, indicating that *E. histolytica* still poses a risk to people of all ages.

The study suggests strengthening sanitary infrastructure, conducting routine school-based screenings, and boosting health education on cleanliness and safe water practices. To assist focused public health actions, more seasonal and region-specific research is also required.

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