

COMPARISON OF SALINE, IODINE AND KOH WET MOUNT PREPARATIONS FOR OCCURRENCE OF PARASITES IN STOOL SAMPLES FROM PATIENTS ATTENDING ICDDR,B

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Abstract: The purpose of the present study was to compare results of saline, iodine, and KOH wet mount preparations executed on each of 1116 stool samples from patients attending ICDDR,B clinical pathology laboratory was compared. *Ascaris lumbricoides* was most prevalent helminth (3.67%) found in KOH wet mount preparation. The protozoan parasites (9.59%) were more prevalent than helminths (8.24%) and *Giardia sp* (4.03%) shows the higher infection found by KOH wet mount preparation. Females are more infected by parasites than males found in observations by all the three methods (saline, iodine and KOH wet mount preparation). It was also observed that the young (1-10 years) were more infected than older age group (above 51). In seasonal variation, parasitic infestation was higher during May (27%) and the prevalence was lower in December (10%). KOH was found to be effective in destroying the faecal debris, faecal mass and other artifacts without affecting many of the parasitic ova, cysts and larvae in the stool smears. KOH for wet mount smear in examining the stool samples for detection of intestinal helminths may be routinely used which is simple, cost-effective and could be afforded in resource poor setting.

Key words: Prevalence, saline, iodine, KOH preparation, seasonal variation

INTRODUCTION

Intestinal parasites are widely prevalent as the cause of diarrhoea especially among children and often associated with morbidity and mortality in under developed countries. Parasites of diarrhoeal etiology are widespread, infecting a significant proportion of the human population in third-world countries (Vignesh *et al.* 2007), especially across the Asian subcontinent. The prevalence of intestinal parasitosis is very high in Bangladesh (Muazzem and Ali 1969, Islam 1984). With an ever-increasing population leading to overcrowding and unhygienic practices these parasites pose a serious threat that is compounded by limited resources. About 80% people live in the rural areas (Chowdhury 1979, 1981). Correct identification of a parasite is the first step to ensure a combat against these parasitism and infectious diseases.

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Faeces, the most frequent specimens, are usually examined in the laboratory by microscopic examination, which essentially consists of direct wet mount preparation, concentration, and permanent staining. Microscopic observation of wet mounts remains the most widely used method for identifying ova and cyst of parasites from stool specimens (Valentine and Lalitha 2001). The specific diagnosis of the condition is made by demonstration of protozoan cysts or trophozoite; helminthes ova or less frequently adult worms or larvae in the stool smears by microscopy (Parija 1993).

Wet mount preparation of stool is extensively used in stool microscopy in a parasitological laboratory for the diagnosis of intestinal parasitic infections (Parija and Srinivasa 1999). Saline, quensel stain, Nairs buffered methylene blue, Lugols iodine solution, D'Antonie' iodine stain and lacto-phenol cotton blue are the agents frequently used in the wet mount preparation of the stool (Parija and Prabhakar 1995). In contrary, if a very thick stool smear is made by taking relatively a large volume of stool, the smear become too thick to be examined. This is due to a dense background formed by faecal artifacts thereby masking the parasitic ova and cyst that are present, thus making their detection very difficult (Parija 1996).

Potassium hydroxide (KOH) is widely used in the wet mount preparation of various clinical specimens for demonstration of fungi and fungal elements, skin scales, hair stuffs, and nail scraping or other specimen with a few drops of 10% KOH on a microscope glass slide. The KOH digests protinaceous debris and bleaches pigments, loosens the sclerotic materials but without damaging the clinical materials which remain unaffected by this treatment appear in the examination by microscopy (Al-Doory 1990).

The main objectives were to detect, identification and determine the prevalence of protozoa and helminth parasites in different months, age-groups, in male and female patients attending ICDDR,B.

MATERIAL AND METHODS

Study area and study period: The study was conducted among the diarrheal patients who submitted stool sample in Clinical laboratory of ICDDR,B. The study period was April 2011 to March 2012.

