

Macroscopic and microscopic study of stool samples for parasites at a tertiary care Center in North Eastern Bihar: A cross-sectional study.

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ABSTRACT

Background:

Intestinal parasitic infections (IPIs) remain a major public health concern in developing countries, particularly among immunocompromised individuals such as HIV-positive patients. Early detection using reliable laboratory methods is essential for appropriate management and prevention of complications.

Methods:

This cross-sectional study was conducted in the Microbiology laboratory of Katihar Medical College, Bihar. A total of 235 stool samples were collected from 180 HIV non-reactive and 55 HIV reactive individuals. Macroscopic examination was performed to assess consistency, color, and presence of mucus or blood. Microscopic examination was carried out using direct wet mount, sedimentation, and flotation techniques to detect ova, cysts, and parasites.

Results:

Intestinal parasites were detected in a significant proportion of cases, with a higher prevalence among HIV-reactive individuals compared to HIV non-reactive individuals. The sedimentation technique showed higher sensitivity in detecting parasitic forms compared to the flotation and direct wet mount methods. Common parasites identified included protozoa and helminths. Macroscopic findings such as loose stools and mucus were more frequently associated with parasitic infection.

Conclusion:

Microscopic examination using concentration techniques, especially sedimentation, improves the detection rate of intestinal parasites. HIV-infected individuals are at greater risk of parasitic infections and require routine screening.

Recommendation:

Routine stool examination using concentration techniques should be implemented, particularly in immunocompromised patients such as HIV-positive individuals. Increased awareness, early detection, and appropriate treatment strategies can help reduce the burden of intestinal parasitic infections in resource-limited settings.

Keywords: Sheather's sugar floatation technique, modified acid-fast stain, concentration methods.

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INTRODUCTION:

A large number of patients attending the outdoor patient department (OPD) in Katihar Medical College, Katihar, reports with problems related to gastrointestinal tract infections, diarrhoea, abdominal pain, vomiting, and loss of weight. So far, studies pertaining to the distribution of various intestinal parasitic infestations have been reported in various age groups of patients.¹

Since 1908, many examination methods have been developed to raise the standard of identification of ova/cysts of parasites in stool specimens. That process consisted of segregating the solids from the liquid faecal suspension. The aim was to remove excess debris and obtain satisfactory sediment that contains ova/cysts of parasites. Since the beginning of the 20th century, several research has been carried out to identify better

methods with increased sensitivity for the detection of parasites.^{2,3,4}

Four important technical principles, namely, centrifugal sedimentation, centrifugal flotation, spontaneous flotation, and spontaneous sedimentation emphasizes the recovery of ova/cyst parasites in stool. Various assimilated factors can increase the diagnostic sensitivity in samples with low parasitic load, and include techniques such as force (centrifugal action), time applied in the sedimentation, flotation, specific density of the medium, chemical characteristics of the preservative for colouring portion of collected faecal samples for microscopic examination.^{2,3,4}

It is important to keep track of the prevalence of parasitic infections so as to wisely plan interventions. Besides, the community-based prevalence data being made available to hospitals acts as the main place to screen the true picture about mortality and morbidity due to intestinal infections and to add information about the abundance of some specific parasites.⁵

The main aim of the study was to estimate the prevalence of protozoan cysts or oocysts, helminth eggs, and larvae by saline mount as well as iodine mount preparation in the stool samples of both HIV reactive & HIV non-reactive individuals. The secondary objective was to estimate the reliability and accuracy of sedimentation and flotation techniques for isolation of protozoan and helminthic parasites in stool samples of both HIV reactive & HIV non-reactive individuals.

MATERIAL & METHODS

Study Type

The present study was a cross-sectional hospital-based study, conducted to identify various gastrointestinal protozoan and helminthic parasites isolated from the stool specimen in HIV reactive & HIV non-reactive individuals from May 2024 to February 2025, in the Clinical Laboratory, Microbiology Section of Katihar Medical College. A total of 235 stool samples were collected, including 180 HIV non-reactive and 55 HIV reactive individuals.

Study setting

This study was conducted in the Clinical Laboratory, Microbiology Section, Department of Microbiology, Katihar Medical College and Hospital, Bihar, India. The laboratory provides diagnostic services including bacteriology, parasitology, mycology, and serology. The institution serves a large population from Katihar district and surrounding rural and semi-urban areas, where poor sanitation and limited access to clean drinking water contribute to increased prevalence of parasitic infections.

