ORIGINAL ARTICLE

Efficacy of Stool Concentration Techniques versus Direct Wet Mount Microscopy in Detecting Intestinal Parasites: A Cross-Sectional Study at a Tertiary Care Hospital in Western Nepal

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ABSTRACT

Introduction: Intestinal parasitic infections remain a significant public health problem in Nepal. Accurate diagnosis guides effective treatment and control. Direct wet mount microscopy, though commonly used, has limited sensitivity. Concentration techniques such as zinc sulfate flotation and formal-ether sedimentation may improve diagnostic results, but data comparing their efficacy in western Nepal are scarce. **Aims:** To compare the efficacy of concentration techniques with the routine method for diagnosing intestinal parasitosis. **Methods:** A cross-sectional study was conducted among 383 patients attending the inpatient and outpatient departments of Nepalgunj Medical College Teaching Hospital, Kohalpur, Nepal. Stool samples were examined by three methods: direct wet mount (saline and iodine), zinc sulfate flotation, and formal-ether sedimentation. Detection rates of the three methods were compared. Demographic and clinical data were collected using a semi-structured questionnaire. **Results:** The overall detection rates were 11.75% (45/383) for direct wet mount, 14.88% (57/383) for zinc sulfate flotation, and 17.75% (68/383) for formal-ether sedimentation. Formal-ether sedimentation detected 23 more positives than wet mount (51.1% increase) and 12 more than zinc sulfate flotation (a 21.05% increase). *Entamoeba histolytica* (32 cases) and *Ascaris lumbricoides* (27 cases) were the predominant parasites. Mixed infections (7 cases) were detected exclusively by formal-ether sedimentation. Ascaris lumbricoides was predominantly seen in individuals under 20 years (93.75%), while *E. histolytica* occurred across all age groups, with the highest in those over 60 years. **Conclusion:** Formal-ether sedimentation was the most sensitive method for detecting intestinal parasites, followed by zinc sulfate flotation. Both concentration techniques significantly outperformed direct wet mount microscopy. Routine use of concentration techniques, particularly formal-ether sedimentation, is recommended for accurate diagnosis of intestinal parasitosis in clinical laboratories.

Keywords: Diagnostic efficacy; Formal-ether sedimentation; Intestinal parasites; Wet mount; Zinc sulfate flotation

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INTRODUCTION

Intestinal parasitic infection is a common problem and has serious medical and public health impacts in developing countries like Nepal.[1] An estimated 3.5 billion people have intestinal parasite infections, of whom 450 million are symptomatic, and

over 200,000 die each year, according to estimates from the World Health Organization (WHO).[2] Intestinal parasitosis (IPIs) pose a significant threat to global health by increasing clinical morbidity and death.[3] The most prevalent intestinal parasites causing gastrointestinal diseases are helminths such as T*aenia saginata, Hymenolepis nana, Ascaris lumbricoides, Strongyloides stercoralis, Trichuris trichiura, Enterobius vermicularis*, and hookworms, predominantly Necator americanus and Ancylostoma duodenale, and among the protozoans, Giardia lamblia and Entamoeba histolytica.[4] Various environmental, geographical, and socioeconomic factors influence variations in the prevalence and distribution of IPIs. Globally, parasitic infections pose a serious threat to public health, especially in underdeveloped nations.[5] Protozoan parasites, especially *Giardia lamblia, Entamoeba histolytica,* and *Cryptosporidium*, and helminthic parasites are among the most common causes of intestinal morbidity in infants and are prevalent in developing countries like Nepal, along with helminthic parasites.[6] To date, no study from western Nepal has systematically compared the efficacy of routine wet mount microscopy with stool concentration techniques for diagnosing intestinal parasitosis, representing a critical knowledge gap that this study aims to address. Hence, this study was conducted to compare the efficacy of different concentration techniques with the conventional technique used in diagnosing intestinal parasitic infections in patients attending Nepalgunj Medical College Teaching Hospital, Kohalpur, Nepal.

