ORIGINAL RESEARCH

Study of Intestinal protozoans parasite in symptomatic cases by direct wet mount and modified ZN staining Methods

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ABSTRACT
This study investigated the prevalence of intestinal protozoan parasites in symptomatic cases using direct wet mount and modified ZN staining methods. A cross-sectional study was conducted on 422 participants at a tertiary care hospital over 12 months. The overall prevalence of intestinal protozoan parasites was 62.8%, with *Entamoeba histolytica/dispar* (16.1%) being the most common, followed by *Giardia lamblia* (12.6%) and *Blastocystis hominis* (9.7%). Direct wet mount showed higher sensitivity (82.3%) compared to modified ZN staining (74.7%), but combining both methods improved overall detection. Children under 15 years and individuals from low socioeconomic backgrounds had significantly higher odds of infection. Diarrhea, abdominal pain, and weight loss were strongly associated with protozoan infections. The study highlights the significant burden of intestinal protozoan parasites and the importance of using multiple diagnostic techniques. The findings emphasize the need for targeted interventions, particularly among vulnerable populations, and underscore the continued public health importance of these infections. This study contributes valuable information for public health interventions and clinical management strategies in addressing intestinal protozoan infections.

Keywords: Protozoan Infections, *Entamoeba histolytica*, *Giardia lamblia*, Socioeconomic Conditions, Immunocompromised Individuals.

INTRODUCTION

Intestinal protozoan parasites are a significant cause of gastrointestinal disorders worldwide, particularly in developing countries. These microscopic organisms can lead to a range of symptoms, from mild discomfort to severe, life-threatening conditions. The study of intestinal protozoan parasites in symptomatic cases is crucial for accurate diagnosis, effective treatment, and the development of preventive strategies. Protozoan parasites that commonly infect the human intestinal tract include *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*, and *Blastocystis hominis*. These organisms can cause a variety of clinical manifestations, such as diarrhea, abdominal pain, nausea, vomiting, and malnutrition. In immunocompromised individuals, these infections can be particularly severe and even life-threatening (Stark et al., 2018).

The diagnosis of intestinal protozoan infections relies heavily on laboratory techniques for the detection and identification of these parasites. Two commonly used methods are direct wet mount examination and modified Ziehl-Neelsen (ZN) staining. The direct wet mount is a simple, rapid, and cost-effective technique that allows for the visualization of motile trophozoites and cysts of protozoan parasites. This method is particularly useful for detecting Entamoeba and Giardia species (Garcia, 2016). Modified ZN staining, on the other hand, is specifically used for the detection of acid-fast organisms, including Cryptosporidium, Cyclospora, and Isospora. This technique involves the use of carbol fuchsin as the primary stain, followed by decolorization with acid-alcohol and counterstaining with methylene blue. The modified ZN stain enhances the visibility of oocysts, making them appear bright pink against a blue background (Checkley et al., 2015).S

The prevalence and distribution of intestinal protozoan parasites vary greatly depending on geographical location, socioeconomic conditions, and environmental factors. In many developing countries, poor sanitation, inadequate water treatment, and limited access to healthcare contribute to the high prevalence of these infections. Studies have shown

that intestinal protozoan parasites are a significant cause of morbidity and mortality, particularly among children and immunocompromised individuals (Osman et al., 2016). The impact of intestinal protozoan infections extends beyond individual health, affecting communities and economies. Chronic infections can lead to malnutrition, growth stunting in children, and reduced cognitive function. Furthermore, these infections can exacerbate existing health conditions and increase susceptibility to other diseases. The economic burden of intestinal protozoan infections is substantial, encompassing healthcare costs, lost productivity, and the expense of preventive measures (Bartelt & Sartor, 2015).

Recent advances in molecular techniques have revolutionized the detection and characterization of intestinal protozoan parasites. Polymerase chain reaction (PCR) and other nucleic acid-based methods offer higher sensitivity and specificity compared to traditional microscopy techniques. However, these molecular methods are often too expensive and technically demanding for routine use in resource-limited settings, where the burden of intestinal protozoan infections is highest (Ryan et al., 2017). The study of intestinal protozoan parasites in symptomatic cases is essential for several reasons. Firstly, it provides valuable epidemiological data on the prevalence and distribution of these parasites in specific populations. This information is crucial for public health planning and the allocation of resources for control and prevention strategies. Secondly, accurate diagnosis of protozoan infections is necessary for appropriate treatment, as different species may require different therapeutic approaches (Speich et al., 2013).

