

Microscopic Detection of the *Entamoeba histolytica*/dispar Complex in Stool Specimens from Two Clinical Laboratories in Al-Abyar and Toulmetha, Northeastern Libya: A Retrospective Descriptive Study

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الكشف المجهرى عن مجموعة الإنتاميبا هستوليتيكا / ديسبار في عينات براز مقدمة إلى مختبرين طبيين في الأبيار وظلميثة، شمال شرق ليبيا: دراسة وصفية استيعادية

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Abstract:

Background: Stool microscopy can detect morphologically compatible *Entamoeba histolytica*/dispar organisms but cannot reliably identify pathogenic *E. histolytica* at the species level. This study describes the proportion of stool specimens with such findings in two laboratories in northeastern Libya. Methods: A retrospective descriptive analysis was based on 517 laboratory stool-specimen records: 410 from Al-Salam Medical Laboratory, Al-Abyar, and 107 from Toulmetha District Hospital Laboratory. The original manuscript describes macroscopic examination and direct saline and Lugol iodine wet-mount microscopy. Results: Intestinal parasites were recorded in 147 of 517 specimens (28.43%). The *E. histolytica*/dispar morphological complex was detected in 31 specimens (6.00% of all examined specimens and 21.09% of parasite-positive specimens). Detection was recorded in 24/410 specimens (5.85%) in Al-Abyar and 7/107 (6.54%) in Toulmetha. Of the 31 positive specimens, 20 were from females (64.52%) and 11 from males (35.48%); 12 were recorded in summer, eight in autumn, seven in winter, and four in spring. These distributions cannot establish sex-specific or seasonal differences in positivity without the corresponding numbers examined in each category. Conclusion: Approximately 6% of examined specimens were microscopy-positive for the *E. histolytica*/dispar morphological complex, but pathogenic *E. histolytica* infection was not confirmed. Species-specific antigen testing, or PCR is warranted for confirmation. The findings do not estimate community prevalence.

Keywords: *Entamoeba histolytica*/dispar, stool microscopy, laboratory-based study, positive samples, Al-Abyar, Toulmetha, Libya.

المخلص:

الخلفية: لا يستطيع الفحص المجهرى التقليدي التمييز بصورة موثوقة بين الطفيلي الممرض *Entamoeba histolytica* والطفيليات المشابهة له شكلياً، ومنها *E. dispar*. الهدف: وصف نسبة عينات البراز الموجبة مجهرياً لمجموعة *E. histolytica*/dispar في مختبرين طبيين شمال شرق ليبيا. المنهجية: دراسة وصفية استيعادية شملت 517

سجلاً لعينة براز؛ منها 410 عينات من مختبر السلام الطبي بالأبيار، و107 عينات من مختبر مستشفى طلميثة. اعتمدت الدراسة، وفق الوصف الأصلي، على الفحص العياني والفحص المجهرى المباشر بالمحلول الملحي ومحلول لوجول. النتائج: كُشف عن طفيليات معوية في 147 عينة (28.43%)، وعن مجموعة *E. histolytica/dispar* في 31 عينة (6.00% من إجمالي العينات و21.09% من العينات الموجبة للطفيليات). بلغت نسبة العينات الموجبة في مختبر الأبيار 5.85% (410/24)، وفي مختبر طلميثة 6.54% (107/7). ومن بين العينات الإيجابية الـ31، سُجّلت 20 عينة لإناث و11 لذكور، وتوزعت الحالات على الصيف (12)، والخريف (8)، والشتاء (7)، والربيع (4). ولا تُثبت هذه الأعداد فروقاً بين الجنسين أو الفصول دون معرفة العدد الإجمالي المفحوص بكل فئة. الخلاصة: تمثل نسبة 6.00% إيجابية العينات المفحوصة للمجموعة الشكلية، ولا تمثل انتشار العدوى المؤكدة بين السكان، كما يلزم اختبار نوعي بالمستضد أو تفاعل البوليميراز المتسلسل للتأكيد.

الكلمات المفتاحية: الفحص المجهرى للبراز، إيجابية العينات، الأبيار، طلميثة، ليبيا.

Introduction:

Intestinal parasitic infections remain relevant to public health, particularly where safe water, sanitation, and access to diagnostic services are limited. Laboratory-based studies can help describe the spectrum of intestinal parasites among individuals whose stool specimens are submitted for testing, although such findings are not population prevalence estimates (Gashout et al., 2017; Omar & Abdelal, 2022).