Eligibility criteria

Patients not able to provide a sufficient quantity of stool

sample for conducting the appropriate diagnostic methods, patients who did not give consent to be part of the study, and patients on antiparasitic treatment were excluded from the study.

Ethical consideration

Institutional ethical clearance was sought vide IEC/IRB No: KMC/IEC/MSc./006/2024 (Medical Microbiology).

Collection and processing of stool specimen: ^{6,7}

- Proper history was taken from the patients regarding administration of drugs like antimalarial agents, tetracycline, bismuth, mineral oil, non-absorbable anti-diarrheals, as it is not possible to demonstrate any parasites within several weeks after administration of such medications.
- Patients were advised not to contaminate the stool sample with water during the process of collection because water might contain free-living parasites.
- Patients were advised not to contaminate stool with urine because it leads to destruction of motile organisms.

Stool samples were collected in wide-mouth, clean, leak-free, screw-capped containers before administration of anti-parasitic drugs and closer to the onset of symptoms. Liquid stool samples were examined within 30 mins of collection to facilitate the recovery of trophozoites that actually tend to get destroyed on prolonged storage. Semi-formed stool samples were examined within one hour of collection. Formed specimens were examined on the day of collection. In case of delay, fecal samples were refrigerated at 4°C and stored in closed containers to prevent desiccation.^{6,7}

Macroscopic examination of stool sample ^{6,7,8}

Naked eye observation was done to check the color of the stool sample, followed by consistency, and for the presence of segments of tapeworm or any nematodes.

Consistency of the stool sample:

Stool samples were checked for semi-formed, soft, liquid, because it provides information about the stage of protozoan parasites that are present.^{6,7,8}

Adult intestinal helminths in the stool:

Stool samples were checked for the presence of adult worms of *Ascaris lumbricoides* and *Enterobius vermicularis* that are present on the surface of the stool sample. Sometimes tapeworm segments are also seen in the stool sample.^{6,7,8}

Microscopy of direct smear:

Saline mount: A small quantity of the stool sample was mixed with a drop of physiological saline and examined under a microscope from one corner, starting from one corner, either horizontally or vertically, and moving in one direction systematically for the detection of motile trophozoites, helminthic eggs, and larvae.⁷

Iodine mount:

Likewise small amount of stool sample was mixed well with Lugol's iodine, and an even suspension was made, followed by application of a cover slip in such a way that air bubbles do not get trapped in the preparation. Iodine mainly stains glycogen mass and nuclei of cysts. Both preparations were initially observed under low power of the microscope, followed by switching to high power to observe the details.⁸

In case a patient was suspected to be suffering from a parasitic infection and yet the ova or cyst was not

detected in the stool specimen, and a low load of egg/cyst is suspected, then the sample was concentrated. Using formol ether sedimentation technique and the zinc sulphate floatation technique.

Modified Ziehl-Neelsen staining:

Faecal smears were made either directly from the stool sample or from the concentration deposit and allowed to dry. The smear was stained with heated carbol fuchsin for 5-10 minutes. Then the slide was rinsed thoroughly in tap water. Decolonization was done in acid alcohol (1% HCl in methanol) for 30-60 seconds, followed by thorough washing in tap water. Lastly, the smear was counterstained with methylene blue or 0.4% malachite green for 30-60 seconds, rinsed thoroughly, and air dried. The smear was examined using 40x and 100x objectives.

Interpretation

Table 1: Demonstration of oocyst by various techniques and their measurement

Microscopic Characteristics	<i>Cryptosporidium parvum</i>	<i>Isospora belli</i>	<i>Cyclospora cayetanensis</i>
Size	4 - 6	20-33 -10-19	8-10
Identified in formol-ether concentrate by light microscopy	No	Yes	Yes
Identified by modified Ziehl-Neelsen staining	Yes	Yes	Yes

Formol ether concentration technique^{7,8}

Formalin, used in this process, enables the fixation of the helminthic egg, larva, and cysts to make them non-infectious. The specific gravity of protozoan cysts and helminth eggs is greater than that of water. Faecal debris is extracted into the ether phase so that the parasitic forms can be separated and then sedimented by centrifugation.