Furthermore, studying the clinical presentation of intestinal protozoan infections can help improve our understanding of the pathogenesis of these organisms. This knowledge can lead to the development of new diagnostic tools, more effective treatments, and potentially vaccines. Additionally, investigating the relationship between specific protozoan species and symptom severity can help clinicians make more informed decisions about patient management (Efstratiou et al., 2017). The use of both direct wet mount and modified ZN staining methods in this study allows for a comprehensive approach to parasite detection. While the direct wet mount is effective for identifying a wide range of protozoan species, the modified ZN stain provides enhanced detection of certain acid-fast organisms that might be missed by wet mount alone. Combining these techniques can improve diagnostic accuracy and provide a more complete picture of the parasitic burden in symptomatic individuals (Squire & Ryan, 2017).

The global health community has recognized the importance of addressing intestinal protozoan infections as part of broader efforts to improve water, sanitation, and hygiene (WASH) practices. The World Health Organization (WHO) has included several protozoan parasites in its list of neglected tropical diseases, emphasizing the need for increased research, surveillance, and control efforts (WHO, 2020). The study of intestinal protozoan parasites in symptomatic cases using direct wet mount and modified ZN staining methods is a crucial area of research with significant implications for public health. By improving our understanding of these infections, their diagnosis, and their clinical impact, we can work towards more effective control strategies and ultimately reduce the global burden of intestinal protozoan diseases.

The aim of this study was to investigate the prevalence and types of intestinal protozoan parasites in symptomatic cases using direct wet mount and modified ZN staining methods, and to compare the efficacy of these two diagnostic techniques.

METHODOLOGY

Study Design: This study employed a cross-sectional design to investigate the prevalence of intestinal protozoan parasites in symptomatic cases. The cross-sectional approach allowed for the assessment of the current status of protozoan infections in the study population at a specific point in time.

Study Site: The study was conducted at the Department of Microbiology, Government Medical College, Bettiah, West Champaran, Bihar, India.

Study Duration: The study was carried out over 12 months, from April 2021 to March 2022. This duration was selected to account for potential seasonal variations in parasitic infections and to ensure an adequate sample size.

Sampling and Sample Size: Consecutive sampling technique was used to enroll participants who met the inclusion criteria. The sample size was calculated using the formula for estimating a population proportion with specified absolute precision. Assuming a prevalence of 50% (to maximize the sample size), a confidence level of 95%, and an absolute precision of 5%, the minimum required sample size was determined to be 384. To account for potential dropouts or incomplete data, the sample size was increased by 10%, resulting in a final target of 422 participants.

Inclusion and Exclusion Criteria: The study included patients aged 5 years and above who presented with gastrointestinal symptoms such as diarrhea, abdominal pain, nausea, or vomiting for at least three days. Patients who had received antiparasitic treatment within the past month, those with bloody diarrhea suggestive of bacterial infection, and individuals unable to provide informed consent were excluded from the study.

A structured questionnaire was used to collect demographic information and clinical data from the

participants. The questionnaire included items on age, gender, socioeconomic status, source of drinking water, sanitation facilities, and specific gastrointestinal symptoms. Trained research assistants administered the questionnaire through face-to-face interviews. For parasitological examination, stool samples were collected from each participant in clean, wide-mouthed, leak-proof containers. Participants were instructed on proper sample collection techniques to ensure the quality of specimens. Each stool sample was divided into two portions: one for direct wet mount examination and another for modified ZN staining.

Direct Wet Mount Examination: A small amount of stool sample was mixed with a drop of normal saline on a clean glass slide. A coverslip was placed on the mixture, and the preparation was examined under a light microscope using 10x and 40x objectives. The entire coverslip area was systematically scanned for the presence of protozoan trophozoites and cysts. Iodine staining was also performed to enhance the visibility of cyst morphology.

Modified ZN Staining: Thin smears were prepared from the stool samples and allowed to air dry. The smears were then fixed with methanol for 3 minutes. Carbol fuchsin stain was poured to the smear and heated until the fumes appear. After cooling, the smear was washed with tap water and decolorized with 1% acid-alcohol for 1 minute. Methylene blue was used as a counterstain for 1 minute. The stained smears were examined under oil immersion (100x objective) for the presence of acid-fast oocysts.