Sample Collection and Processing: A total of 1116 fresh stool specimens were examined microscopically in the Clinical pathology laboratory of ICDDR,B for the detection of intestinal parasite.

Urine free stool samples were collected in a clean and leak proof, transparent container. No antiseptic or de-worming medication was given before

taking these samples. Examination of specimen was performed as soon as possible, within one hour of collection. In most instances liquid stool or those specimen with mucus and or blood was examined on a priority basis.

Saline wet mount preparation: A minute portion of the faeces was diluted with normal saline (0.9%) on a microscopic glass slide, a cover slip was then gently put over it so as to spread out the emulsion into a thin transparent layer.

Iodine wet mount preparation: Iodine wet mounts were prepared by mixing a small volume of stool to a drop of Lugols iodine on the glass slide that contain saline and placed a cover slip on it.

KOH wet mount preparation: A thick stool smear was prepared by adding more volume of stool to a drop of 10% KOH on a microscopic slide and placing a cover slip on the smear. 10% KOH was prepared by mixing 10 gm of potassium hydroxide pellet in 100 ml of distilled water. The wet mounts of stool sample were examined at different time interval. Like 30 min 1 and 2 hr preparation of stool smears with KOH for observe the changes of morphological feature of intestinal parasites.

Stool microscopy: Three different wet mounts preparation of stool was observed in three different microscopes and examined initially by using a low power (10X) objective, then again by using a high power objective (40X) for confirmation of presumptive and positive finding.

Data processing and analysis: After completion of entire data collection were tabulated and calculated. The tabulation was done manually as well as SPSS software version 12.5. For calculation electronic calculator was also used. Picture of the cyst of protozoa and eggs of ova of helminth were photographed with a camera fitted to a microscope in the clinical pathology laboratory, ICDDR,B.

Identification of trophozoite, cysts, oocysts, egg/ova: The trophozoite, cyst, oocysts, egg/ova as observed under microscope were identified by the following descriptions and pictures published by WHO (1980), Schmidt and Roberts (1989).

RESULTS AND DISCUSSION

The present cross sectional study was conducted to find out the effectiveness of KOH for detection of intestinal parasite which is usually clinically used for demonstration of fungi and fungal elements. Five species of gastrointestinal helminth parasites and four species of protozoan parasites were found.

Helminth ova encountered were *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Enterobius vermicularis*, *Trichuris trichiura* and encountered larvae

was *Strongyloides stercoralis*. Cyst or trophozoites of protozoan species were *Giardia lamblia*, *Entamoeba histolytica*, *Endolimax nana*, and *Entamoeba coli*. The presence of cysts, trophozoites and ova of the above parasites in the stool indicated the presence of adult or mature stages of these parasites in the individuals.

Out of 1116 stool samples only 33 were positive for *Ascaris lumbricoides* and the prevalence was 2.96%, 18 samples were positive for *Ancylostoma duodenale* and prevalence was 1.61%, 7 samples were positive for *Enterobius vermicularis* and the prevalence was 0.63%, 10 sample were positive for *Trichuris trichiura* where prevalence was 0.89%, 5 samples were positive for *Strongyloides stercoralis* and the prevalence was 0.45%. Total 73 samples were positive for helminths by iodine wet mount preparation (Fig. 1).