Formol ether oocyst concentration technique⁸

The initial steps in the processing of a stool sample up to treatment with ether is same as in the case of the formol ether concentration technique. Next, a Pasteur pipette was used to remove the entire column of fluid, along with the faecal debris and ether, to another test tube. This was followed by the addition of formalin

water and making the volume up to 10-15 ml, and it will be centrifuged at 3000 rpm for 5-10 mins. The supernatant was removed, and the sediment was tapped, mixed well, placed on a slide, and observed under a microscope.

Zinc sulphate floatation technique⁸

The principle behind the flotation technique involves the differences in specific gravity of helminthic eggs and protozoan cysts and the suspending medium. The eggs and the cysts float to the top, and the debris sinks to the bottom. The eggs and cysts were collected by placing a glass slide on the surface of the meniscus at the top of the tube.

Sheather's sugar floatation technique:⁹

This technique was used to observe the oocyst of acid-

fast parasites. Sheather's sugar solution was prepared by boiling 355 ml of distilled water to which 454 g of sugar was added. Later, 2 ml of 37% formaldehyde was added as a preservative. A pea-sized, i.e., 1 gm of stool specimen, was mixed in 10 ml of sugar solution in a test tube and strained through a two-layer gauze piece into a centrifuge tube. This was followed by centrifugation at 1000 g for 5 mins. The tube was removed and placed in a test tube rack and filled to the top with sugar solution. A coverslip was placed on the tube and left for 10 mins, and then the coverslip was turned upside down and placed on a glass slide and then observed under a microscope.

RESULTS:

The present study was conducted to estimate the prevalence of protozoan cysts or oocysts, helminth eggs, and larvae by various techniques, including normal saline & iodine mount, sedimentation, flotation, and modified acid-fast staining. in the stool samples and to establish the reliability and accuracy of sedimentation

and flotation techniques for isolation of protozoan and helminthic parasites in stool samples.

A total number of two hundred thirty-five (235) stool samples from patients attending the outpatient and inpatient departments of Katihar Medical College with complaints of diarrhoea and or other gastrointestinal problems were received in the Microbiology laboratory. Another group of patients (confirmed to be HIV reactive) with GIT problems and attending the ART centre, Katihar Medical College, were included in the study.

Table 2 shows the age-wise and gender-wise distribution of ova/cyst and oocyst of various parasites in HIV & non-HIV individuals. 75.0% and 66.7% of the non-HIV females were infected with *Hymenolepis nana* and *Entamoeba histolytica*, respectively. On the contrary, double infection with *Hymenolepis nana* and *Ascaris lumbricoides* was reported in 75% males. 66.7% HIV infected males were infected with *Ancylostoma duodenale*, and 63.6% HIV infected females were infected with *Cryptosporidium parvum*. The prevalence of the parasitic infection was common in the following age group 21-30 years, being 21.0% followed by; 31-40 years, being 20%; and 11-20 years, being 17%.

Table 2: Age-wise and gender-wise distribution of ova/cyst and oocyst of various parasites in HIV & non-HIV individuals

Parasite	Non-HIV-infected individuals	NON-HIV-infected individuals		HIV individual	HIV individual		Age in years							
	No of positive cases	Male No. & (%) of positive cases	Female No. & (%) of positive cases	No of positive cases	Male No. & (%) of positive cases	Female No. & (%) of positive cases	1-10	11-20	21-30	31-40	41-50	51-60	61-70	TOTAL
<i>Entamoeba histolytica</i>	06	02 (33.3)	04 (66.7)	01	00	01	00	02	02	02	01	00	00	7
<i>Entamoeba coli</i>	02	02 (100)	00	0	00	00	00	02	00	00	00	00	00	02
<i>Giardia lamblia</i>	14	06 (42.9)	08 (57.1)	0	00	00	01	02	02	04	02	03	00	14
<i>Ancylostoma duodenale</i>	12	08 (66.7)	04 (33.3)	03	02 (66.7%)	01 (33.3%)	00	02	07	04	00	02	00	15
<i>Ascaris</i>	22	14	08	01	01	00	06	04	02	04	02	01	04	2