Statistical Analysis: Data from the questionnaires and laboratory results were entered into a Microsoft Excel spreadsheet and subsequently transferred to SPSS version 25 for analysis. Descriptive statistics were used to summarize the demographic characteristics of the study population and the prevalence of intestinal protozoan parasites. Frequencies and percentages were calculated for categorical variables, while means and standard deviations were computed for continuous variables.

The prevalence of intestinal protozoan parasites was calculated as the proportion of positive cases among the total number of samples examined. Separate prevalence rates were determined for each protozoan species identified. The performance of direct wet mount and modified ZN staining methods was compared using McNemar's test for paired nominal data. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for each method, considering the combined results of both techniques as the gold standard.

Chi-square tests or Fisher's exact tests (when expected cell frequencies were less than 5) were used to assess the association between parasitic infections and various demographic and clinical factors. Logistic regression analysis was performed to identify potential risk factors for protozoan infections. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to quantify the strength of associations. A p-value of less than 0.05 was considered statistically significant for all analyses.

Ethical Considerations

The study protocol was reviewed and approved by the Institutional Ethics Committee of the GMC, Bettiah where the research was conducted.

RESULTS

Table 1: Demographic characteristics of study participants (N=422)

Characteristic	Number (%)
Age group (years)	
May-14	84 (19.9%)
15-24	97 (23.0%)
25-44	156 (37.0%)
45-64	65 (15.4%)
≥65	20 (4.7%)
Gender	
Male	198 (46.9%)
Female	224 (53.1%)
Socioeconomic status	
Low	183 (43.4%)
Middle	201 (47.6%)
High	38 (9.0%)

This table presents the demographic characteristics of the 422 study participants. The largest age group was 25-44 years (37.0%), followed by 15-24 years (23.0%). There was a slightly higher proportion of females (53.1%) compared to males (46.9%). The majority of participants were from middle (47.6%) or low (43.4%) socioeconomic status, with only 9.0% from high socioeconomic backgrounds. This demographic distribution provides important context for interpreting the study results, particularly in relation to age-related and socioeconomic factors that may influence the prevalence of intestinal protozoan parasites.

Table 2: Prevalence of intestinal protozoan parasites detected by combined methods (N=422)

Parasite species	Number of positive cases (%)
Entamoeba histolytica/dispar	68 (16.1%)
Giardia lamblia	53 (12.6%)
Cryptosporidium parvum	22 (5.2%)
Blastocystis hominis	41 (9.7%)
Entamoeba coli	29 (6.9%)
Other protozoa	15 (3.6%)
Mixed infections	37 (8.8%)
Total positive cases	265 (62.8%)

Table 2 shows the prevalence of intestinal protozoan parasites detected by combined methods. The overall prevalence was high at 62.8%. *Entamoeba histolytica/dispar* was the most common parasite (16.1%), followed by *Giardia lamblia* (12.6%) and *Blastocystis hominis* (9.7%). *Cryptosporidium parvum* was found in 5.2% of cases. Mixed infections were observed in 8.8% of participants. This distribution of parasites provides valuable epidemiological data and highlights the significant burden of these infections in the study population. The high prevalence underscores the need for effective diagnostic and treatment strategies.

Table 3: Comparison of diagnostic methods for detecting intestinal protozoan parasites (N=422)

Method	Positive cases (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Direct wet mount	218 (51.7%)	82.30%	100%	100%	75.50%
Modified ZN staining	198 (46.9%)	74.70%	100%	100%	69.60%
Combined methods	265 (62.8%)	100%	100%	100%	100%

Table 3 compares the performance of direct wet mount and modified ZN staining methods for detecting intestinal protozoan parasites. Direct wet mount showed higher sensitivity (82.30%) compared to modified ZN staining (74.70%), while both methods demonstrated 100% specificity. The combined use of both methods increased the detection rate to 62.8% of all cases. This comparison highlights the strengths of each method and demonstrates the value of using multiple diagnostic techniques to improve overall detection rates. The results suggest that a combination of methods may be optimal for comprehensive parasite detection in clinical settings.

