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Comparison of Different Concentration Techniques of the Stool Examination for Detecting Intestinal Parasites.

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ABSTRACT

Intestinal parasitic infestations have a high prevalence in tropical and subtropical countries which accounts for significant morbidity in humans. Several concentration techniques are available to detect intestinal parasites; however, effective and better technique has a paramount importance in early detection. To evaluate a various concentration methods in comparison with the gold standard Formol-ether concentration technique in detecting intestinal parasites. A total of 400 stool samples were collected from hospital based population. Samples were processed and examined using salt flotation, zinc sulphate flotation, formol-ethyl acetate, formol-petrol, formol-acetone and formol-ether concentration methods. Formol-ether detected 34% of parasites followed by formol-petrol (33.5%), formol-ethyl acetate (28.75%), zinc sulphate flotation (27%), salt flotation and direct smear had 23.5% and 23.25% respectively. Formol-ether and formol-petrol concentration techniques gave almost similar parasite recovery rate which indicates that these two techniques are effective method for examination of stool specimens.

Keywords: Stool concentration techniques, formol-petrol concentration, formol-acetone concentration, formol-ether concentration, zinc-flotation, salt flotation

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INTRODUCTION

Intestinal parasitic infections are among the most prevalent infections in humans mainly in developing countries [1]. These infections cause chronic conditions, which may progress to serious diseases. Intestinal parasitic infections are globally endemic and have been described as constituting the greatest single worldwide cause of illness and disease [2].The World Health Organization (WHO) estimates that 3.5 billion people worldwide are infested with some type of intestinal parasite, and as many as 450 million of them are sick as a result of these infections (3). Most of these infections are as a result of poverty, poor standard of living, poverty, poor sanitation, unhygienic conditions, low socio-economic status, low literacy and lack efficient diagnostic facilities [4, 5].

In this HIV era, mixed intestinal parasitic infections are common phenomenon and there is a need to include an inexpensive, effective technique as a routine diagnostic method in all hospitals and research institutes. Many methods are available for the detection of intestinal parasites but the choice of a particular technique will depend on various factors such as its effectiveness, level of knowledge in identification, its affordability and ease to carry out. The methods range from simple microscopy to PCR and DNA probes. The methods like DNA probes, PCR and direct fluorescent antibody methods [6] are highly sensitive but affordability remains a major concern in the developing countries. Hence the conventional methods like direct wet mount and iodine mount remains the main the gold standard diagnostic method in detecting intestinal parasites because of its simplicity and affordability. However if the density of the parasite in the faeces is low, direct smear method is not an ideal choice for detecting the parasite [7].The detection of parasites in the faecal specimens is enhanced by the use of concentration procedures.

Various concentration techniques like simple slat floatation, Zinc sulphate centrifugal floatation, formol-ether concentration and formol-ethyl acetate, formol-petrol concentration are employed for the diagnosis and the epidemiologic surveillance of parasitic infections in humans. The objective of this study is to compare 4 different concentration techniques with the conventional technique in diagnosing parasitic infections.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology during the period of six months from April 2010 to September 2010. A total of 400 stool samples were obtained from both outpatients and inpatients of Saveetha Medical College & Hospital and also in the Rural Health Centre, Kuthambakkam.

Ethical approval from the Ethics Committee of the University was obtained. All study subjects were informed about the study and written informed consent was obtained. Symptomatic and asymptomatic patients are included in the study. Specimens were collected in sterile containers and transported to the Department of Microbiology immediately. The specimens contaminated with water or patient's urine, or retrieved from the toilet bowl are rejected and repeat sample was collected after proper instructions. The stool specimens from the patient had been taking non-absorbable anti-diarrheal drugs, mineral oil based laxatives, or antimicrobials within 1 week were rejected. All thespecimens were examined using various techniques.

Each stool specimen was examined by the following techniques.