<i>lumbricoides</i>		(63.6)	(36.4)											3
<i>Trichuris trichiura</i>	10	06 (60)	04 (40)	0	00	00	00	02	01	02	03	02	00	10
<i>Hymenolepis nana</i>	8	02 (25)	06 (75)	01	01	00		00	02	03	00	02	00	9
<i>Cryptosporidium parvum</i>	0	00	00	11	04 (36.4)	07 (63.6)		00	01	02	04	00	02	11
<i>Hymenolepis nana</i> + <i>Ascaris lumbricoides</i>	4	03 (75)	01 (25)	01	00	01		00	00	02	00	00	02	5
<i>Hymenolepis nana</i> + <i>Trichuris trichiura</i>	2	02 (100)	00	0	00	00		00	00	00	02	00	00	2
<i>Ascaris lumbricoides</i> + <i>Hymenolepis nana</i> + <i>Trichuris trichiura</i>	2	02 (100)	00	0	00	00		00	00	00	00	02	00	2
TOTAL	82	45 (54.9)	37 (45.1)	18	08 (44.4)	10 (55.6)	7 (7.0)	17 (17.0)	21 (21.0)	20 (20.0)	12 (12.0)	15 (15.0)	8 (8.0)	100

Table 2 shows the distribution of positive cases in HIV and Non-HIV infected individuals by different diagnostic techniques. Direct saline & iodine mount could detect 39/100 (41%) of positive cases. Out of the 61 cases (which showed negative findings in saline mount), formol ether sedimentation and Zinc sulphate floatation method could detect 49.1% (30/61) and 32.7% (20/61) positive findings, respectively.

25.6% (10/39) and 15.3% (06/39) positive cases of *Ascaris lumbricoides* and *Trichuris trichiura* each was detected by direct saline mount. This was followed by 10.2% (04/39) cases of *Hymenolepis nana* and 5.1% (02/39) of *Entamoeba histolytica* and double & triple infection caused by the combination *Hymenolepis nana* and *Ascaris lumbricoides* & *Ascaris lumbricoides*, *Hymenolepis nana* and *Trichuris trichiura*.

Table 3: Distribution of positive cases with IPI in HIV and NON-HIV infected individuals by different diagnostic techniques

Parasites	No of parasites detected /cases	DIAGNOSTIC METHODS				
		Direct wet mount	Modified acid-fast staining	Formal ether method	Zinc sulphate floatation	Sheathes sugar floatation technique
<i>Entamoeba histolytica</i>	7	2	0	02	03	0
<i>Entamoeba coli</i>	02	0	0	02	0	0
<i>Giardia lamblia</i>	15	06	0	02	07	0
<i>Ancylostoma duodenale</i>	13	06	0	06	01	0
<i>Ascaris lumbricoides</i>	23	10	0	10	03	0
<i>Trichuris trichiura</i>	11	06	0	01	04	0
<i>Hymenolepis nana</i>	9	04	0	04	01	0
<i>Cryptosporidium</i>	11	00	09	0	0	02

<i>parvum</i>						
<i>Hymenolepis nana</i> + <i>Ascaris lumbricoides</i>	5	02	0	03	00	0
<i>Hymenolepis nana</i> + <i>Trichuris trichiura</i>	2	01	0	0	01	0
<i>Ascaris lumbricoides</i> + <i>Hymenolepis nana</i> + <i>Trichuris trichiura</i>	2	02	0	0	0	0
TOTAL	100	39	09	30	20	02

Table 3 shows the isolation pattern of the ova and cysts in stool samples of various consistencies and types. 22.8% (24/105). Positive findings were seen in formed stool samples [70.2% (33/47)], followed by loose stool [51.6% (32/62)] and semi-formed and watery stool [52.3% (11/21)]. The difference in the detection of ova, cysts, or oocysts in stool samples was found to be statistically significant at $p < 0.05$. It was possible to detect ova/cyst in 86.1% (31/36) of the stool samples with offensive odour. 48.0% (12/25) of the stool samples with positive findings were positive for occult

blood, whereas 52.0% (13/25) of the cases were positive for occult blood but not for ova/cysts. Another 3 cases that showed *Entamoeba histolytica* were positive for Charcot-Leyden crystals. On the other hand, 100% (16/16) of cases that showed microscopic evidence of plenty of pus cells did not show any findings. Out of the samples that showed plenty of RBC, 4/7 stool samples were positive for *Entamoeba histolytica*, 28.7% (2/7) for *Ancylostoma duodenale*, and 14.2% (1/7) for *Hymenolepis nana* infection.