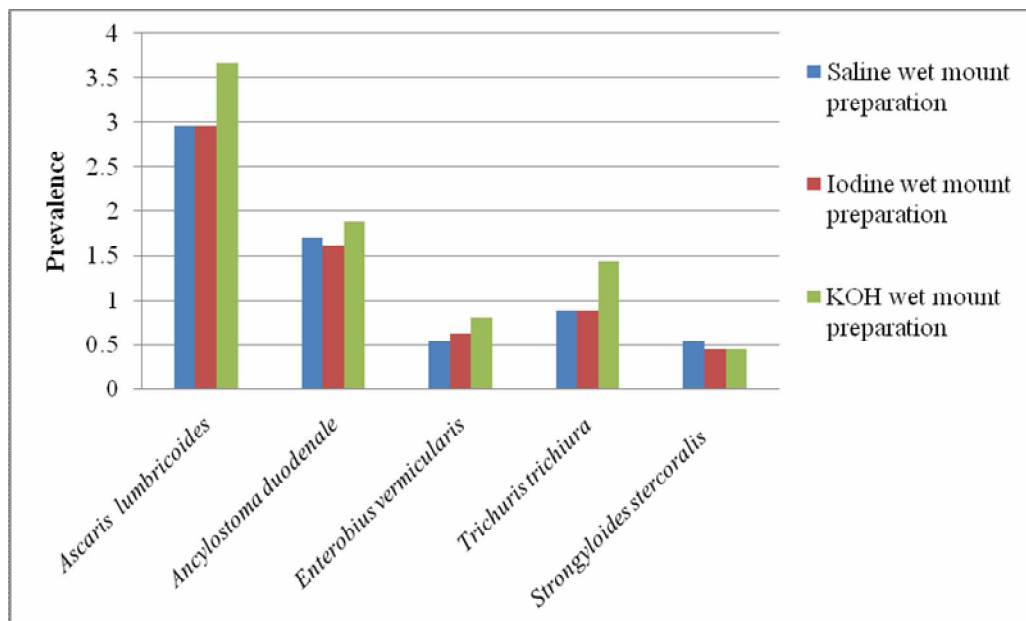


Fig. 1. Comparative prevalence of helminths by different types of wet mount preparations.

The prevalence of *Ascaris lumbricoides* was 2.96% detected by saline wet mount preparation, 2.96 % prevalence of *Ascaris lumbricoides* confirmed by iodine wet mount method and KOH wet mount method detected 3.67% prevalence of *Ascaris lumbricoides* in total samples. The prevalence of *Ancylostoma duodenale* was 1.70% detected by saline wet mount preparation, 1.61% prevalence of *Ancylostoma duodenale* confirmed by iodine wet mount method, KOH wet mount method detected 1.88% prevalence of *Ancylostoma duodenale* in total samples. In case of *Enterobius vermicularis* the prevalence

was 0.54, 0.63 and 0.81% detected by saline, iodine and KOH wet mount preparation. The prevalence of *Trichuris trichiura* was 0.89% by saline method, 0.89% by iodine, and 0.43% by KOH wet mount preparation. The prevalence of *Strongyloides stercoralis* was 0.54% detected by saline wet mount preparation, 0.45% detected by iodine wet mount, and 0.45% by KOH wet mount preparation (Fig. 2).