METHODS

A hospital-based cross-sectional study was conducted at Nepalgunj Medical College and Teaching Hospital (NGMCTH), Kohalpur, Nepal. The study was carried out from June 2025 to April 2026. The institutional review committee of NGMC approved the study protocol [66/081-82, dated 9 June 2025]. The informed consent (written and verbal) was collected from each participant.

A convenient sampling method was used; the sample size was calculated using the formula:

SAMPLE SIZE

$n = z^2 \times p\ (1-p)/e^2$

Where,

n = required sample size

z = confidence level at 95% (Standard value of 1.96)

p = estimated prevalence of was 17.8 %, analyzing the past studies[7]

e = margin of error at 5% (Standard value 0.05).

Thus, the required sample size was calculated as

Sample size (n) = $z^2 \times p\ (1-p)/e^2$ = (1.96)2 x 0.178(1-0.178)/(0.05)2 =225

Adding 10% in the case of unlabeled, mishandled, and inadequate-quality specimens, 10 % of 225; therefore, the estimated sample size in our study was 248. However, a total of 383 patients were enrolled during the study period.

The study included 383 patients attending the inpatient and outpatient departments of Nepalgunj Medical College and Teaching Hospital (NGMCTH), Kohalpur, Nepal. Participants were enrolled based on the presence of acute or chronic gastrointestinal symptoms and clinical suspicion of intestinal parasitosis. Sociodemographic data (age, sex, ethnicity), source of drinking water, hand hygiene practices, knowledge of parasitosis and sanitation, pet ownership (cats/dogs), use of protective footwear, and bowel habits were collected using a semi-structured questionnaire. Patients who had been taking anti-helminthic drugs within the past six months were excluded from the study.

Patient was instructed to collect approximately 5 g of stool in a clean, dry, disinfectant-free, wide-mouthed plastic container without contamination by urine. The collected stool samples were transported to the Department of Microbiology, Nepalgunj Medical College Teaching Hospital, Kolhapur, for analysis.

Laboratory Processing and Parasitological Examination

Macroscopic and Microscopic Examination

Stool specimens were subjected to immediate macroscopic examination to assess consistency, color, and the presence of blood, mucus, or adult worms. Subsequently, microscopic examination was performed using the direct wet mount technique (saline and iodine preparations) to identify protozoan cysts, trophozoites, and helminthic ova.

Sample Preservation and Concentration

Following initial screening, all fecal samples were preserved in 10% formalin to maintain the morphological integrity of the parasitic stages. These preserved specimens were subsequently utilized for concentration techniques, specifically zinc sulfate floatation and formal ether sedimentation, to enhance the diagnostic yield and detect low-density infections.

Stool examination techniques:

1. Macroscopic examination: The color, consistency, and nature of the faces were recorded. The stool specimens were examined for the presence of worms such as *Ascaris, Enterobius*, proglottids of *Taenia*, adult Hookworm, and *Trichuris*, either with the naked eye or with a hand lens.

2. Direct microscopic examination by using saline and iodine preparations: The stool sample was emulsified with a few drops of saline or iodine solution on a microscopic slide. A cover slip was placed on the suspension from above, ensuring that the preparation was free of air bubbles and microscopic debris.

Stool concentration

3. Zinc sulfate centrifugal floatation: Approximately 1 g of feces was emulsified in 10 parts of normal saline and strained

through wire gauze. The filtrate was collected in a Wassermann tube and centrifuged at 2,500 rpm. The supernatant was discarded, and the sediment was re-suspended in saline; this process was repeated until the supernatant became clear. The sediment was then mixed with 3–4 mL of 33% zinc sulfate solution, and the tube was filled with the same solution to within 0.5 inch of the rim. Several loopfuls of the supernatant were collected using a bacteriological loop for examination of the parasite.[8]

