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Comparison of Microscopy and ELISA for the Detection of *Entamoeba histolytica* in Stool Samples of Pediatric Patients with Acute Gastroenteritis

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Abstract: ***Background:*** *Amoebiasis caused by Entamoeba histolytica remains an important intestinal parasitic infection, particularly in developing countries. Conventional stool microscopy is inexpensive and widely available but has limitations in sensitivity and species-specific identification. Antigen-detection enzyme-linked immunosorbent assay (ELISA) provides an alternative diagnostic approach.* ***Objective:*** *To compare the diagnostic performance of conventional stool microscopy and ELISA for the detection of E. histolytica in stool samples obtained from pediatric patients presenting with acute gastroenteritis.* ***Methods:*** *A prospective observational study was conducted over a period of six months in the Department of Microbiology, SGT University, Gurugram, Haryana, India. A total of 79 stool samples from patients aged 1-18 years presenting with symptoms of acute gastroenteritis were examined. Direct wet-mount microscopy using normal saline and iodine preparations was performed for detection of Entamoeba cysts and trophozoites. All samples were additionally tested using the Serazym® Entamoeba histolytica ELISA, which detects the serine-rich E. histolytica protein (SREHP). Microscopy was used as the reference standard for calculation of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).* ***Results:*** *Microscopy detected 2 positive samples (2.53%), whereas ELISA detected 3 positive samples (3.79%). Compared with microscopy, ELISA demonstrated a sensitivity of 100%, specificity of 98.7%, PPV of 66.7%, and NPV of 100%. One sample was positive by ELISA but negative by microscopy.* ***Conclusion:*** *In this study, ELISA demonstrated high diagnostic performance compared with conventional microscopy for the detection of E. histolytica. The additional ELISA-positive result highlights the potential value of antigen detection in samples that may not be identified by microscopy. However, the small number of positive cases and use of microscopy as the reference standard limit the precision and interpretation of the diagnostic accuracy estimates. Larger studies using an independent reference method are warranted.*

Keywords: Entamoeba histolytica; amoebiasis; microscopy; enzyme-linked immunosorbent assay; ELISA; SREHP; stool examination; diagnostic accuracy; acute gastroenteritis.

1. Introduction

Amoebiasis is an intestinal and extraintestinal parasitic disease caused by the protozoan parasite *Entamoeba histolytica*. It remains an important public health problem worldwide, particularly in resource-limited settings where inadequate sanitation and unsafe water contribute to transmission. The clinical spectrum ranges from asymptomatic intestinal infection to invasive intestinal disease, including amoebic dysentery, and extraintestinal manifestations such as amoebic liver abscess.[1, 2, 3]

Transmission occurs predominantly through ingestion of infective cysts in contaminated food or water. Although many infections remain asymptomatic, invasive disease can result in substantial morbidity and, in severe cases, mortality. [1, 3] The burden of amoebiasis remains significant globally, with disease occurring particularly in endemic regions and among populations exposed to inadequate sanitation and hygiene. [1, 2]

Accurate identification of *E. histolytica* is important because the morphologically similar species *E. dispar* and *E. moshkovskii* may be detected during routine microscopic examination but cannot be reliably distinguished from *E. histolytica* on morphology alone. [4, 5, 6]

Conventional stool microscopy remains widely used because it is inexpensive, relatively simple, and accessible in routine diagnostic laboratories. However, its diagnostic performance is affected by specimen quality, parasite burden, intermittent shedding, timing of examination, and observer expertise. Furthermore, microscopy cannot reliably differentiate *E. histolytica* from morphologically similar *Entamoeba* species, which may lead to overdiagnosis and unnecessary treatment. [4, 5]

Antigen-detection methods, including ELISA, have therefore been developed to provide more specific detection of *E. histolytica*. Studies comparing microscopy with antigen detection have demonstrated that antigen-based assays can identify infections that may not be detected by microscopy alone. [7, 6]

The Serazym® *Entamoeba histolytica* assay is designed to detect the serine-rich 30-kDa membrane protein (SREHP), providing species-specific antigen detection. Uslu et al. evaluated Serazym® alongside other diagnostic methods and specifically described its use for detection of the serine-rich 30-kDa membrane protein of *E. histolytica*.[11]

Previous studies, including investigations in children with diarrhea, have demonstrated the potential advantages of antigen detection over conventional microscopy. [8, 12] Comparative studies have also reported improved diagnostic yield using antigen-based and molecular approaches for intestinal protozoal infections.[9, 10, 13, 14]

Despite the availability of these diagnostic methods, microscopy continues to be used extensively in routine

clinical microbiology laboratories because of its low cost and accessibility. Therefore, evaluating the performance of ELISA in comparison with routine microscopy remains clinically relevant, particularly in pediatric patients presenting with gastrointestinal symptoms.

