

Original article**Laboratory evaluation of different concentration techniques of Stool examination for Intestinal Parasitic Infection at a Tertiary Care Center, Kanpur (UP)****Sujatha R¹, Deepak S¹, Nashra A²**

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Abstract

Background- Intestinal parasitic infections are still one of the major health concerns in developing countries. In India, malnutrition, unhygienic conditions, the improper disposal of sewage, the non-availability of potable water supplies in the rural and the urban areas, the indifferent attitude of the population towards personal hygiene, their low socio-economic status and the low literacy rates are responsible for the high rates of intestinal parasitic infections. Therefore, the aim of this study was to assess the prevalence of intestinal parasitic infection and evaluation of different concentration techniques of the stool examination for their diagnosis.

Material And Method - A total of 154 consecutive stool sample received during January 2018 to June 2018 were included in the study. All stool samples were processed by direct wet mount, salt floatation method and formalin-ether concentration method and the results were compared and analyzed. **Result-** The prevalence rate of the study was (31) 20.1%. Males were having 64.5% of intestinal parasitic infection and females were having 35%. Middle age group (20-30) year had the highest prevalence of the parasitic infection. The isolates were *Taenia* species (35%), *A. lumbricoides* (32%), Hook worm (16%), *H. nana* (12%) and *T. trichuria*(3%). The evaluation of intestinal parasite by different methods showed with formal-ether concentration (83%), zinc sulphate centrifugal floatation(70%), simple salt floatation (61%) and wet mount

(48%) preparation respectively. **Conclusion-** In this study, formal-ether concentration technique has showed a high sensitivity and specificity. Its implementation along with routine method, will improve the diagnostic accuracy of stool examination to detect the intestinal parasites.

Keyword- Intestinal Parasite, Concentration techniques, Simple salt floatation, Zinc sulphate, Formal-ether floatation

Introduction

Parasitic infections are a major public health problem worldwide, particularly in the developing countries [1]. The prevalence of the intestinal parasitic infections varies from one region to another and it also depends largely on the diagnostic methods which are employed and the number of stool examinations which are done. In India, malnutrition, unhygienic conditions, the improper disposal of sewage and the non-availability of potable water supplies in the rural and the urban areas are responsible for the high rate of intestinal parasitic infections [2]. Globally, as many as 500 million people may harbour *Entamoeba histolytica* and several thousands die each year as a consequence of fulminating colitis or amoebic liver abscesses [3]. The number of people who are affected by *Giardia lamblia*, whipworm, roundworm and hookworm in the developing world has been estimated to be 200, 500, 700 and 800 million respectively [4].

Due to low density of parasites in the faeces, direct wet mount method can miss parasites (ova, cysts and larvae) and the detection can be enhanced through using concentration techniques. The present study was conducted with an aim to determine the prevalence of intestinal parasitic infection in a tribal tertiary care hospital and to compare the stool concentration techniques for detection and identification of intestinal parasites.[5]

Material and Methods

Stool Sample collection

A total of 154 consecutive stool sample received during January 2018 to June 2018 were included in the study. All stool samples were processed by direct wet mount, salt floatation method and formalin-ether concentration method and the results were compared and analyzed.

In The Laboratory The specimen was subjected to **(1) Macroscopic examination** was done and the color, consistency nature presence of mucus and blood were noted and also observation was made for presence of adult helminthic worms with the help of hand lens. **(2) Microscopic examination** was done using direct preparation of (saline and iodine wet mount) for detection of trophozoites and cysts of protozoa and ova of helminths. Modified Ziehl Neelson technique was done after methanol fixation for the specimens suspected to have been taken from HIV Patients for detection of coccidian parasites like cryptosporidium and isospora.

Concentration Techniques Performed

(1). Brine concentration flotation technique : saturated solution of NaCl (brine solution) is prepared and a small amount of faeces is mixed with 2ml of brine solution in a bijou bottle. More brine solutions added till the brim of bijou bottle while stirring. Drops of brim solution are added to the surface of bottle without over spilling. A clean glass slide is placed over the solution surface and left for 30 minutes exactly. The slide is lifted in single hand motion and examined under Microscope.