Statistical Analysis

4. Formal-ether concentration: Approximately 1 g of the stool sample was emulsified with 7 mL of 10% formal saline and allowed to fix for 10 minutes. The suspension was strained through wire gauze, and the filtrate was mixed with 3 mL of ether, then centrifuged at 2,000 rpm for 2 minutes. The supernatant was carefully discarded, and the sediment was examined for parasites using a wet mount preparation.[9]

Statistical Method

Software SPSS Windows version 25.0 was used for the statistical analysis. Descriptive statistics (frequencies, percentages) summarized participant characteristics and parasite detection rates. Diagnostic performance of stool concentration techniques versus direct wet mount was assessed by calculating sensitivity and specificity, with 95% confidence intervals (CIs) for each. Paired comparisons of detection proportions were performed using McNemar's Chi-square test (significance at $p < 0.05$).

RESULTS

A total of 383 patients attending the inpatient and outpatient departments of Nepalgunj Medical College Teaching Hospital (NGMCTH), Kohalpur, Banke, Nepal, were included in this cross-sectional study. Participants were enrolled based on the presence of acute or chronic gastrointestinal symptoms and clinical suspicion of intestinal parasitosis. The most represented age group was 11–20 years (27.15%), followed by <10 years (20.37%) and >60 years (17.75%). The smallest proportion of participants was in the 31–40-year age group (7.05%). Females constituted a larger proportion of the study population (56.7%, n=217) compared to males (43.3%, n=166). The chi-square test was used to assess the association between age and sex. The results were not statistically significant $p > 0.05$), suggesting that the proportion of males and females was similar across all age groups. The lack of significance may be influenced by the relatively small sample size in certain age groups (e.g., 31–40 years, n=27) or by unmeasured confounding variables such as occupation, healthcare-seeking behaviour, or sex-based differences in hospital admission rates across age groups. (Table I)

Age Group (years)	Male (n)	Female (n)	Total (n)	Percentage (%)
<10	29	49	78	20.37
11–20	50	54	104	27.15
21–30	18	16	34	8.88
31–40	13	14	27	7.05
41–50	18	22	40	10.44
51–60	11	21	32	8.36
>60	27	41	68	17.75
Total	166	217	383	100.00
($\chi2 = 6.85$, df = 6, p=0.335)				

Table I: Age and sex distribution of study participants (N=383)

Parasite Species (Wet Mount)	<10	11–20	21–30	31–40	41–50	51–60	>60	Total (n)	% of Positive Cases
Entamoeba histolytica (cyst)	2	3	4	2	0	4	8	23	51.1
Ascaris lumbricoides	8	7	1	0	0	0	0	16	35.6
Hymenolepis nana (ova)	2	1	0	0	0	0	0	3	6.7
Taenia species (ova)	0	0	0	0	1	0	0	1	2.2
Hookworm (ova)	0	0	0	1	0	0	0	1	2.2
Giardia lamblia	1	0	0	0	0	0	0	1	2.2
Total	13	11	5	3	1	4	8	45	100

Table II: Age-specific distribution of intestinal parasites detected by wet mount microscopy (N=45)

Entamoeba histolytica was the most commonly detected parasite, accounting for more than half of all infections (51.1%, n=23). It appeared across a wide age range, with the highest number of cases (8) observed among individuals over 60 years old. *Ascaris lumbricoides* was the second most prevalent parasite (35.6%, n=16); notably, 15 of its 16 cases (93.8%) occurred in individuals under 20 years of age, suggesting a strong predilection for younger populations. Other parasites: *Hymenolepis nana* (6.7%, n=3), *Taenia* species (2.2%, n=1), hookworm (2.2%, n=1), and *Giardia lamblia* (2.2%, n=1) were rarely encountered. Age-wise, the highest number of positive cases was observed in children under 10 years (13 cases), followed by the 11–20 years age group (11 cases). Very few infections were detected in middle-aged adults aged 31 to 60 years (Table II).