The present study was undertaken to compare conventional stool microscopy and ELISA for the detection of *E. histolytica* in stool samples from pediatric patients presenting with acute gastroenteritis.

Objective

To compare the diagnostic performance of conventional stool microscopy and ELISA for the detection of *E. histolytica* in stool samples from pediatric patients presenting with acute gastroenteritis.

2. Materials and Methods

2.1 Study Design and Setting

This prospective observational study was conducted over a period of six months in the Department of Microbiology, Faculty of Medicine and Health Sciences, SGT University, Gurugram, Haryana, India.

2.2 Study Population

A total of 79 stool samples were collected from patients aged 1–18 years presenting with symptoms of acute gastroenteritis, including diarrhea, abdominal pain, vomiting, and fever.

Patients with prior antiparasitic treatment were excluded to minimize the possibility of false- negative results.

2.3 Sample Collection and Processing

Fresh stool samples were collected in sterile, wide-mouthed, leak-proof containers and processed within one hour of collection. Macroscopic examination was performed to assess stool consistency, color, and the presence of blood or mucus.

2.4 Microscopic Examination

Each stool sample was subjected to direct wet-mount examination using normal saline and iodine preparations. The preparations were examined microscopically under low-power (10X) and high-power (40X) objectives for the detection of cysts and trophozoites of *Entamoeba* species. Identification was based on standard morphological characteristics.[4, 5]

2.5 ELISA for Antigen Detection

All stool samples were additionally analyzed using the commercially available Serazym® *Entamoeba histolytica* ELISA. The assay detects the serine-rich *E. histolytica* protein (SREHP), a species-specific antigen.[11]

The assay was performed according to the manufacturer's instructions. Optical density (OD) values were measured using an ELISA reader, and results were classified as positive or negative according to the manufacturer's specified cut-off.

2.6 Reference Standard

For the present analysis, conventional microscopy was used as the reference standard for comparison with ELISA. The choice of microscopy as the reference standard was based on its use as the routine diagnostic method in the study setting. However, because microscopy has recognized limitations in the detection and species-specific identification of *E. histolytica*, diagnostic accuracy estimates should be interpreted as performance of ELISA relative to microscopy rather than as definitive estimates against an independent gold standard.[4, 5]

2.7 Statistical Analysis

A 2 × 2 contingency table was constructed by comparing ELISA results with microscopy results.

Diagnostic performance was evaluated using:

- Sensitivity
- Specificity
- Positive predictive value (PPV)
- Negative predictive value (NPV)

Sensitivity was calculated as:
TP/ (TP + FN) × 100

Specificity was calculated as:
TN/ (TN + FP) × 100

PPV was calculated as:
TP/ (TP + FP) × 100

NPV was calculated as:
TN/ (TN + FN) × 100

Diagnostic accuracy was reported in accordance with principles of diagnostic-accuracy reporting recommended by the STARD 2015 guideline.[15]

3. Results

A total of **79 stool samples** were analysed.

Microscopy detected *Entamoeba* cysts/trophozoites consistent with a positive result in **2 samples (2.53%)**, whereas ELISA detected **3 positive samples (3.79%)**.

3.1 Comparison of Microscopy and ELISA

Table 1: Cross-tabulation of ELISA results against microscopy

ELISA result	Microscopy positive	Microscopy negative	Total
Positive	2	1	3
Negative	0	76	76
Total	2	77	79

Thus:

- True positive (TP) = 2
- False positive (FP) = 1
- False negative (FN) = 0
- True negative (TN) = 76

3.2 Diagnostic Performance

Table 2: Diagnostic performance of ELISA compared with microscopy

Diagnostic parameter	Result
Sensitivity	100%
Specificity	98.7%
Positive predictive value	66.7%
Negative predictive value	100%

The sensitivity of ELISA was 100%, while specificity was 98.7%. The PPV was 66.7%, and the NPV was 100%.

One sample produced a positive ELISA result while being negative by microscopy. Because microscopy was used as the reference standard, this discordant result cannot be definitively classified as a true infection without an independent confirmatory method such as PCR.

4. Discussion

The present study evaluated the performance of an antigen-detection ELISA compared with conventional stool microscopy for the detection of *E. histolytica* in pediatric patients presenting with acute gastroenteritis. Among the 79 stool samples examined, microscopy identified 2 positive samples, whereas ELISA identified 3 positive samples.