Table 4: Association between demographic factors and protozoan infection (N=422)

Factor	Infected (%)	Not infected (%)	OR (95% CI)	p-value
Age group				
<15 years	62 (73.8%)	22 (26.2%)	1.86 (1.09-3.17)	0.022
≥15 years	203 (60.1%)	135 (39.9%)		
Gender				
Male	128 (64.6%)	70 (35.4%)	1.15 (0.78-1.69)	0.483
Female	137 (61.2%)	87 (38.8%)		
Socioeconomic status				
Low	132 (72.1%)	51 (27.9%)	2.31 (1.53-3.49)	<0.001
Middle/High	133 (55.6%)	106 (44.4%)		

Table 4 presents the association between demographic factors and protozoan infection. Children under 15 years had significantly higher odds of infection compared to older individuals (OR: 1.86, p=0.022). Low socioeconomic status was strongly associated with increased infection risk (OR: 2.31, p<0.001). Gender did not show a significant association with infection rates. These findings highlight important risk factors for protozoan infections, particularly age and socioeconomic status. The results suggest that targeted interventions focusing on children and low-income populations may be most effective in reducing the burden of these infections.

Table 5: Clinical symptoms associated with protozoan infection (N=422)

Symptom	Infected (%)	Not infected (%)	OR (95% CI)	p-value
Diarrhea	198 (74.7%)	67 (25.3%)	3.65 (2.43-5.48)	<0.001
Abdominal pain	187 (70.6%)	78 (29.4%)	2.39 (1.62-3.53)	<0.001
Nausea/vomiting	142 (66.4%)	72 (33.6%)	1.32 (0.90-1.94)	0.156
Weight loss	89 (76.1%)	28 (23.9%)	2.22 (1.38-3.57)	0.001

Table 5 analyzes the clinical symptoms associated with protozoan infection. Diarrhea showed the strongest association (OR: 3.65, p<0.001), followed by abdominal pain (OR: 2.39, p<0.001) and weight

loss (OR: 2.22, p=0.001). Nausea/vomiting had a weaker, non-significant association (OR: 1.32, p=0.156). These findings provide valuable information for clinical diagnosis, highlighting the key symptoms that should raise suspicion of protozoan infection. The strong association with diarrhea emphasizes the importance of considering these parasites in the differential diagnosis of persistent diarrheal illnesses.

DISCUSSION

The present study revealed a high prevalence (62.8%) of intestinal protozoan parasites among symptomatic cases in the study population. This finding is consistent with several studies conducted in developing countries, where intestinal protozoan infections remain a significant public health concern. For instance, a study by Osman et al. (2016) in Lebanon reported a prevalence of 85% among schoolchildren, while Gebretsadik et al. (2020) found a prevalence of 62.3% in Ethiopia.

Entamoeba histolytica/dispar was the most prevalent parasite (16.1%), followed by *Giardia lamblia* (12.6%) and *Blastocystis hominis (9.7%).* These results align with the findings of Tulu et al. (2014), who reported *E. histolytica/dispar* as the predominant species in Ethiopia. However, some studies have found *G. lamblia* to be more prevalent, such as Squire and Ryan (2017) in their review of African studies. The variation in species distribution could be attributed to differences in geographical locations, environmental factors, and socioeconomic conditions.

The prevalence of *Cryptosporidium parvum* (5.2%) in our study is lower than that reported by Checkley et al. (2015) in their global review, where prevalence ranged from 15-25% in symptomatic cases in developing countries. This discrepancy might be due to differences in study populations, seasonal variations, or the sensitivity of diagnostic methods used.

Our study compared the performance of direct wet mount and modified ZN staining methods for the detection of intestinal protozoan parasites. The direct wet mount method showed higher sensitivity (82.3%) compared to modified ZN staining (74.7%). This finding is in line with the study by Shimelis and Tadesse (2014), who reported that direct microscopy outperformed other methods for the detection of most intestinal parasites. However, the modified ZN staining method was particularly effective in detecting Cryptosporidium oocysts, which might be missed by direct wet mount alone. This observation supports the recommendation by Garcia (2016) to use a combination of diagnostic techniques for comprehensive parasite detection.

The combined use of both methods in our study yielded a higher overall detection rate (62.8%) compared to either method alone. This emphasizes the importance of using multiple diagnostic approaches to improve the accuracy of intestinal protozoan parasite detection, as suggested by Ryan et al. (2017) in their review of molecular epidemiology and typing of intestinal protozoa.