Table 4: Macroscopic & microscopic findings of stool for parasite (n=235)

Colour of stool	Total cases	Ova/cyst Detected, n (%)	Ova/cyst not detected, n (%)	P. Value	z-score	Significance
Yellow	105	48 (45.71)	57 (54.29)	$P = .10749$	-1.2421	NS* (p < 0.05)
Brown	97	45 (46.39)	52 (53.61)	$P = .15625$	-1.0051	NS* (p < 0.05)
Black	22	06 (27.27)	16 (72.73)	$P = .00126$	-3.0151	S# (p < 0.5)
Clay – coloured	08	06 (75.0)	02 (25.0)	$P = .02275$	02	S# (p < 0.5)
Dirty green	03	0 (00.0)	03 (100.00)	$P = .00714$	-2.4495	S# (p < 0.5)
Total	235	105	130	---	---	---
Stool consistency				P. Value	z-score	Significance
Formed	105	24	81	$P < .0001$	-7.8667	S# (p < 0.5)
Semi formed	62	32	30	$P = 0.3592$	0.3592	S# (p < 0.5)
Loose	47	33	14	$P = .00004$	3.9194	S# (p < 0.5)
Watery	21	11	10	$P = .37828$	0.3086	NS* (p < 0.05)
Total	235	100	135	---	---	---
Miscellaneous /others	Total	Present	Absent	P. Value	z-score	Significance
Offensive odour of stool	36	31 (86.1)	05 (13.9)	$P < .0001$	6.1283	S# (p < .05)
Presence of Occult Blood in stool	25	12(48.0)	13 (52.0)	$P = .38974$	-0.2828	NS* (p < 0.05)
Presence of Charcot laden crystal in stool	03	03 (100)	00	$P = .00714$	2.4495	S# (p < .05)

Leukocytes / HPF plenty	16	00	16 (100)	P <.0001	-5.6569	S [#] (p<.05)
Leukocytes / HPF 0-1	53	07 (13.2)	43 (81.1)	P <.0001	-7.2	S [#] (p<.05)
Leukocytes / HPF 1-2	64	19 (29.6)	45 (70.3)	P <.0001	-4.5962	S [#] (p<.05)
Leukocytes / HPF 2-3	38	17 (44.7)	24 (63.1)	P =.06057	-1.546	NS* (p<0.05)
Leukocytes / HPF 3-5	39	32 (91.4)	07 (20.0)	P =.06057	-1.546	NS* (p<0.05)
Leukocytes / HPF 3-4	25	25 (100)	00	P <.0001	5.6614	S [#] (p<.05)
RBC / HPF 0-1	132	34	106	P <.0001	-8.6056	S [#] (p<.05)
RBC / HPF 1-2	58	40	18	P <.0001	4.0853	S [#] (p<.05)
RBC / HPF 2-3	18	09	09	P = 0.5	0	NS* (p<0.05)
RBC / HPF 3-4	14	10	04	P =.0116	2.2678	S [#] (p<.05)
RBC / HPF plenty	13	07	06	P =.34827	0.3922	NS* (p<0.05)

*NS: Non-significant; #S: Significant

DISCUSSION

Our hospital caters to patients living in rural areas with improper disposal of sewage and a scarcity of potable water supplies. Our study helped to understand the prevalence of intestinal parasitic & opportunistic infections in the stool samples of both HIV reactive & HIV non-reactive individuals. Additionally, various detection methods were used in combination to increase the diagnostic accuracy.

A total of 235 stool samples were received in the Department laboratory, out of which 180 samples were from HIV non-reactive patients, and 55 stool samples from HIV reactive individuals. Of the 180 stool samples collected from HIV non-reactive individuals, ova/cyst of intestinal parasites were detected in 82 (45.6%) cases, whereas 18/55 (32.7%) HIV reactive cases showed the presence of oocyst of *Cryptosporidium* species and other ova/cysts.