Macroscopic examination:

The colour, consistency, presence of blood and mucuswere recorded. The stool specimens were examined for the presence of parasitic structures such as proglottids, scolices, adult tapeworm, trichuris, enterobius, ascaris, or hookworm with the help naked eye and with the aid of a hand lens.

Direct microscopic examination by using saline and iodine preparations:

On a microscopic slide, one drop of 0.85% NaCl is placed on the left side of the slide and one drop of iodine on the right side of the slide. A small amount of fecal specimen was thoroughly emulsified in saline and iodine using an applicator stick. The sample should be spread thinly enough that newsprint can barely be read

when the slide is placed on top of text. A 22mm cover slip was placed at an angle into the edge of the emulsified fecal drop taking care that the preparation was free of air bubbles. The entire cover slip with overlapping fields was scanned systematically scan with the 10x objective and with 40X objective for more detailed study of any suspect eggs or protozoa.

Concentration techniques:

Simple salt floatation: Briefly, about 1gm of faeces was emulsified with 3-4 ml of saturated salt solution in a 20ml conical glass test tube. It was stirred well and more salt solution was added till the container was nearly full, with the stirring being continued. Any coarse matter which floated up was removed and the tube was placed on a levelled surface with a glass slide being placed over the top of the tube, which was in contact with the fluid. It was allowed to stand for 30 minutes. The slide was removed and observed for the presence of eggs/cysts [8].

Zinc sulphate centrifugal floatation:0.5 to1g of the stool specimen was emulsified in 10 parts of tap water and it was strained through awire gauze. The filtrate was collected in a Wassermann tube and centrifuged at 2,500 rpm for 2 min. The supernatant was discarded and the sediment was re-suspended in water. This step was repeated till the supernatant became clear. To the sediment, 3-4 ml of 33% Zinc sulphate solution was added, it was mixed well and it was filled with ZnSO4 solution, about half an inch of the rim. Several loopfull of the supernatant fluid were removed with a bacteriological loop and they were observed for parasites [8].

Formol-ether concentration: 0.5 to 1g of stool was emulsified in 7ml of 10% formol saline and it was kept for 10 minutes for fixation. It was then strained through a wire gauze. The filtrate was added to 3 ml of ether and it was centrifuged at 2000 rpm for 2 minutes. It was allowed to settle. The supernatant was removed and capillary tubes were used for drawing the sediment from the centrifuge tube and delivering it onto the slide. Saline and iodine wet mounts were prepared from the concentrated sedimentand examined [9].

Formol-ethyl acetate concentration: Samples of stool varying from 0.09 to 0.17 g of feces were placed in 1-ml centrifuge tubes. By using a micropipetting device, 0.5 ml of 10% Formalin was added to the specimens. This achieved a range of formalin to stool ratio of between 3:1 and 5:1. The specimens were stirred, and 0.25 ml of ethyl acetate was added to the mixture. The tubes were capped andshaken for 30 s. They were then centrifuged at 400 x g for 1 min. Four layersresulted after centrifugation: excess ethyl acetate, a "cloudlike" layer of debris, formalin, and the sediment. The supernatant was decanted and a saline and iodine wet mounts were prepared from the concentrated sediment and examined [10].

Formol-petrol concentration technique: 1 gram of stool sample was emulsified in 10mls of normal saline in a centrifuge tube and spun at 3000 rpm for 10 minutes. The supernatant was discarded. This process was done two times to wash the stool sample. Then the sediment was resuspended in 7mls of formol saline and 3mls of petrol (super) was added and the mixture stoppered with a rubber bung and shaken vigorously. It was spun at 3000 rpm for 10 minutes. It separated into three portions. The first from the bottom was the sediment, followed by a layer of formol saline in the middle and at the top were coarse stool particles, petrol and fats. The layers above the sediment were carefully aspirated and discarded using pasture pipette. The sediment was examined under the microscope for the presence of parasites using saline and iodine mount [7].