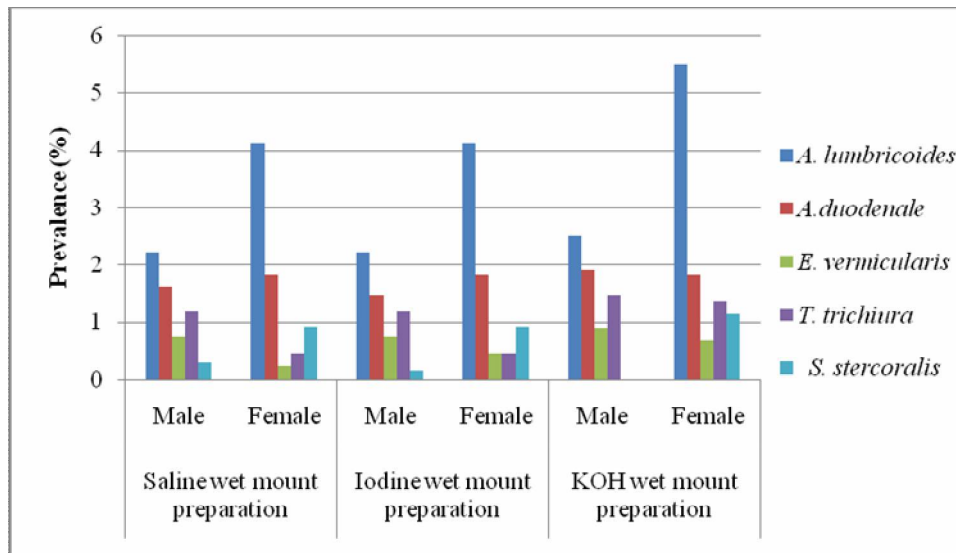


Fig. 2. Comparative prevalence of different helminths in different sex groups by different types of wet mount preparations.

The comparative prevalence of different helminthes in different host sex by saline wet mount, iodine wet mount, and KOH wet mount preparation of stool where the prevalence of *Ascaris lumbricoides* and of *A. duodenale* was higher in female than male found in saline and iodine preparation. In case of *E. vermicularis* and *T. trichiura* males were more infected than female found in different wet mount preparation. *S. stercoralis* was found only in female host by KOH preparations. But it was statistically found that overall there was no relationship among the helminth parasites and sex of the host.

The comparative prevalence of different helminths in different age groups by different wet mount preparation, it was found that in case of *Ascaris lumbricoides* the higher prevalence found in age group 1-10 years. For *Ancylostoma duodenale*, higher prevalence was found in the age group of 11-20 years. In case of *E. vermicularis* and *T. trichiura* higher prevalence was found in age group 1-10 years. Regarding *Strongyloides stercoralis*, the positive samples were found in the age group of 11-20 years (Table 1).

Table 1. Comparative prevalence of different helminths in different age groups by different wet mount preparation

Age group (years)	Name of helminth parasites																			
	<i>A. lumbricooides</i>				<i>A. duodenale</i>				<i>E. vermicularis</i>				<i>T. trichiura</i>				<i>S. stercoralis</i>			
	S	I	KOH	S	S	I	KOH	S	S	I	KOH	S	S	I	KOH	S	S	I	KOH	S
1-10	5.12	5.12	6.12	2.15	2.15	2.15	2.48	0.99	0.99	1.16	1.49	1.65	1.65	1.65	2.64	0	0	0	0	0
11-20	2.78	2.78	5.55	8.33	6.94	8.33	8.33	0	0	0	0	0	0	0	0	8.33	6.94	6.94	6.94	6.94
21-30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31-40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41-50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
>51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

p Value*8: SWMP-0.001; IWMP- 0.001; KOH WMP-0.000336, S=Saline; I=Iodine.

Table 2. Comparative prevalence of different protozoan in different age groups by different wet mount preparation

Age group (years)	Name of the parasites															
	<i>G. lamblia</i>				<i>E. histolytica</i>				<i>E. coli</i>				<i>E. nana</i>			
	Saline	Iodine	KOH	Saline	KOH	Iodine	Saline	KOH	Saline	Iodine	Saline	KOH	Saline	Iodine	Saline	KOH
1-10	5.78	5.78	6.77	2.48	2.48	3.14	2.64	0	0	0	0	0	0	0	0	0
11-20	5.55	4.16	5.55	6.94	6.94	6.94	6.94	6.94	8.33	8.33	11.11	11.11	0	0	0	0
21-30	0	0	0	0	0	0	0	5.12	5.98	6.84	6.84	6.84	0	0	0	5.12
31-40	0	0	0	0	0	0	0	0	0	0	0	0	5.55	5.55	5.55	8.88
41-50	0	0	0	0	0	0	0	0	0	0	0	0	5.49	5.49	5.49	6.59
>51	0	0	0	3.55	3.55	0	3.55	0	0	0	0	0	5.67	3.54	3.54	0