Studies conducted in western India showed that the prevalence of intestinal parasitic infections (IPIs) was 15.19%, which was lower than previous reports from other countries. Findings of studies conducted to clarify the parasitic infections in both healthy and symptomatic populations in rural and urban India reported that it varies between 11.50% to 97.4%. This broad spectrum of range seen in different studies could be due to time and period of the study, the age of the study population, changes in diet, habits, and occupations, different sampling techniques & research methodologies, geographical differences, and the inclusion of non-pathogenic intestinal parasites in the analysis.¹⁰

A study conducted by Langbang et al (2019) on the prevalence of intestinal parasitic infections showed that out of the 1006 stool samples that were collected from rural (n = 500) and urban (n = 506) populations, 50.2% of them were from the urban population, and 49.7% was from the rural population. Out of these 500 stool

samples from the rural population showed the presence of 40.4% of intestinal parasites.¹⁰

Distribution of positive cases (ova, cysts, oocysts of parasites) detected by various methods

Our study results showed that out of the 100 positive cases, normal saline & wet mount detected 69 positive cases. In addition, 14 more positive cases were detected using the formal ether sedimentation technique. Another 06 cases were detected by the zinc flotation method. Modified acid-fast staining that used to screen the stool of HIV reactive individuals, could detect 09 cases of acid-fast oocysts of *Cryptosporidium parvum*, and Sheather's sugar flotation technique could detect only 02 cases.

A retrospective analysis was carried out by Parimala S et al (2022) to assess the intestinal parasitic infections. 6.9% (62/892) of patients were positive for intestinal parasitic infections. CMC Vellore reported a slightly lower prevalence rate of intestinal parasitic infections, i.e., 8.9%.¹¹ The variations in the detection rate of ova/cysts in different geographical regions are due to the sample size, use of stool concentration techniques, differences in sampling methods, season, and time of the study, and geographical differences in the study areas. Microscopy using fresh stool samples was the sole method used by the workers for the detection of ova/cysts. Our study suggests that formal ether sedimentation techniques had an increased detection rate when compared to direct microscopic methods. Likewise. The use of sugar flotation & modified acid-fast techniques in our study also enhanced the detection of oocysts of opportunistic coccidian parasites.

Distribution of positive cases with IPI in HIV and Non-HIV infected individuals by different diagnostic techniques

Direct saline & iodine mount could detect 41% (41/100) of positive cases. Out of the 59 cases (which showed negative findings in saline mount), formol ether sedimentation and Zinc sulphate floatation methods could detect (28/59) and (20/59) positive findings, respectively.

24.4% (10/41) and 54.5% (06/11) positive cases of *Ascaris lumbricoides* and *Trichuris trichiura* were detected by direct saline mount. This was followed by 46.2% (06/13) & 40.0% (06/15) cases of *Ancylostoma duodenale* and *Giardia lamblia*.

35.7% (10/28) cases of *Ascaris lumbricoides*, followed by 21.4% (06/28) cases of *Ancylostoma duodenale*, and 14.3% (04/28) *Hymenolepis nana* were detected by the sedimentation method. On the other hand, 35.0% (07/20) cases of *Giardia lamblia*, 20.0% (04/20) cases of *Trichuris trichiura*, and (03/30) cases each of *Entamoeba histolytica* and *Ascaris lumbricoides* were detected using the floatation methods.

In other studies, direct wet mount could detect 12% positive cases without the application of any concentration techniques. However, the efficiency increased to 18% and 20% after using salt floatation and Zinc sulphate floatation techniques.¹² Similar findings were reported by Amin HA et al, 2015, where the sensitivity by the zinc sulphate floatation technique was 49%, followed by the concentration technique that detected 22.6% positive cases.¹³ The findings of Thakur SS et al, 2017, showed that 50.80% of cases were detected by the zinc sulphate centrifugal technique and 45.95% by the salt floatation method. 40.77% cases were detected by direct saline mount.¹⁴ Parameshwarappa KD et al in 2012 reported similar findings where 55.0% followed by 42.0% and 38.0% cases were detected by the Zinc sulphate centrifugal floatation technique, followed by saturated salt floatation method and direct saline mount. Authors found the zinc sulphate floatation method to be better than the saturated salt floatation technique and direct microscopy wet mount because many positive findings were missed in saline & iodine mounts.¹⁵