RESULTS

A total of 400 stool specimens were examined, out of which 135(33.75%) samples were positive for intestinal parasitic infestation. Of the 400 samples, 268 samples were from males of which 98 samples were positive for intestinal parasites and remaining 132 samples were from female patients with 37(28%) positivity. Distribution of parasites among different age groups was shown in Table 1. Age group between 21-30 years had the highest prevalence of the parasitic infestations. Prevalence of parasitic infections in the study population is shown in Table 2.The percentage of parasite recovery under different examination techniques is shown in Table 3.

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Table 1: Distribution of parasites among different age groups

Age group	Distribution of positivity	Percentage
1 to 12 yrs	17	12.5%
13 to 20 yrs	30	22.2%
21 to 30 yrs	34	25.2%
31 to 40 yrs	22	16.3%
41 to 50 yrs	14	10.3%
> 50 yrs	12	9%

Table 2: Prevalence of parasitic infections in the study population

PARASITE	Male	Female	Total
Giardia lamblia	17	3	20(5%)
Entamoeba coli	18	6	24(6%)
Entamoebahistolytica	8	2	10(2.5%)
Isospora belli	-	1	1(0.25%)
Ancylostomaduodenale	37	17	54(13.5%)
Taenia species	4	2	6(1.5%)
Trichuristrichiura	3	3	6(1.5%)
Ascarislumbricoides	1	3	4(1%)
Strongyloidesstercoralis	10	-	10(2.5%)

Table 3: Different parasites and their percentage of recovery in different examination methods

Parasites		Concentration methods				
	Saline and Iodine Method	Salt floatation	Zinc sulphate floatation	Formol-ether	Formol-Ethyl acetate	Formol-petrol
Giardia lamblia	15 (3.7%)	17 (4.2%)	19 (4.7%)	20 (5%)	18 (4.5%)	20 (5%)
Entamoeba coli	18 (4.5%)	21 (5.2%)	24 (6%)	24 (6%)	20 (5%)	23 (5.75%)
Entamoebahistolytica	6 (1.5%)	9 (2.2%)	9 (2.2%)	10(2.5%)	7 (1.7%)	9 (2.25%)
Isospora belli	-	-	-	1 (0.2%)	-	1 (0.2%)
Ancylostomaduodenale	38 (9.5%)	45 (11.25%)	53 (13.25%)	54 (13.5%)	50 (12.5%)	53 (13.25%)
Taenia species	4 (1%)	-	-	6 (1.5%)	5 (1.25%)	6 (1.5%)
Trichuristrichiura	3 (0.75%)	2 (0.5%)	3 (0.75%)	6 (1.5%)	4 (1%)	6 (1.5%)
Ascarislumbricoides	2 (0.5%)	-	-	4 (1%)	3 (0.75%)	6 (1.5%)
Strongyloidesstercoralis	5 (1.25%)	-	-	10 (2.5%)	8 (2%)	10 (2.5%)
Total	**93 (23.25%)**	**94 (23.5%)**	**108 (27%)**	**135 (34%)**	**115 (28.75%)**	**134 (33.5%)**

DISCUSSION

Human parasitism is a global problem and the prevalence rates of intestinal parasites exhibit wide variation from country to country; between geographic areas, communities and ethnic groups even seasonal variations are also known [2]. The variations in prevalence rate of the parastitic infestations could be due the choice of stool examination and detection method. A variety of situations may arise that will make difficult or prevent obtaining a stool sample adequate for detection using standard routine method like stool microscopy. Advance made in the parasitology techniques have led to the formulation of effective strategy against parasitic diseases. Various studies have been done in the different parts of the world to detect specific methods.

The fecal concentration methods play a vital role in increased recovery of eggs and larvae of helminths and the cysts of protozoa. The increased yield of positive findings with all types of fecal parasites, the relatively clean deposit, and the enhanced visibility of the structural detail of cysts obtained by concentration method justify its use as a routine diagnostic procedure in various hospitals and research institutes. Thereby exact prevalence rate of parasitic infection can be obtained in the study population.