Our study found a significant association between age and protozoan infection, with children under 15 years having higher odds of infection (OR: 1.86, 95% CI: 1.09-3.17) compared to older individuals. This finding is consistent with numerous studies, including that of Osman et al. (2016), which reported higher prevalence rates among children. The increased susceptibility of children to protozoan infections may be attributed to their developing immune systems and higher exposure risk due to poor hygiene practices. Socioeconomic status was also significantly associated with protozoan infections, with individuals from low socioeconomic backgrounds having higher odds of infection (OR: 2.31, 95% CI: 1.53-3.49). This result aligns with the findings of Speich et al. (2013), who reported a strong correlation between poverty and parasitic infections. Low socioeconomic status often correlates with poor sanitation, limited access to clean water, and inadequate health education, all of which contribute to increased risk of protozoan infections.

Interestingly, our study did not find a significant association between gender and protozoan infection (OR: 1.15, 95% CI: 0.78-1.69). This contrasts with some studies, such as that by Gebretsadik et al. (2020), which reported higher infection rates among females. The lack of gender disparity in our study might suggest that exposure risks are similar for both males and females in the study population.

The analysis of clinical symptoms revealed strong associations between certain gastrointestinal manifestations and protozoan infections. Diarrhea was the most strongly associated symptom (OR: 3.65, 95% CI: 2.43-5.48), followed by abdominal pain (OR: 2.39, 95% CI: 1.62-3.53) and weight loss (OR: 2.22, 95% CI: 1.38-3.57). These findings are consistent with the clinical presentation of intestinal protozoan infections described by Stark et al. (2018) in their review. The strong association between diarrhea and protozoan infection underscores the importance of considering these parasites in the differential diagnosis of persistent diarrheal illnesses. Bartelt and Sartor (2015) highlighted the role of *Giardia lamblia* in chronic diarrhea and malabsorption, which aligns with our observations. Interestingly, nausea and vomiting showed a weaker and non-significant association with protozoan infection in our study (OR: 1.32, 95% CI: 0.90-1.94). This suggests that while these symptoms may occur in protozoan infections, they are less specific and may be influenced by other factors or concurrent infections. The significant association between weight loss and protozoan infection (OR: 2.22, 95% CI: 1.38-3.57) highlights the potential nutritional consequences of these infections, particularly in chronic cases. This finding supports the observations of Burgess and Petri (2016), who discussed the impact of intestinal protozoa on nutrient absorption and overall nutritional status.

The high prevalence of intestinal protozoan parasites observed in our study, coupled with the significant associations with socioeconomic factors and clinical symptoms, underscores the continued public health importance of these infections. The findings align with the World Health Organization's classification of several protozoan parasites as neglected tropical diseases (WHO, 2020), emphasizing the need for increased attention and resources in control efforts. The higher prevalence among children and individuals from low socioeconomic backgrounds highlights the need for targeted interventions. Efstratiou et al. (2017) emphasized the importance of improving water, sanitation, and hygiene (WASH) practices to reduce the transmission of waterborne protozoan parasites. Our findings support the implementation of such measures, particularly in vulnerable populations.

The comparison of diagnostic methods in our study demonstrates the value of combining different techniques for accurate parasite detection. This has implications for clinical practice and epidemiological surveillance. As suggested by Squire and Ryan (2017), there is a need for continued research into more sensitive and specific diagnostic tools, particularly those that can be applied in resource-limited settings. While our study provides valuable insights into the prevalence and clinical significance of intestinal protozoan parasites, it has some limitations. The cross-sectional design limits our ability to establish causal relationships between risk factors and infections. Additionally, the study was conducted in a single centre, which may limit the generalizability of the findings to other populations.

Future research should consider longitudinal studies to better understand the dynamics of protozoan infections and their long-term health impacts. The integration of molecular diagnostic techniques, as discussed by Ryan et al. (2017), could provide more accurate species identification and insight into genetic diversity of parasites. Furthermore, investigating the role of the gut microbiome in modulating susceptibility to protozoan infections, as suggested by Burgess and Petri (2016), represents an exciting avenue for future research. Such studies could lead to novel approaches for prevention and treatment of intestinal protozoan infections.

CONCLUSION

Our study highlights the significant burden of intestinal protozoan parasites in symptomatic cases and underscores the importance of comprehensive diagnostic approaches. The findings contribute to the growing body of evidence on the epidemiology and clinical significance of these parasites, providing valuable information for public health interventions and clinical management strategies.

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