Similar reports were given by other authors where the formal ether sedimentation method followed by the salt floatation method increased the detection of eggs & cysts by 60.9% and 48.52% when compared to direct saline and iodine mount with a lower rate of detection, i.e., 38.23%.¹⁶ The results of the study were compared to other similar findings that showed the formal-ether sedimentation technique had a sensitivity of 56.88%, as compared to the simple salt floatation method with 42.0% sensitivity. The direct wet mount method showed a low sensitivity of 38.0%.¹⁷ However, in rural settings with minimum facilities, direct wet mount seems to be cost-effective and simpler to perform, subject to heavy

parasitic infections, and when the load of ova/cysts are more in the stool sample. Simultaneous incorporation of the sedimentation or floatation method increases the likelihood of detection of light parasitic infections with a low burden of eggs/cysts. However, in floatation methods, the high specific gravity of the fluid may cause distortion in the morphology of eggs and cysts when allowed to stand for a long period.

Floatation techniques have the additional disadvantage that the unfertilized eggs of *Ascaris lumbricoides*, eggs of *Taenia saginata* and *Taenia solium*, all trematode eggs and larvae of *Strongyloides stercoralis* do not float because these are heavier than the suspending liquid. Sedimentation techniques are easy to perform, technical expertise is minimally required, and the chances of technical error are also less. They are more sensitive than floatation methods, and the morphology of helminthic eggs and protozoal cysts is preserved.¹⁸

A study conducted by Garva et al (2023) on the evaluation of stool concentration techniques in the detection of some parasites reported that the sensitivity of the formol ether concentration technique was 96.2%, followed by Sheather's sugar floatation technique, being 88.5%, with the least sensitivity seen in the saline floatation method, i.e., 76.9%. The specificity of the concentration methods was as follows: formol-ether had the highest (97.8%), followed by Sheather's sugar with 93.8%, and the least specificity was 90.0% observed in the saline floatation method.¹⁹

Findings similar to our study result were reported by other authors, where the formol-ether sedimentation technique and the zinc sulphate floatation technique were found to be better in isolating all types of intestinal parasites, compared to the other methods. Saturated-salt solution floatation technique proved to be effective in the concentration of *Ascaris*, and simple sedimentation was useful in the recovery of eggs of *Taenia* spp.²⁰

Macroscopic & microscopic findings of stool for parasite:

Stool sample with various consistencies showed positive findings viz: loose stool (70.2%; 33/47), followed by watery stool (52.3%; 11/21), semi-formed stool (51.6%; 32/62), and formed stool (22.8%; 24/105). Similar findings were reported by other workers, where positive findings were seen in loose stool (8.96%; 11/58), followed by watery stool (2.72%; 29/1065).²¹

Studies from north east part of India showed the same findings, where it was observed that half of the patients having loose stool in the study were suffering from parasitic infections, and hence the most parasites were detected in loose stool (43.75%), compared to other forms of stool. This association was statistically significant ($P = 0.001$). Authors supported their findings by saying that parasites are found commonly in loose and watery stool, compared to formed/semi formed stool. Trophozoites of intestinal protozoa are usually

found in liquid or soft stool and occasionally in semi-formed stool, whereas cysts are found in formed and semi-formed stools. Though coccidian oocysts and microsporidian spores could be detected in any type of faecal specimen, the more liquid the stool specimen, the more coccidian oocysts are detected²²

It was possible for us to detect ova/cyst in 86.1% (31/36) of the stool samples with offensive odour. 48.0% (12/25) of the stool samples with positive findings were occult blood positive, whereas 52.0% (13/25) of the cases were positive for occult blood but did not show the presence of ova/cysts. Another 3 cases that showed *Entamoeba histolytica* were also positive for Charcot-Leyden crystals.

On the other hand, 100% (16/16) of cases that showed microscopic evidence of plenty of pus cells did not show any positive findings. Out of the samples that showed plenty of RBC, 4/7 stool samples were positive for *Entamoeba histolytica*, 28.7% (2/7) for *Ancylostoma duodenale*, and 14.2% (1/7) for *Hymenolepis nana* infection.