Table III shows that among 57 parasite-positive cases detected by zinc sulfate flotation, *Entamoeba histolytica* (45.6%, n=26) and *Ascaris lumbricoides* (40.4%, n=23) were the dominant species. *A. lumbricoides* was almost exclusively confined to individuals under 20 years (95.7%), whereas *E. histolytica* occurred across all ages, peaking in those over 60 years. Other parasites (hookworm, *Trichuris trichiura, Hymenolepis nana, Taenia*, and *Giardia*) constituted only 14.03% of cases combined. The highest positivity was observed in the 11–20 years age group (n=19), followed by under 10 years (n=13) and over 60 years (n=9), with minimal infections in middle-aged adults.

Parasite Species (Zinc Sulfate)	<10	11–20	21–30	31–40	41–50	51–60	>60	Total (n)	% of Positive Cases
Entamoeba histolytica (cyst)	2	5	4	2	0	5	8	26	45.6
Ascaris lumbricoides	9	13	1	0	0	0	0	23	40.4
Hookworm (ova)	0	1	0	1	1	0	0	3	5.3
Trichuris trichuria (ova)	1	0	0	0	0	0	1	2	3.5
Hymenolepis nana (ova)	0	0	0	1	0	0	0	1	1.7
Taenia species (ova)	0	0	0	0	1	0	0	1	1.7
Giardia lamblia (cyst)	1	0	0	0	0	0	0	1	1.7
Total	13	19	5	4	2	5	9	57	100.0

Table III: Age-wise distribution of intestinal parasites detected by zinc sulfate flotation technique (N=57)

Parasite Species (Sedimentation)	<10	11–20	21–30	31–40	41–50	51–60	>60	Total (n)	% of Positive Cases
Entamoeba histolytica (cyst)	2	6	3	2	0	5	8	26	38.2
Ascaris lumbricoides (ova)	8	12	1	0	0	0	0	21	30.9
Hymenolepis nana (ova)	3	1	0	1	0	1	0	6	8.8
Hookworm (ova)	0	2	0	1	1	0	0	4	5.9
A. lumbricoides + H. nana	1	3	0	0	0	0	0	4	5.9

Giardia lamblia (cyst)	1	0	0	0	0	1	0	2	2.9
Trichuris trichiura (ova)	1	0	0	0	0	0	0	1	1.5
Taenia species (ova)	0	0	0	0	1	0	0	1	1.5
A. lumbricoides + E. histolytica	0	0	1	0	0	0	0	1	1.5
A. lumbricoides + T. trichiura	0	1	0	0	0	0	0	1	1.5
E. histolytica + H. nana	0	0	0	0	0	1	0	1	1.5
Total	16	25	5	4	2	8	8	68	100

Table IV: Age-stratified distribution of intestinal parasites detected by formal-ether sedimentation technique (N=68)

To determine the total number of infected individuals, a combination of all three methods was considered the reference standard. Out of 383 participants, 77 (20.10%) tested positive by at least one method. Out of 383 participants, 77 (20.10%) tested positive by at least one method. Table V compares the detection rates of the three diagnostic methods. The formal-ether sedimentation technique detected the highest number of positives (68 cases, 88.3% of total positives), followed by zinc sulphate flotation (57 cases, 74.0% of total positives). Direct wet mount microscopy detected only 45 cases (58.4% of total positives), missing nearly 42% of infections identified by concentration techniques (Total V)

Diagnostic Method	Positive Cases (n)	Detection Rate (%)	Percentage of Total Positives*
Direct Wet Mount	45	11.75	58.4
Zinc Sulfate Flotation	57	14.88	74.0
Formal-Ether Sedimentation	68	17.75	88.3
Combined (All Methods)	77	20.10	100.0

Table V: Comparison of intestinal parasite detection rates by three diagnostic methods (N=383)

Compared to direct wet mount, formal-ether sedimentation detected 30 additional positives (66.7% increase), while zinc sulfate flotation detected 17 additional positives (37.8% increase). When compared to zinc sulfate flotation, formal-ether sedimentation detected 16 additional positives (28.07% increase) (Table VI).