The findings are consistent with the recognized limitations of conventional microscopy. Although microscopy remains an accessible and inexpensive diagnostic method, its performance can be affected by specimen quality, parasite burden, timing of examination, intermittent shedding, and observer expertise. In addition, morphology-based examination cannot reliably distinguish *E. histolytica* from morphologically similar species such as *E. dispar* and *E. moshkovskii*.[4]

The inability of microscopy to distinguish *E. histolytica* from morphologically similar species has important clinical implications. Molecular studies have demonstrated that microscopy may overestimate the occurrence of pathogenic *E. histolytica* when morphologically similar organisms are identified without species-specific confirmation.[6, 14]

In contrast, antigen-detection methods provide a more specific approach to the identification of *E. histolytica*. Previous studies have demonstrated the usefulness of ELISA and other antigen-based assays for the detection of *E. histolytica* in stool specimens.[7, 9]

The present study used the Serazym® *E. histolytica* assay, which detects the serine-rich 30- kDa membrane protein (SREHP). Uslu et al. specifically evaluated Serazym® *E. histolytica* antigen testing alongside other diagnostic methods and demonstrated the value of species- specific antigen detection in specimens initially identified microscopically as *E. histolytica/E. dispar*.[11]

The detection of 3 ELISA-positive samples compared with 2 microscopy-positive samples in the present study resulted in a sensitivity of 100% and specificity of 98.7% when microscopy was used as the reference standard. Similar studies have demonstrated that antigen detection can identify samples that may be missed by microscopy. In a study involving children with diarrhea, ELISA detected additional *E. histolytica* infections among samples that were negative by microscopy.[12] Studies comparing microscopy and ELISA have similarly highlighted the limitations of microscopy and the potential value of antigen detection.[8, 9]

The PPV observed in the present study was 66.7%. The relatively low PPV should be interpreted in the context of the small sample size and the low number of positive cases. Predictive values are influenced by disease prevalence, and the low number of positive samples in the present study limits the precision of the PPV estimate.

The NPV was 100% in the present study. However, because only two microscopy-positive samples were identified, the precision of the sensitivity and NPV estimates is limited. These values should therefore not be interpreted as definitive estimates of the performance of ELISA against true infection.

One sample was positive by ELISA but negative by microscopy. This finding may reflect the ability of antigen detection to identify a sample not detected by conventional microscopy; however, because no independent confirmatory test was performed, the true infection status of this discordant sample cannot be established. Previous diagnostic studies have used molecular methods to resolve discrepant antigen-test and microscopy results, highlighting the value of an independent reference method in diagnostic-accuracy studies.[13, 16]

Molecular diagnostic approaches, including PCR, provide species-specific detection and can distinguish *E. histolytica* from morphologically similar *Entamoeba* species.[6, 14] However, molecular testing may not be routinely available in all clinical laboratories because of cost, infrastructure, and technical requirements. Consequently, antigen-detection methods may provide a practical intermediate approach between conventional microscopy and molecular diagnosis in laboratories where PCR is not readily available.[9, 17]

The findings of the present study support the potential usefulness of incorporating antigen detection into the diagnostic evaluation of suspected intestinal amoebiasis. Nevertheless, microscopy remains valuable because of its affordability, accessibility, and ability to detect a range of intestinal parasites. A complementary diagnostic strategy may therefore be useful, particularly in settings where molecular testing is unavailable.

4.1 Strengths of the Study

A strength of the present study is its prospective design and direct comparison of conventional microscopy with a

commercially available *E. histolytica*-specific antigen-detection assay in clinical stool specimens. The study was also conducted in a routine microbiology laboratory setting, making the findings relevant to real-world diagnostic practice.

4.2 Limitations of the Study

The study has several limitations. First, the sample size was relatively small, with only 79 stool samples and two microscopy-positive cases. This limits the precision of the diagnostic accuracy estimates, particularly sensitivity. Second, microscopy was used as the reference standard despite its recognized limitations for detecting and specifically identifying *E. histolytica*. Third, molecular confirmation by PCR was not performed to resolve the discordant ELISA- positive/microscopy-negative sample. Therefore, the additional ELISA-positive result cannot be definitively classified as a true positive in the absence of an independent reference method. Larger prospective studies incorporating PCR or another validated independent reference method are required to establish the diagnostic performance of ELISA more robustly.

5. Conclusion

In this prospective study of 79 stool samples from pediatric patients with acute gastroenteritis, ELISA detected one additional positive sample compared with conventional microscopy and demonstrated high sensitivity and specificity when microscopy was used as the reference standard.