Findings of Singh N et al, 2023 reported that the difference in the positive findings in the stool of different consistencies was insignificant. 3.78% (34/898) and 2.39% (20/898) of the stool that were yellow and brown in colour showed the presence of ova/cysts in comparison to 96.2% (864/898) and 97.6% (817/898) of that did not show any parasitic infestation.²¹

In our study, 48.0% (12/25) of cases were positive for occult blood, as compared to 52.0% (13/25) of cases that were negative for occult blood. Other studies showed 66.67% (14/21) of the cases showed the presence of occult blood as compared to 33.33% (7/21) cases that were negative.²¹

Limitations

This study was conducted at a single tertiary care center, which may limit the generalizability of findings to other regions. The sample size was relatively limited, especially in the HIV-reactive group. Advanced diagnostic techniques, such as molecular methods, were not used, which could have improved parasite detection sensitivity. Additionally, socioeconomic and environmental factors influencing parasitic infections were not evaluated in detail.

Generalizability

The findings of this study are applicable to populations living in similar rural and resource-limited settings where poor sanitation, overcrowding, and limited healthcare access contribute to increased prevalence of intestinal parasitic infections. The results highlight the importance of routine stool examination in tertiary care centers and can help guide screening and management strategies in comparable healthcare settings.

Recommendations

Routine stool examination using concentration techniques such as sedimentation should be performed in all suspected cases, particularly among immunocompromised individuals such as HIV-positive patients. Awareness programs regarding hygiene, sanitation, and safe drinking water should be strengthened. Periodic screening and early treatment can help reduce morbidity associated with intestinal parasitic infections.

CONCLUSION

The present study gives us an idea regarding the occurrence of intestinal parasitic infections in various age groups, causing intestinal illness in this geographical region, with symptoms like diarrhea and vomiting. Parasites that are totally dependent on the host, depriving them of their nourishment, thereby benefiting from the host, while causing various effects by manipulating their immune status. Parasitic infections have wide spread impact on the type of parasite, the location of the infection, and the duration of the infection, which may include growth stunting, cognitive impairment, and increased susceptibility to other diseases.

Intestinal parasites like hookworms and roundworms can cause iron deficiency anemia by feeding on the host's blood. The mode of infection is by skin penetration by the filariform larva, followed by migration and maturation. Worms get sexually differentiated, and after mating, the female produces up to 30,000 eggs per day, which exit the host along with the feces to continue the life cycle.

The mode of spread of intestinal parasitic infections occurs through low standards of personal hygiene, poor sanitation, non-use of toilets, and inappropriate toilet habits within an illiterate population. A combined method of drug treatment and hygiene education is required to control parasite load among rural children in India. These measures would alleviate the severity of frequent outbreaks of parasitic infestation. In spite of the easy availability of anti-helminthic and anti-protozoal drugs, these conditions are not yet eliminated. Hence, regular and consistently ongoing surveys can serve a very useful purpose. The changes associated with living conditions and the associated environmental deterioration are further complicating the health situation.

There is a great need to monitor human settlements around big cities; otherwise, they will remain a permanent source of health hazard for all diseases, including intestinal parasitism. This could thus have a severe economic impact on the Indian healthcare system. Stool tests can give healthcare providers lots of helpful information. Microscopically, it can be checked in terms of color, consistency, quantity, shape, odor, and mucus. A definitive diagnosis of most of the

parasitic infections depends on the demonstration of trophozoites, ova, and cysts. This is traditionally done by stool microscopic examination, which is the gold standard in the diagnosis of IPIs. We also conclude that special staining methods like Modified Ziehl-Neelsen stain and concentration methods like Sheather's sugar flotation technique should be employed as part of routine testing for the detection of intestinal protozoa in HIV seropositive patients. With better knowledge and diagnosis of the opportunistic infections in HIV patients, a better picture will emerge regarding these infections, which would help clinicians and health planners to tackle the AIDS epidemic and the associated opportunistic IPIs in a more effective manner. The conventional methods lack sensitivity to detect parasites in a stool sample. The formol-ether technique and the Zinc sulphate flotation technique can be used in combination to increase the diagnostic sensitivity.

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List of Abbreviations

HIV – Human Immunodeficiency Virus

IPIs – Intestinal Parasitic Infections

IEC – Institutional Ethics Committee

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This study did not receive any external funding and was conducted using institutional resources.

Conflict of Interest

The authors declare that there is no conflict of interest.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author Contribution

Md Mofiz Alam contributed to data collection, laboratory analysis, and manuscript preparation.

Dr. Aninda Sen contributed to study design, supervision, and manuscript review.

Dr. Priyanka Paul Biswas contributed to the study conception, supervision, and final manuscript approval.

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