The comparative prevalence of different protozoan infection by different wet mount preparation it was found that the prevalence of *Giardia lamblia* was 3.49% by saline wet mount preparation, 3.41% by iodine wet mount preparation and KOH was 4.03% prevalence. The prevalence of *Entamoeba histolytica* was 2.24% by saline wet mount preparation, 2.15% prevalence by iodine wet mount preparation, and 2.33% prevalence by KOH wet mount method. The prevalence of *Entamoeba coli* was 0.98, 1.16 and 1.43% by saline, iodine and KOH wet mount preparation. The prevalence of *Endolimax nana* was 1.61, 1.34 and 1.79% by saline, iodine, and KOH wet mount preparation (Fig. 3).

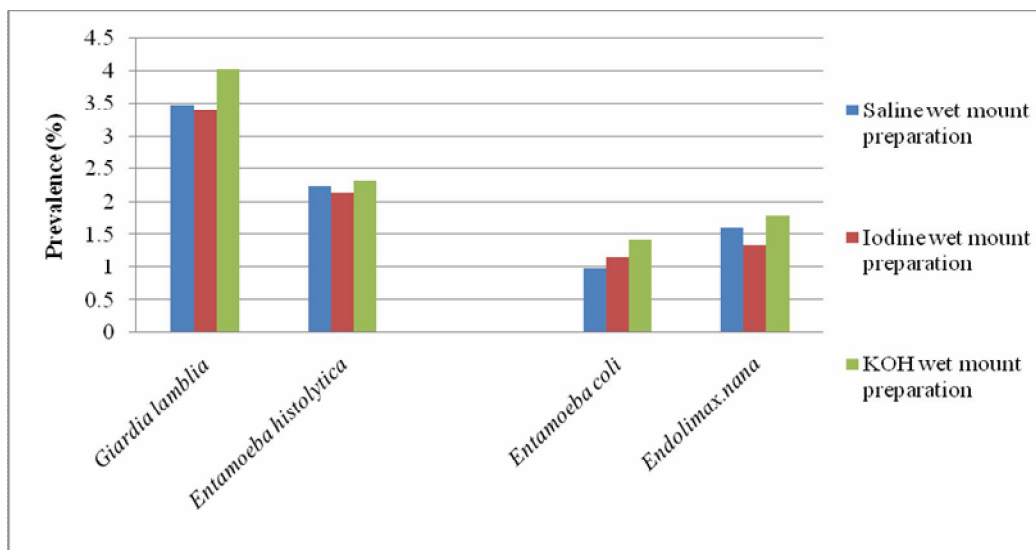


Fig. 3. Comparative prevalence of different protozoa by different types of wet mount preparations.

The comparative prevalence of different helminthes in different sex groups by saline wet mount, iodine wet mount, and KOH wet mount preparation of stool where, the prevalence of *Ascaris lumbricoides* and of *A. duodenale* was higher in female than male found in saline, iodine and KOH wet mount preparation. In case of *E. vermicularis* and *T. trichiura* males were more infected than females found in different wet mount preparation. *S. stercoralis* found only in female host by KOH preparations. But it was statistically found that overall there was no relationship among the helminth parasites and sex of the host (Fig. 4).

The comparative prevalence of different protozoan parasites in different age groups by different wet mount preparation where it is found that in case of *G. lamblia* higher prevalence was found in age group 1-10 years. In *E. histolytica*, and *E. coli* the higher prevalence were found in the age group 11-20. For *E. nana* the higher prevalence were found in age group above 51 years. In saline wet

mount preparation and 31-40 years age group were more infected found in iodine and KOH preparation (Table 2).

The difference in prevalence of infestation was noticeable between the months of the study period (April 11-March 12). The prevalence of infection was highest in the month of May (27%) and was lowest in December (10%). In the remaining month the prevalence fluctuated from moderate to low (Fig. 5).