The findings suggest that antigen detection may provide useful additional diagnostic information in suspected *E. histolytica* infection, particularly when conventional microscopy is negative. However, the small number of positive cases and the use of microscopy as the reference standard limit the certainty with which the diagnostic accuracy estimates can be generalized.

Further studies involving larger sample sizes and an independent reference method, preferably species-specific molecular confirmation, are recommended to validate these findings and determine the clinical utility of ELISA in routine diagnostic laboratories.

References

[1] Shirley DA, Farr L, Watanabe K, Moonah S. A review of the global burden, new diagnostics, and current therapeutics for amebiasis. InOpen forum infectious diseases 2018 Jul (Vol. 5, No. 7, p. ofy161). US: Oxford University Press.
[2] Nasrallah J, Akhoundi M, Haouchine D, Marteau A, Mantelet S, Wind P, Benamouzig R, Bouchaud O, Dhote R, Izri A. Updates on the worldwide burden of amoebiasis: A case series and literature review. Journal of infection and public health. 2022 Oct 1;15(10):1134-41.
[3] Kantor M, Abrantes A, Estevez A, Schiller A, Torrent J, Gascon J, Hernandez R, Ochner C. Entamoeba histolytica: updates in clinical manifestation, pathogenesis, and vaccine development. Canadian Journal of Gastroenterology and Hepatology. 2018;2018(1):4601420.
[4] Tanyuksel M, Petri Jr WA. Laboratory diagnosis of amebiasis. Clinical microbiology reviews. 2003 Oct;16(4):713-29.
[5] Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness J. Laboratory diagnostic techniques for Entamoeba species. Clinical microbiology reviews. 2007 Jul;20(3):511- 32.
[6] Ali IK, Clark CG, Petri Jr WA. Molecular epidemiology of amebiasis. Infection, Genetics and Evolution. 2008 Sep 1;8(5):698-707.
[7] Leo M, Haque R, Kabir M, Roy S, Lahlou RM, Mondal D, Tannich E, Petri Jr WA. Evaluation of Entamoeba histolytica antigen and antibody point-of-care tests for the rapid diagnosis of amebiasis. Journal of clinical microbiology. 2006 Dec;44(12):4569- 71.
[8] Mohanty S, Sharma N, Deb M. Microscopy versus enzyme linked immunosorbent assay test for detection of Entamoeba histolytica infection in stool samples. Tropical parasitology. 2014 Jul;4(2):136.
[9] Alver O, Topaç T, Töre O. Evaluation of Two Methods (Nativ-Lugol Preparation and Enzyme-Linked Immunosorbent Assay) for Detection of Entamoeba histolytica in Stool Samples. Türkiye Parazitolojii Dergisi. 2015 Sep 1;39(3):185.
[10] Mengeloğlu FZ, Aktaş E, Külah C, Cömert FB. Detection of Entamoeba histolytica in stool specimens with the ELISA method. Turkiye Parazitolojii Dergisi. 2009 Jan 1;33(1):1-3.
[11] Uslu H, Aktas O, Uyanik MH. Comparison of various methods in the diagnosis of Entamoeba histolytica in stool and serum specimens. The Eurasian journal of medicine. 2016 Jun;48(2):124.
[12] Koltas IS, Demirhindi H, Hazar S, Ozcan K. Importance of the detection of amoebic antigens in stool samples for the diagnosis of Entamoeba histolytica infection, among children in southern Turkey. Annals of Tropical Medicine & Parasitology. 2007 Mar 1;101(2):143-50.
[13] Verkerke HP, Hanbury B, Siddique A, Samie A, Haque R, Herbein J, Petri Jr WA. Multisite clinical evaluation of a rapid test for Entamoeba histolytica in stool. Journal of Clinical Microbiology. 2015 Feb;53(2):493-7.
[14] Parija SC, Garg A, Pushpa K, Khairnar K, Priya T. Polymerase chain reaction confirmation of diagnosis of intestinal amebiasis in Puducherry. Indian Journal of Gastroenterology. 2010 Jul;29(4):140-2.
[15] Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, Lijmer JG, Moher D, Rennie D, De Vet HC, Kressel HY. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. Radiology. 2015 Dec;277(3):826-32.
[16] Bruijnesteijn van Coppenraet LE, Wallinga JA, Ruijs GJ, Bruins MJ, Verweij JJ. Parasitological diagnosis combining an internally controlled real-time PCR assay for the detection of four protozoa in stool samples with a testing algorithm for microscopy. Clinical Microbiology and Infection. 2009 Sep;15(9):869-74.
[17] Dacal E, Köster PC, Carmena D. Diagnóstico