Comparison	Method A Positives	Method B Positives	Concordant Positives	Discordant Positives (Missed by Method A)	% Increase in Detection (Method B vs. A)
Wet Mount vs. Zinc Sulfate	45	57	40	17	37.8%
Wet Mount vs. Formal-Ether	45	68	38	30	66.7%
Zinc Sulfate vs. Formal-Ether	57	68	52	16	28.07%

Table VI: Pairwise comparison of diagnostic methods for intestinal parasite detection

Using the combined results of all three methods as the reference standard (77 total positives), the relative sensitivity of each method was calculated. Table VII shows that formal-ether sedimentation had the highest relative sensitivity (88.3%), followed by zinc sulfate flotation (74.0%). Direct wet mount had the lowest sensitivity (58.4%), missing 32 of 77 infections.

Diagnostic Method	True Positives (n)	False Negatives (n)	Relative Sensitivity (%)
Direct Wet Mount	45	32	58.4
Zinc Sulfate Flotation	57	20	74.0
Formal-Ether Sedimentation	68	9	88.3
Combined (All Methods)	77	20.10	100.0

Table VII: Relative sensitivity of diagnostic methods using combined results as reference (N=77 total positives)

DISCUSSION

Parasitic infestations are the major causes of morbidity and mortality in developing countries like Nepal. Detecting medically important parasites poses a significant challenge for clinical microbiology laboratories in healthcare settings, requiring skilled differentiation among them. Although saline and iodine wet mounts are routinely employed for ova and cyst detection, their low sensitivity frequently results in false negatives, thereby compromising treatment outcomes, exacerbating clinical symptoms, and facilitating community transmission of infection. This diagnostic limitation can be effectively addressed using stool concentration techniques, which enable the detection of parasites at low abundance that would otherwise be missed by direct wet-mount examination.[10,11] Hence, in our study, we evaluated the effectiveness of two concentration techniques in combination with routine wet mounts for diagnosing intestinal parasites in specimens identified as positive or negative for the first time. To our knowledge, this is the first study of its kind in our region.

In this cross-sectional study involving 383 patients attending a tertiary care hospital in western Nepal, the formal-ether sedimentation technique has detected the highest number of intestinal parasite infections (68 cases, 17.75%), followed by zinc sulfate flotation (57 cases, 14.88%) and direct wet mount microscopy (45 cases, 11.75%). The two concentration techniques together identified 23–30 additional positive cases that would have been missed by routine wet mount examination alone. *Entamoeba histolytica* and *Ascaris lumbricoides* were the dominant parasites across all methods. Notably, mixed infections (n=7) were detected exclusively by the formal-ether sedimentation technique.

The overall prevalence observed in this study (17.75% by the most sensitive method) is consistent with findings from other parts of Nepal and neighboring countries. Studies have reported prevalence rates of 15.17% in Biratnagar[12], 10.96 % in Janakpur[13], 14.9% in China[14], and 13.3% in India.[15] However, our rate is considerably lower than reports from Sudan (35.5%)[16], Yemen (58.7%)[17] and Brazil (46%).[18] This relatively lower prevalence in our study population may be attributed to improved sanitation facilities, greater awareness of hygiene, government-led deworming programs, and overall improvements in health services in the region over recent years.