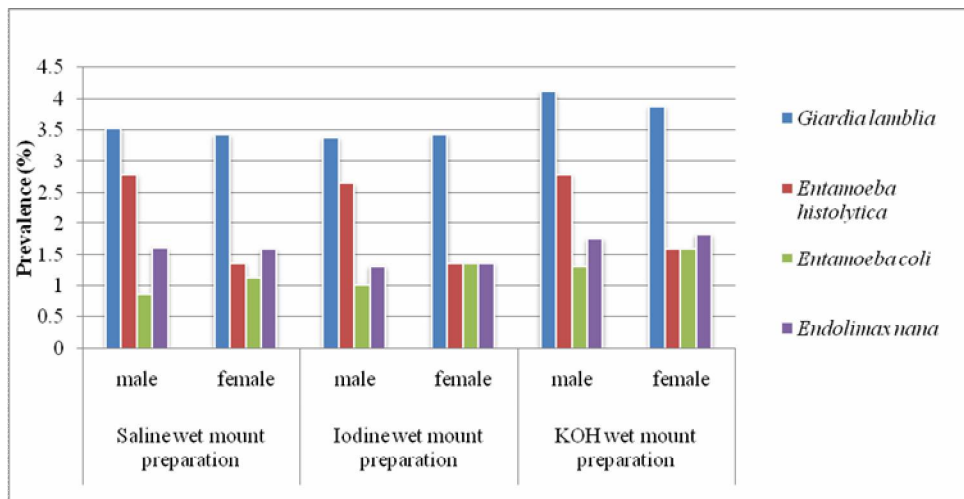


Fig. 4. Comparative prevalence of protozoa in different sex groups by different types of wet mount preparations.



Fig. 5. Monthly variations in prevalence of infestation of total gastrointestinal parasites.

A total of 1116 stool samples were examined from male and female patients and high detection rate was observed by potassium hydroxide wet mount preparation ($p < 0.05$) by McNemar test except *S. stercoralis*. Qualitatively, all parasites which were detected by the routine methods (iodine, saline and KOH

wet mount method) were also detected by the KOH wet mount preparation but with different rates.

Begum and Rahman (1975) studied intestinal helminths and protozoa. They reported five species of protozoa (*E. histolytica*, *E. coli*, *Endolimax nana*, *I. butchlii* and *G. lamblia*) and four species of helminths (*A. lumbricoides*, hookworm, *T. trichiura* and *E. vermicularis*). Khaled *et al.* (1983) found eight species of parasites including *E. histolytica*, *E. coli*, *G. lamblia*, *A. lumbricoides*, *A. duodenale*, *E. vermicularis*, *T. trichiura* and *H. nana*. Islam (1984) reported *A. lumbricoides*, *A. duodenale*, *E. vermicularis*, *T. trichiura* in the patients of Barisal Medical College and Hospital. Nahar (2011) and Khatun (2000) also reported same, which was similar to present study. The detection of *A. lumbricoides* was found to be higher by using KOH wet mount preparation ($p < 0.05$). Muttalib *et al.* (1976), Saha and Chowdhury (1981) and Reinthaler *et al.* (1988) recorded prevalence of *A. lumbricoides* from Bangladesh and southwest Nigeria, respectively.

Table 3. Detection of stool parasites with saline, iodine and KOH wet mount preparations

