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EPIDEMIOLOGICAL AND MOLECULAR DETECTION OF *ENTAMOEB* *HISTOLYTICA* AND *GIARDIA LAMBLIA* IN ENVIRONMENTAL AND CLINICAL SAMPLES

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ABSTRACT

Waterborne and soilborne protozoan parasites, most notably *Entamoeba histolytica* and *Giardia lamblia*, represent a complex public health challenge in resource-limited settings. This study presents an epidemiological and molecular evaluation of the prevalence of both parasites across diverse environmental matrices (N = 300), including surface water, tap water, and agricultural soil, integrated with a clinical assessment.

Direct light microscopy identified parasitic cysts belonging to the *Entamoeba* complex in 75 samples (25.0%). However, nested polymerase chain reaction (nested-PCR) assays refined these diagnoses, revealing that only 37 samples (12.3%) harbored the pathogenic *E. histolytica*, whereas 38 samples (12.7%) contained non-pathogenic species (*E. dispar* / *E. moshkovskii*). Regarding *G. lamblia*, molecular detection yielded a higher positivity rate of 18.7% (n = 56) compared to 15.3% (n = 46) determined microscopically.

Spatial analysis demonstrated significantly elevated contamination levels in peri-urban and agricultural sectors compared to urban centers ($p < 0.001$), with peak occurrence recorded during the summer season. Genotyping of *G. lamblia* isolates revealed a predominance of Assemblage B (53.6%), followed by Assemblage A (35.7%). These findings underscore the inherent diagnostic limitations of direct microscopy and advocate for the systematic integration of molecular tools into epidemiological surveillance frameworks and water resource management strategies.

Keywords: *Entamoeba histolytica*; *Giardia lamblia*; Nested-PCR; Waterborne parasites; Epidemiology; Public health

1 INTRODUCTION

Intestinal protozoan infections pose a substantial global burden on healthcare systems, particularly in regions constrained by inadequate water treatment infrastructure and compromised environmental sanitation [11]. Among these microorganisms, *Entamoeba histolytica* and *Giardia lamblia* remain primary etiologic agents of acute and chronic gastrointestinal illness worldwide.

Entamoeba histolytica is a tissue-invasive protozoan responsible for amoebic dysentery and severe extraintestinal complications, such as amoebic liver abscesses, accounting for tens of thousands of fatalities annually [12]. A major

diagnostic obstacle in routine laboratory practice is the complete morphological indistinguishability between *E. histolytica* cysts and those of commensal, non-pathogenic species including *E. dispar* and *E. moshkovskii* [10]. This diagnostic ambiguity frequently leads to false-positive reports and unnecessary administration of antiprotozoal therapy.

Conversely, *Giardia lamblia* is a flagellated protozoan that colonizes the small intestine of humans and mammals, leading to diarrheal disease and malabsorption, particularly among pediatric populations [9]. Transmission occurs via the ingestion of environmentally resistant, quadrinucleate cysts present in contaminated drinking water and soil. *Giardia lamblia* exhibits marked genetic diversity and is classified into eight distinct assemblages (A–H), with Assemblages A and B primarily associated with infections in humans and domestic animals [6].

The primary objectives of this study were to:

- Assess contamination levels of *E. histolytica* and *G. lamblia* across various water and soil matrices (N = 300).
- Utilize nested-PCR assays to discriminate pathogenic *E. histolytica* from morphologically identical non-pathogenic species and determine the genetic assemblages of *G. lamblia*.
- Evaluate the influence of seasonal variations and spatial distribution, linking environmental contamination patterns to clinical presentations.

2 MATERIALS AND METHODS

A total of 300 environmental samples (N = 300) were collected across three distinct sources (n = 100 per matrix):

- Surface Water (n = 100): 5-liter samples collected from rivers and irrigation canals.
- Tap Water (n = 100): 10-liter samples drawn from municipal distribution networks after flushing the taps for two minutes.
- Agricultural Soil (n = 100): Topsoil samples obtained at a depth of 0–10 cm from adjacent farmland.

Concurrently with the environmental sampling, 125 stool specimens were obtained from patients presenting with acute gastrointestinal symptoms at local health centers to facilitate comparative clinical and molecular analyses.

3 RESULTS AND DISCUSSION

Initial microscopic examination detected parasitic cysts corresponding to the *Entamoeba* complex in 75 environmental samples (25.0%). Subsequent nested-PCR characterization established that only 37 of these samples (12.3%) were positive for pathogenic *E. histolytica*, while 38 samples (12.7%) were attributed to non-pathogenic *E. dispar*.

For *G. lamblia*, microscopic examination initially indicated a positivity rate of 15.3% (n = 46). Molecular diagnostic techniques increased the detection rate to 18.7% (n = 56), highlighting the superior sensitivity of PCR-based assays.