Entamoeba histolytica is a pathogenic intestinal protozoan that can cause intestinal and extraintestinal disease. Transmission is primarily fecal–oral. Nevertheless, routine microscopic examination of stool can produce morphologically indistinguishable findings for *E. histolytica*, *E. dispar*, and other related *Entamoeba* species. Molecular tests or appropriately species-specific antigen assays are therefore needed for reliable identification of pathogenic *E. histolytica* (Centers for Disease Control and Prevention [CDC], n.d.).

Retrospective analyses of routine laboratory records are practical for describing diagnostic findings and their distribution across testing sites. Published studies from Libya and neighboring settings also demonstrate that positivity figures differ across laboratories, sampling frames, and diagnostic procedures, making direct numerical comparisons potentially misleading (Amer et al., 2018; Gashout et al., 2017; Kiani et al., 2015).

The present report describes records from Al-Salam Medical Laboratory in Al-Abyar and Toulmetha District Hospital Laboratory, two laboratories in northeastern Libya. It focuses on the proportion of tested stool specimens with microscopy-compatible *E. histolytica/dispar* findings, their distribution by specimen source, and the descriptive composition of positive specimens by sex and season. No causes of transmission or population-level risk factors are inferred from these aggregate data.

Objectives:

The primary objective was to estimate the proportion of stool specimens with microscopy-compatible *E. histolytica/dispar* findings among specimens recorded at the two selected laboratories. Secondary objectives were to describe the positivity proportion in each laboratory and to summarize the sex and seasonal distribution of the 31 microscopy-positive specimens, without presenting these case distributions as sex-specific or seasonal risks.

Materials and Methods:

- Study design and setting:** This was a retrospective descriptive study of laboratory stool-specimen records obtained from Al-Salam Medical Laboratory in Al-Abyar and Toulmetha District Hospital Laboratory in northeastern Libya. The source manuscript describes a combined set of 517 specimen records. Each laboratory was analyzed separately for the sample-positivity proportion, and the total was used for the pooled descriptive proportion.
- Study records, selection, and unit of analysis:** The dataset summarized in the manuscript comprised 410 stool-specimen records from Al-Abyar and 107 from Toulmetha. The unit of analysis is referred to as a stool specimen/record, not necessarily a unique patient. Accordingly, the 517 specimens must not be described as 517 distinct individuals unless the laboratory registers confirm one specimen per person. The manuscript did not provide a documented selection process, exclusion count, or duplicate-record assessment. Reporting these items is consistent with observational-study recommendations (von Elm et al., 2007).
- Stool examination and species identification:** According to the original method description, stool samples underwent gross inspection and direct wet-mount microscopic examination in physiological saline and Lugol iodine. The microscopy findings were recorded as *E. histolytica/dispar* on morphological grounds. This designation does not establish that all positive specimens contained pathogenic *E. histolytica*. The source description does not demonstrate

confirmatory antigen testing, PCR, or differentiation from other morphologically similar *Entamoeba* species (CDC, n.d.).

4. **Variables and statistical analysis:** Only descriptive counts and percentages supported by the reported aggregate data were calculated. Three denominators were distinguished: the full specimen count (n=517), the subset positive for any intestinal parasite (n=147), and the specimens recorded as *E. histolytica/dispar* positive (n=31). Within-laboratory positivity used the total number of specimens examined at each laboratory as its denominator. Sex and seasonal percentages describe only the composition of the 31 positive specimens because totals examined by sex and season were not reported. No chi-square tests, p-values, sex-specific rates, or seasonal positivity rates are claimed.
5. **Ethics and data protection:** The report uses aggregated laboratory findings without publishing patient names or identifiers. Documentation of ethics approval or exemption, permission to use the laboratory records, and the confidentiality safeguards adopted for the original record review must be supplied by the investigators before journal submission.

Results:

1. **Overall specimen findings:** Among 517 tested stool specimens, 147 (28.43%) had at least one intestinal parasite recorded. Microscopy-compatible *E. histolytica/dispar* findings were reported in 31 specimens, equivalent to 6.00% of all tested specimens and 21.09% of the 147 specimens positive for any intestinal parasite. These percentages have different denominators and must not be summed (Table 1; Figure 1).

Table (1): Specimen-level positive findings in the combined dataset.