molecular de parasitosis intestinales. Enfermedades infecciosas y microbiologia clinica. 2020 Jan 1; 38: 24-31.
[18] Ali IK, Clark CG, Petri Jr WA. Molecular epidemiology of amebiasis. Infection, Genetics and Evolution. 2008 Sep 1;8(5):698-707.
[19] Alver O, Topaç T, Töre O. Evaluation of Two Methods (Nativ-Lugol Preparation and Enzyme-Linked Immunosorbent Assay) for Detection of Entamoeba histolytica in Stool Samples. Türkiye Parazitolojii Dergisi. 2015 Sep 1;39(3):185.
[20] Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, Lijmer JG, Moher D, Rennie D, De Vet HC, Kressel HY. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. Radiology. 2015 Dec;277(3):826-32.
[21] Bruijnesteijn van Coppenraet LE, Wallinga JA, Ruijs GJ, Bruins MJ, Verweij JJ. Parasitological diagnosis combining an internally controlled real-time PCR assay for the detection of four protozoa in stool samples with a testing algorithm for microscopy. Clinical Microbiology and Infection. 2009 Sep;15(9):869-74.
[22] Dacal E, Köster PC, Carmena D. Diagnóstico molecular de parasitosis intestinales. Enfermedades infecciosas y microbiologia clinica. 2020 Jan 1; 38: 24-31.
[23] Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness J. Laboratory diagnostic techniques for Entamoeba species. Clinical microbiology reviews. 2007 Jul;20(3):511-32.
[24] Kantor M, Abrantes A, Estevez A, Schiller A, Torrent J, Gascon J, Hernandez R, Ochner C. Entamoeba histolytica: updates in clinical manifestation, pathogenesis, and vaccine development. Canadian Journal of Gastroenterology and Hepatology. 2018;2018(1):4601420.
[25] Koltas IS, Demirhindi H, Hazar S, Ozcan K. Importance of the detection of amoebic antigens in stool samples for the diagnosis of Entamoeba histolytica infection, among children in southern Turkey. Annals of Tropical Medicine & Parasitology. 2007 Mar 1;101(2):143-50.
[26] Leo M, Haque R, Kabir M, Roy S, Lahlou RM, Mondal D, Tannich E, Petri Jr WA. Evaluation of Entamoeba histolytica antigen and antibody point-of-care tests for the rapid diagnosis of amebiasis. Journal of clinical microbiology. 2006 Dec;44(12):4569-71.
[27] Mengeloğlu FZ, Aktaş E, Külah C, Cömert FB. Detection of Entamoeba histolytica in stool specimens with the ELISA method. Turkiye Parazitolojii Dergisi. 2009 Jan 1;33(1):1-3.
[28] Mohanty S, Sharma N, Deb M. Microscopy versus enzyme linked immunosorbent assay test for detection of Entamoeba histolytica infection in stool samples. Tropical parasitology. 2014 Jul;4(2):136.
[29] Nasrallah J, Akhoundi M, Haouchine D, Marteau A, Mantelet S, Wind P, Benamouzig R, Bouchaud O, Dhote R, Izri A. Updates on the worldwide burden of amoebiasis: A case series and literature review. Journal of infection and public health. 2022 Oct 1;15(10):1134-41.
[30] Parija SC, Garg A, Pushpa K, Khairnar K, Priya T. Polymerase chain reaction confirmation of diagnosis of intestinal amebiasis in Puducherry. Indian Journal of Gastroenterology. 2010 Jul;29(4):140-2.
[31] Shirley DA, Farr L, Watanabe K, Moonah S. A review of the global burden, new diagnostics, and current therapeutics for amebiasis. InOpen forum infectious diseases 2018 Jul (Vol. 5, No. 7, p. ofy161). US: Oxford University Press.
[32] Tanyuksel M, Petri Jr WA. Laboratory diagnosis of amebiasis. Clinical microbiology reviews. 2003 Oct;16(4):713-29.
[33] Uslu H, Aktas O, Uyanik MH. Comparison of various methods in the diagnosis of Entamoeba histolytica in stool and serum specimens. The Eurasian journal of medicine. 2016 Jun;48(2):124.
[34] Verkerke HP, Hanbury B, Siddique A, Samie A, Haque R, Herbein J, Petri Jr WA. Multisite clinical evaluation of a rapid test for Entamoeba histolytica in stool. Journal of Clinical Microbiology. 2015 Feb;53(2):493-7.