This study conducted in a tertiary care hospital in South India is focused on the yield of parasites in various stool concentration techniques. Out of 400 samples, 135 were positive for intestinal parasitic infection with a prevalence rate of 34%. Many other studies from India have reported varying rates of intestinal parasitic infections which ranges between 6.68% and37.45% [8, 11, 12]. In the present study, 98(36.6%) male patients and 37(28%) female patients were found to have an intestinal parasitic infection, which shows the incidence of parasitic infection was more in males than females. This observation was in accordance with other studies [8, 12]. Overall 33% of adults were found to be infested with intestinal parasites. Babiker et al [14] has quoted an incidence of 31% among adults study population and this was comparable with our study. Out of 43 children, 17(12.5%) were found to have intestinal parasitic infection which was very less when compared to the study [13] were the prevalence was 42.5%.

The prevalence rates of intestinal parasitic infections may vary from country to country, between geographical areas, communities and even seasonal variations also occurs and to some extent it depends on the method of choice in detecting the parasite in the specimens. We have observed only 23.25% recovery rate by routine direct microscopy method using saline and iodine mount; quite less when compared to other methods. When compared with saturated salt flotation technique and zinc sulphate centrifugal floatation, the latter had high recovery rate (27%). This is in agreement with a similar study conducted in India [8]. The higher recovery rate than the routine method is because the technique concentrated most ova and cysts. Due to centrifugation in a fluid of specific gravity 1.18, the cysts and ova, being lighter than the suspending fluid float to the top, whereas the heavier debris sinks to the bottom. In spite being a simple and efficient method for the recovery of ova, larvae, and protozoan cysts, this method is not suitable for the recovery of eggs from Ascaris, Trichuris, the larvae of Strongyloidesstercoralis and Trematodes. Another drawback of the procedure is that it is not convenient when working with fatty stools. In addition, the morphology of Giardia cysts was better preserved with the sedimentation procedures than with the flotation procedure [15].

The other concentration techniques in the present study were formol ether, formol-petrol, and formol ethyl-acetate method with the recovery rate of 34%, 33.5% and 28.75% respectively. The recovery rate in these methods is higher when compared to the two floatation techniques. This observation agrees favorably with other similar studies [7, 8], which reported superiority of formalin-ether over other concentration procedures. This increased recovery rate by formol-ether can be explained by the fact that the use of formalin fixes and preserves the faecal specimen. Ether decreases the specific gravity of small faecal particles thus causing them to float in the suspension and also dissolves fat. The coarser, non-absorbent elements including eggs and cysts of parasites are left at the bottom.

The recovery rate of formol-ethyl acetate sedimentation technique is 28.75% which was more when compared to the floatation methods, but it was not better than formol- ether and fomol-petrol technique. This may be due to the fact that the thickest interface of ethyl acetate was difficult to remove and they sometime remixed with the sediments and mask the parasites. Even though Formalin-ethyl acetate procedure is a suitablealternative to the Formalin-ether method considering the safety [15], with respect to the recovery rate of parasite it is less when compared to other two methods.

In our observation there is no significant difference ($p > 0.05$) between the parasites detected by formol-ether and formol-petrol concentration techniques, similar findings was reported by Wirkom-Tata et al [7]. Both methods have their own demerits like irritant odours, inflammable property, mutagenicity, neurotoxicity [16] proper care in handling them with the help of biosafety cabinet ensure safety.

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CONCLUSION

Based on these results, we currently employ both routine direct microscopy and formol-ether sedimentation procedures to increase the sensitivity in detecting the parasites in stool specimen. We recommend including any one of the concentration techniques along with routine microscopy method to estimate the exact prevalence rate of the intestinal parasites in a given population.

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