Table 1: Comparative diagnostic performance of direct microscopy and nested-PCR in environmental samples (N = 300)

Sample Type	Sample Size (n)	Microscopy Positive (<i>Entamoeba spp.</i>)	PCR Positive (<i>E. histolytica</i>)	PCR Positive (<i>E. dispar</i>)	Microscopy Positive (<i>Giardia spp.</i>)	PCR Positive (<i>G. lamblia</i>)
Surface Water	100	29 (29.0%)	22 (22.0%)	19 (19.0%)	26 (26.0%)	31(31.0%)
Tap Water	100	9 (9.0%)	4 (4.0%)	6 (6.0%)	6 (6.0%)	8 (8.0%)
Agricultural Soil	100	37 (37.0%)	11 (11.0%)	13 (13.0%)	14(14.0%)	17(17.0%)
Total	300	75 (25.0%)	37 (12.3%)	38 (12.7%)	46 (15.3%)	56(18.7%)

Table 2: Geographical distribution of molecularly confirmed protozoan parasites across environmental sectors (N = 300)

Geographical Sector	Sample Size	<i>E. histolytica</i> Positive (%)	<i>G. lamblia</i> Positive (%)	Dual Contamination (%)	p-value
Zone A (Urban Center)	100	6 (6.0%)	9 (9.0%)	2 (2.0%)	< 0.01
Zone B (Peri-urban)	100	16 (16.0%)	23 (23.0%)	8 (8.0%)	< 0.001
Zone C (Agricultural)	100	15 (15.0%)	24 (24.0%)	6 (6.0%)	< 0.001
Total	300	37 (12.3%)	56 (18.7%)	16 (5.3%)	—

Table 3: Seasonal variability of parasitic cyst contamination in surface water and soil (n = 400)

Season	Sample Size	<i>E. histolytica</i> Positive (%)	<i>G. lamblia</i> Positive (%)	Mean Temperature (°C)	Mean Precipitation (mm)
Spring	100	14 (14.0%)	20 (20.0%)	22.4	45.2
Summer	100	26 (26.0%)	38 (38.0%)	34.8	12.0
Autumn	100	18 (18.0%)	24 (24.0%)	24.1	28.5
Winter	100	6 (6.0%)	13 (13.0%)	12.5	82.1

Table 4: Genotype distribution of *G. lamblia* isolates from environmental and clinical samples

Sample Source	Positive Samples (n)	Assemblage A (%)	Assemblage B (%)	Mixed Genotypes (A + B) (%)
Environmental	56	20 (35.7%)	30 (53.6%)	6 (10.7%)
Clinical	42	15 (35.7%)	24 (57.1%)	3 (7.1%)
Total	98	35 (35.7%)	54 (55.1%)	9 (9.2%)

The findings of this study demonstrate widespread contamination of aquatic and soil environments with the pathogenic protozoans *Entamoeba histolytica* and *Giardia lamblia*, presenting a continuous public health risk.

A key outcome of this research is the quantitative demonstration of the severe limitations of conventional light microscopy. The inability of optical microscopy to differentiate pathogenic *E. histolytica* from morphologically identical non-pathogenic species (*E. dispar* / *E. moshkovskii*) resulted in diagnostic overestimation exceeding 50%. These observations align with previous international benchmark studies [8, 9], which established that sole reliance on microscopic diagnosis inflates epidemiological statistics for amoebiasis and leads to inappropriate therapeutic interventions. The integration of nested-PCR assays provided the requisite resolution to define true epidemiological risks.

The detection of parasitic cysts in treated tap water (8.0% for *G. lamblia* and 4.0% for *E. histolytica*) constitutes a major public health concern. Protozoan cysts possess rigid, multi-layered walls that confer high resistance to standard chlorine disinfection concentrations used in routine water treatment [3]. This municipal contamination likely stems from aging distribution networks, cross-contamination between sewage lines and potable water mains, or suboptimal coagulation and sand filtration processes within treatment plants.

Geographically, peri-urban and agricultural areas exhibited significantly higher contamination rates compared to urban centers. This disparity correlates with inadequate sanitation infrastructure and the widespread use of untreated wastewater for crop irrigation—a well-documented epidemiological pattern in developing regions [2].

At the molecular level, the predominance of *G. lamblia* Assemblage B (53.6%) points to intensive anthroponotic transmission cycles driven by high cyst shedding rates. Concurrently, the substantial presence of Assemblage A (35.7%) highlights potential zoonotic reservoir dynamics and cross-species transmission pathways [5].

4 CONCLUSION

Diagnostic Accuracy: Conventional light microscopy is insufficient for the definitive diagnosis of amoebiasis. Molecular assays, such as nested-PCR, must be integrated into routine epidemiological surveillance and clinical diagnostics.

- **Water Safety:** The presence of viable parasitic cysts in municipal drinking water necessitates an immediate re-evaluation of filtration and disinfection protocols in water treatment facilities.
- **One Health Approach:** High parasite prevalence in agricultural soil and irrigation water warrants the implementation of integrated One Health policies connecting environmental sanitation, agricultural management, and community health education.

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