Name of the parasites		Saline wet mount	Iodine wet mount	KOH wet mount	p value*8
<i>A. lumbricoides</i>	Positive	33 (2.96)	33 (2.96)	41 (3.67)	< 0.001
	Negative	1083 (97.04)	1083 (97.04)	1075 (96.33)	
<i>A. duodenale</i>	Positive	19 (1.70)	18 (1.61)	21(1.88)	< 0.001
	Negative	1097 (98.29)	1098 (98.38)	1095 (98.12)	
<i>E. vermicularis</i>	Positive	6 (0.54)	7 (0.63)	9 (0.81)	< 0.001
	Negative	1110 (99.46)	1109 (99.37)	1107 (99.19)	
<i>T. trichiura</i>	Positive	10 (0.89)	10 (0.89)	16 (1.43)	< 0.001
	Negative	1106 (99.10)	1106 (99.10)	1100 (99.57)	
<i>S. stercoralis</i>	Positive	6 (0.54)	5 (0.45)	5 (0.45)	Nd
	Negative	1110 (99.46)	1111 (99.55)	1111 (99.55)	
<i>G. lamblia</i> cyst	Positive	34 (3.05)	38 (3.41)	45 (4.03)	< 0.001
	Negative	1082 (96.95)	1078 (96.59)	1071 (95.96)	
<i>E. histolytica</i> cyst	Positive	20 (1.79)	24 (2.15)	26 (2.33)	< 0.001
	Negative	1096 (98.21)	1092 (97.85)	1090 (97.67)	
<i>E. coli</i> cyst	Positive	11 (0.98)	13 (1.16)	16 (1.43)	< 0.001
	Negative	1105 (99.04)	1103 (98.83)	1100 (98.47)	
<i>E. nana</i> cyst	Positive	14 (1.25)	15 (1.34)	20 (1.79)	< 0.001
	Negative	1102 (98.74)	1101 (98.65)	1096 (98.21)	

*McNemar Test; 8: Considered for iodine versus KOH wet mount preparation, nd = Not determined

From the earlier and present studies it is observed that the prevalence of *A. lumbricoides* is lower in present than the earlier studies. Children had higher infection with *A. lumbricoides* (Adeyeba and Dipeolu 1984 and Agugua 1983). *E. vermicularis* and *T. trichiura* also showed the same with higher prevalence in 1-10 years age group. The detection of *T. trichiura*, was found to be higher by using KOH wet mount preparations ($p < 0.001$). In *S. stercoralis* detection, iodine and KOH wet mount showed no difference and almost similar detection rate was observed.

From this study, it was observed that, the protozoan parasites were more abundant than helminths and *G. lamblia* was the most prevalent. KOH gave the highest detection (4.03%). Mutallib *et al.* (1976) and Chowdhury (1981) reported higher percentages of *G. lamblia* than that of the present studies result. In the present study it was observed that males and females were almost similarly infected. Reinthaler *et al.* (1988) found higher (4.3%) prevalence in males than females (4.1%). Muttalib *et al.* (1976) found higher 6.35% prevalence of infection with *Giardia* in females than in males. So it can be said that sex is not an attributed factor for *Giardia* infection and statistically they were not significant ($p > 0.05$). *E. histolytica* was 2.24% by saline wet mount preparation, 2.15% prevalence by iodine wet mount preparation, and 2.33% prevalence by KOH wet mount method.

Muttalib *et al.* (1976) observed 40.88% prevalence of intestinal parasites in rural children of Bangladesh. Chowdhury (1979) reported 15.19% prevalence from the people of Dhaka. In the light of present study, the prevalence of *E. histolytica* in our study area is lower than the earlier study. The prevalence was lowest in this diagnostic centre's because may be most of the patients maintain their life in hygienic way. In present studies, found that males were more infected with *E. histolytica* than female. Khatun (2000) stated 17.24% were males and 19.04% were females. In the present study, the higher prevalence of *E. histolytica* was found in the age group 11-20. Adeyeba and Dipeolu (1984) showed 0, 5.4, 10, 3.4% of prevalence in 1-10, 11-20 and 40 years old people, respectively. Schmidh and Roberts (1989) stated that children under 5 years have lower rate of infection with *E. histolytica*.

CONCLUSION

In the present observation, it was observed that KOH has a high detection rate compared to other wet mount preparations. So, the use of KOH preparation along with saline wet mount preparation in a routine pathology laboratory is a good practice. KOH wet mount is simple, the reagent are inexpensive and easily prepared. The procedure also facilitates for better detection and identification of

parasites in the laboratories where permanent stained smear of stool does not form a part of routine stool examination.

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