The predominance of *E. histolytica* and *A. lumbricoides* in our study aligns with global patterns, where these two parasites consistently rank among the most common intestinal pathogens in developing countries.[19–21] The age-wise distribution observed with *A. lumbricoides* predominantly affects children and young adults under 20 years, while *E. histolytica* occurred across all ages, with the highest prevalence among the elderly, reflecting known epidemiological patterns. Children are more susceptible to soil-transmitted helminths due to outdoor play, geophagia, and less developed hygiene practices, while *E. histolytica* infection risk persists throughout adulthood, particularly in settings with contaminated food and water sources. The superiority of concentration techniques over direct wet-mount microscopy is well established in the parasitology literature.[11] Direct wet mount, despite being rapid, inexpensive, and simple to perform, suffers from poor sensitivity because only a small amount of stool is examined, and parasites present in low numbers are easily missed. Our findings confirm this limitation: wet mount microscopy missed 32 cases (42.9% of all infections detected by concentration methods).

The formal-ether sedimentation technique proved to be the most sensitive method in our study, detecting 23 more positives than wet mount (51.1% increase). This technique has several advantages: it is suitable for both protozoan cysts and helminth eggs, effectively removes debris, and concentrates parasites in clean sediment.[22] The ability of formal-ether sedimentation to detect mixed infections is a unique finding in our study, which further supports its utility in routine diagnostics. Mixed infections are clinically important because they may alter disease severity and treatment outcomes.

Zinc sulfate flotation also revealed significant findings, detecting 17 more positives than wet mount. This technique is particularly effective for *Ascaris* eggs and protozoan cysts, as the high specific gravity of the zinc sulfate solution allows parasite elements to float while fecal debris sinks. However, it was less effective than formal-ether sedimentation for detecting *Hymenolepis nana* and hookworm ova, and it failed to detect any mixed infections in our study. This may be due to the flotation process being less suitable for heavier parasite eggs or when parasites are present in low numbers.

Our findings have important implications for clinical microbiology laboratories in resource-limited settings like Nepal. While direct wet mount remains the most commonly used method due to its simplicity and low cost, its low sensitivity leads to false-negative results. Such false negatives can delay appropriate treatment, worsen patient conditions, and contribute to continued transmission of infections in the community[5]. Based on its highest relative sensitivity (88.3%), ability to detect mixed infections, and minimal additional cost and time compared to direct wet mount, we strongly recommend that clinical laboratories, particularly those in tertiary care hospitals, adopt formal-ether sedimentation as the primary routine diagnostic method for intestinal parasites. For laboratories with resource constraints, a stepwise approach initial direct wet mount followed by formal-ether sedimentation for samples with persistent clinical suspicion but negative wet mount could be a pragmatic alternative.

LIMITATIONS

This study is one of the few from western Nepal that directly compares three diagnostic methods for intestinal parasites using the same patient population. The relatively large sample size (N=383) and inclusion of all age groups add to the generalizability of the findings. This study has several limitations. Since individual-level matched data were not available, we could not calculate the exact number of unique positive cases across methods or perform statistical comparisons such as sensitivity, specificity, or pairwise significance testing. A single stool sample was collected from each participant; repeated sampling over consecutive days would likely have increased detection

rates for all methods, especially for parasites with intermittent shedding. Additionally, the absence of a statistically significant association between age and sex, while likely reflecting true population distribution, may also be attributable to limited statistical power in smaller age subgroups or unmeasured demographic confounders. Molecular confirmation (e.g., PCR) was not performed to verify species identification or resolve discrepancies between methods. And the study was conducted at a single tertiary care hospital, so findings may not be generalizable to primary health centers or community settings in rural Nepal.

CONCLUSION

This study demonstrates that stool concentration techniques, particularly formal-ether sedimentation, are substantially more effective than direct wet mount microscopy for detecting intestinal parasites. The formal-ether sedimentation technique detected nearly 52% more positive cases than wet mount and was the only method capable of identifying mixed infections. Given the high burden of intestinal parasitosis in Nepal and the consequences of missed diagnoses, clinical microbiology laboratories should consider adopting concentration techniques as part of their routine diagnostic protocols. Improved diagnosis will lead to better patient outcomes and more effective public health interventions.

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