Indicator	Numerator / denominator	Percentage
Total specimens examined	517 / 517	100.00%
Any intestinal parasite detected	147 / 517	28.43%
<i>E. histolytica/dispar</i> detected (of all specimens)	31 / 517	6.00%
<i>E. histolytica/dispar</i> detected (of parasite-positive specimens)	31 / 147	21.09%

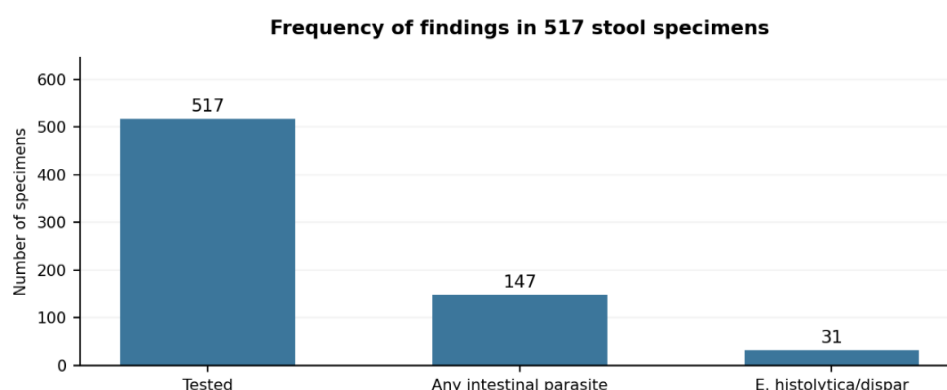


Figure (1): Counts of total tested, parasite-positive, and *E. histolytica/dispar*-positive specimens; nested categories.

2. **Findings by laboratory:** The recorded *E. histolytica/dispar* positivity proportions were 24/410 (5.85%) in Al-Abyar and 7/107 (6.54%) in Toulmetha. The difference is presented descriptively. It does not establish a difference in underlying community risk because the laboratories may serve different patient groups and because the original dataset does not document a formal comparison design (Table 2; Figure 2).

Table (2): *E. histolytica/dispar* microscopy positivity by source laboratory.

Laboratory	Tested specimens	Positive specimens	% Of specimens
Al-Salam Medical Laboratory (Al-Abyar)	410	24	5.85%
Toulmetha District Hospital Laboratory	107	7	6.54%
Combined	517	31	6.00%

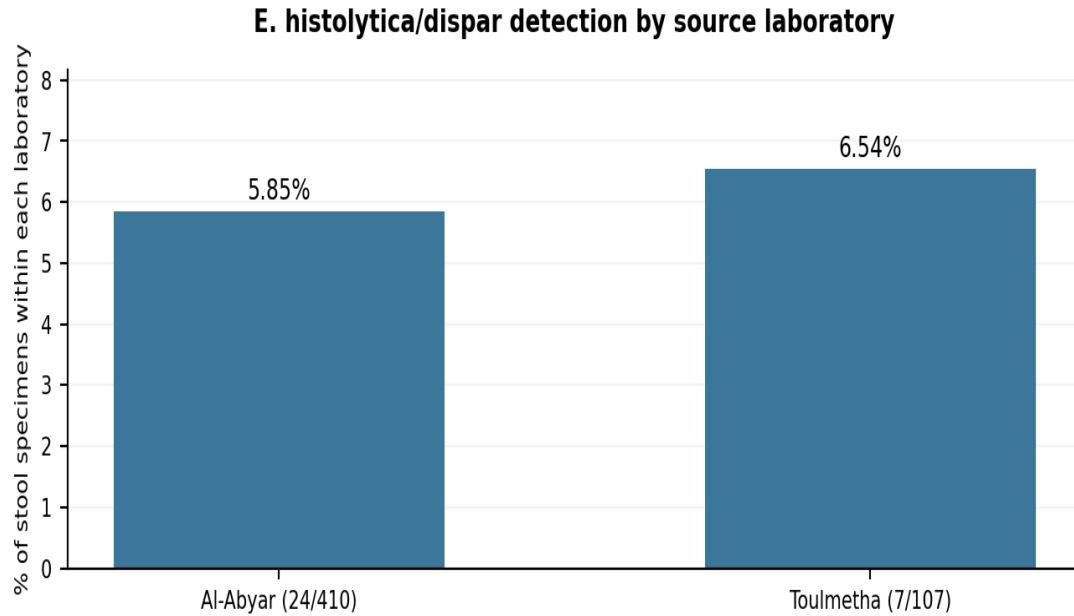


Figure (2): Within-laboratory sample positivity; denominators were 410 and 107, respectively.

- Sex distribution among positive specimens:** Of the 31 specimens with microscopy-compatible *E. histolytica/dispar* findings, 20 (64.52%) were recorded for female patients and 11 (35.48%) for male patients (Table 3; Figure 3). This is a distribution among positive specimens only. The number of all tested specimens from each sex was unavailable; therefore, it is not possible to conclude that female patients had a higher rate of positivity.

Table (3): Sex composition of *E. histolytica/dispar*-positive specimens (n=31).

Recorded sex	Positive specimens	% Among 31 positives
Female	20	64.52%
Male	11	35.48%
Total	31	100.00%

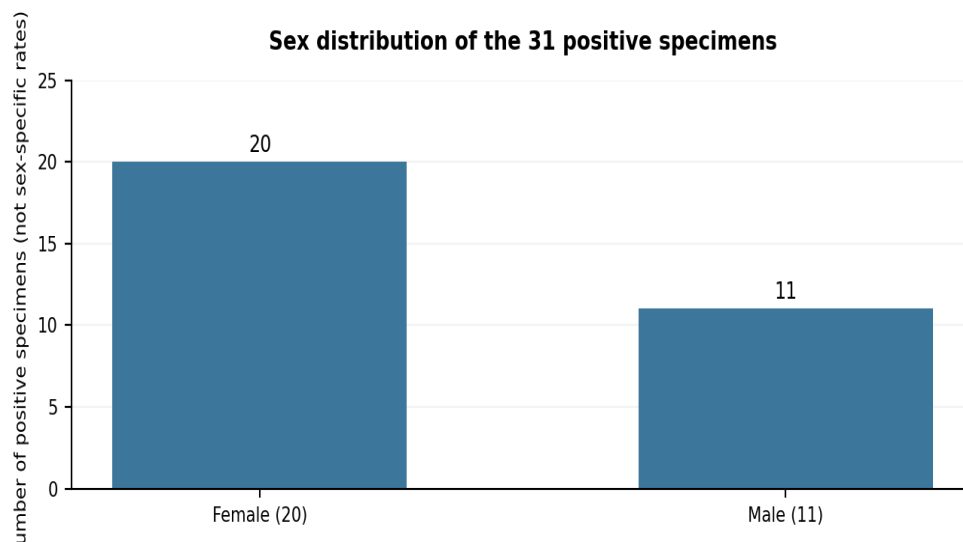


Figure (3): Counts by sex among microscopy-positive specimens (not sex-specific positivity rates).

4. **Seasonal distribution among positive specimens:** Among the 31 *E. histolytica*/dispar-positive specimens, 12 were recorded in summer (38.71%), eight in autumn (25.81%), seven in winter (22.58%), and four in spring (12.90%) (Table 4; Figure 4). These are shares of positive specimens, not rates of positivity during each season. The total number of specimens examined in each season was unavailable.

Table (4): Seasonal composition of *E. histolytica*/dispar-positive specimens (n=31).

Season	Positive specimens	% Among 31 positives
Summer	12	38.71%
Autumn	8	25.81%
Winter	7	22.58%
Spring	4	12.90%
Total	31	100.00%

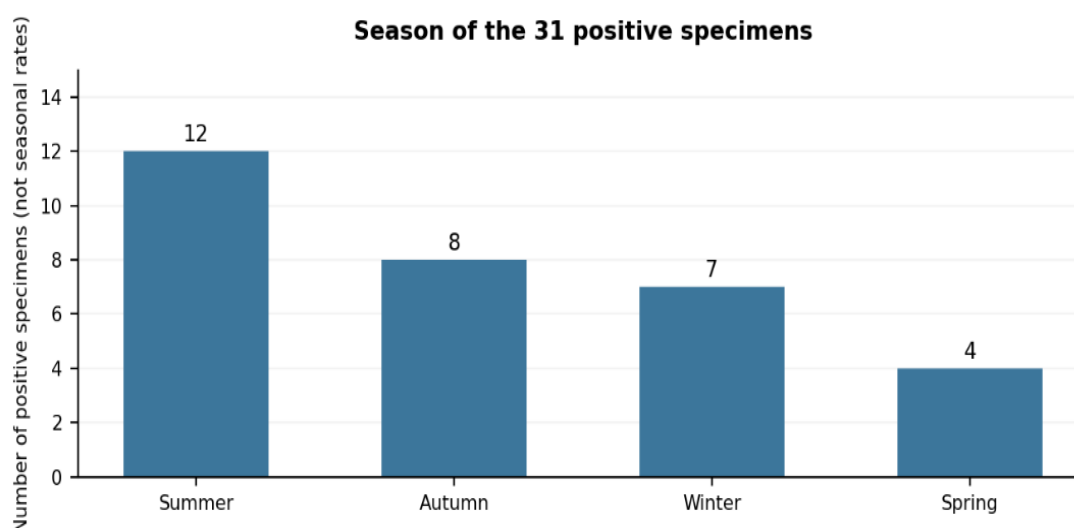


Figure (4): Counts of microscopy-positive specimens by season (not season-specific positivity rates).

Discussion:

The primary result is a 6.00% microscopy-based specimen positivity proportion for the *E. histolytica*/dispar morphological complex across two selected laboratories. The corresponding 21.09% describes the share of parasite-positive specimens attributed to this morphological group, not the rate among the general population. The distinction is particularly important for retrospective laboratory data in which stool testing is linked to healthcare seeking and physician referral.

Published laboratory series have reported varied values in Libya and the wider region. Gashout et al. (2017) documented microscopic findings of *E. histolytica*/*E. dispar* in stool samples at Tripoli Central Hospital, while Omar and Abdelal (2022) analyzed intestinal parasitic findings among patients attending hospitals in northeastern Egypt. Amer et al. (2018) also demonstrated that the observed proportion of intestinal parasites may vary considerably across retrospective hospital datasets. Additional literature from Ethiopian schoolchildren and hospital registers (Abate et al., 2023; Workineh et al., 2022) illustrates further variation in study populations. Differences in setting, study periods, patient selection, and diagnostic methods prevent straightforward numerical comparisons of positivity estimates.

The diagnostic limitation is central: wet-mount morphology does not reliably distinguish *E. histolytica* from *E. dispar* or some other related *Entamoeba* species, so the current findings cannot be interpreted as 31 confirmed cases of pathogenic *E. histolytica* infection. Moreover, it cannot be assumed that the number of true *E. histolytica* infections is precisely known or that a particular correction factor applies. Species-specific antigen assays and molecular methods are recommended where confirmation is needed (CDC, n.d.).

The female majority (20 of 31) reflects the sex composition of positive specimens; it does not demonstrate higher female susceptibility without the number of women and men tested. Similarly, summer contributed the largest number of positive specimens, but without seasonal testing totals this finding cannot establish a summer peak in positivity. Suggested mechanisms involving water quality, food preparation, flies, and outdoor exposures were not measured in this study and cannot be attributed

to the observed distributions. Related literature on parasite epidemiology provides contextual hypotheses but not causal explanations for these data (Al-Malki, 2021; Yimam et al., 2020).

A previously published report by Lameen (2026) also concerns 410 stool samples from Al-Salam Medical Laboratory, Al-Abyar. The current analysis likewise contains an Al-Abyar subset of 410 specimens with 24 *E. histolytica*/dispar findings. Because potential record overlap could materially affect the novelty of this report, the investigators should verify the sampling dates and original case-level records, document any shared dataset, and state the distinct contribution of the two-laboratory analysis clearly. Similarity of totals alone is not proof of duplicate publication.

Strengths and limitations:

The study provides a clear descriptive summary of specimens recorded at two laboratory services and differentiates between overall specimen positivity and positivity among samples containing any parasite. Its limitations include the non-population-based sampling frame; the inability to ascertain whether repeated specimens came from the same patient; reliance on microscopy without species-specific confirmation; missing tested-sample denominators by sex and season; undocumented study dates and eligibility procedures in the source manuscript; and possible differences in patient referral patterns between a private laboratory and a hospital laboratory. These constraints preclude population prevalence, temporal risk, or causal claims.

Conclusion:

Microscopy-compatible *E. histolytica*/dispar organisms were recorded in 31 of 517 examined stool specimens (6.00%) from two clinical laboratories in Al-Abyar and Toulmetha. The 31 specimens represented 21.09% of the 147 specimens positive for any intestinal parasite. Although the positive specimens were more numerous among women and in summer, category-specific positivity could not be assessed with the available denominators. The findings describe specimens submitted for testing; confirmation of pathogenic *E. histolytica* requires species-specific laboratory methods.

Recommendations:

- Where clinically indicated, use a validated species-specific stool antigen assay or molecular test to distinguish pathogenic *E. histolytica* from morphologically similar species.
- Improve routine laboratory registers so that each specimen can be linked securely to a unique patient identifier, sex, date, and laboratory site without compromising confidentiality.
- Record all tested specimens by sex and month or season, so subsequent analyses can estimate category-specific positivity and test predefined associations appropriately.
- Support laboratory quality assurance and public health education on safe drinking water, food hygiene, and sanitation without inferring causal risk factors from this dataset.
- Conduct population-based surveys if estimates representative of Al-Abyar and Toulmetha residents are required.

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Declarations:

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