(2). Zinc sulphate centrifugal floatation technique : 1g of the stool specimen was emulsified in 10 parts of tap water and it was strained through a wire gauze. The filtrate was collected in a Wassermann tube and centrifuged at 2500 rpm. The supernatant was discarded and sediment was re- suspended in water. This step was repeated till the supernatant became clear. To the sediment, 3 to 4 ml of 33% Zinc Sulphate solution was added, it was mixed well and it was filled with ZnSO₄ solution, up to about half an inch of the rim. Several loop full of the supernatant fluid was removed with a bacterial logical loop and they were observed for parasites.

(3). Formol – ether concentration sedimentation technique (Allen and Ridely modification) 1g of stool was emulsified in 7ml of 10% formol saline and it was kept for 10 min for fixation. It was then strained through a wire gauze. The filtrate was added to 3 ml of ether and it was centrifuged at 3000 rpm for 60 seconds and allowed to settle. The supernatant was removed and a wet mount was made of the deposit to look for parasites.

(4). Merthiolate –iodine formalin concentration (MIFC) method : The following solution were prepared weekly and stored in amber coloured bottles ready for use. Solution ‘A’ consisted of tincture metrhiolate 200 ml, distilled water 200 ml, 40% formaldehyde 25 ml and glycerine 5

ml. Solution 'B' consisted of iodine crystals 5 gm, potassium iodide 10 gm and distilled water 100 ml. For each stool specimen, two tubes are prepared - one containing 9.4 ml of solution 'A' and other 0.6 ml of solution 'B', the contents were mixed together immediately before adding to approximately 1 gm of faeces, after the stool specimen emulsified thoroughly. The tube was stoppered and allowed to stand overnight. The contents are mixed again and filtered through surgical gauze the next morning. Ethyl ether is added and tube is shaken vigorously. After keeping the tube for 1 min, centrifugation is carried out at 1800 rpm for 2 min. Four zones are formed with this technique, the faecal plug is separated and the upper three zones decanted. The sediment is thoroughly mixed and a drop was placed on a slide covered with a coverslip, and examined.

Result- The prevalence rate of the study was (31) 20.1%. Males were having 64.5% of intestinal parasitic infection and females were having 35%. Middle age group (20-30) year had the highest prevalence of the parasitic infection. The isolates were *Taenia* species (35%), *A. lumbricoides* (32%), Hook worm (16%), *H. nana* (12%) and *T. trichuria*(3%). The evaluation of intestinal parasite by different methods showed with formal-ether concentration (83%), zinc sulphate centrifugal floatation(70%), simple salt floatation (61%) and wet mount (48%) preparation respectively.

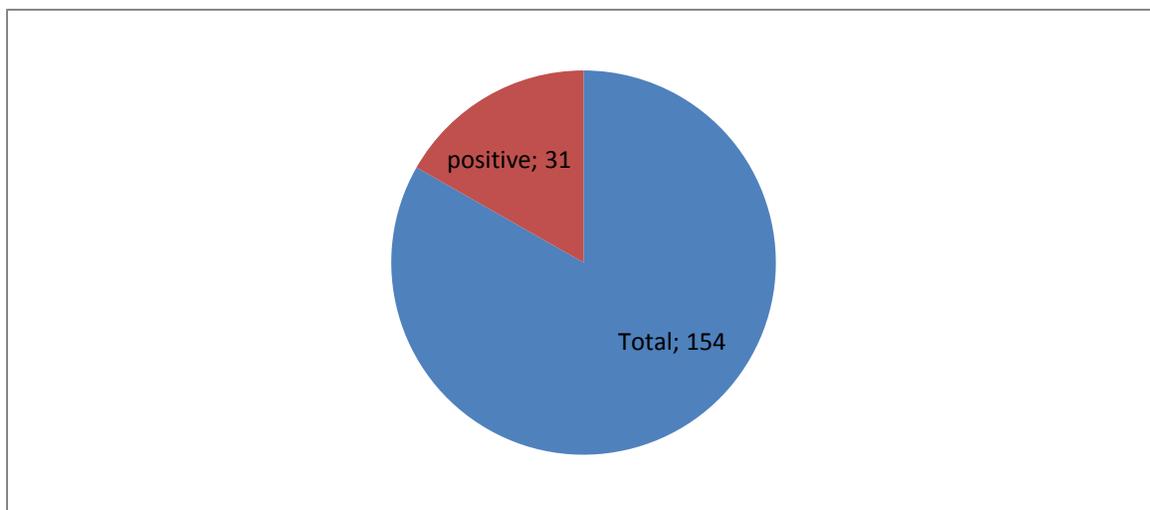


Fig 1 :- Total no. of sample n=154

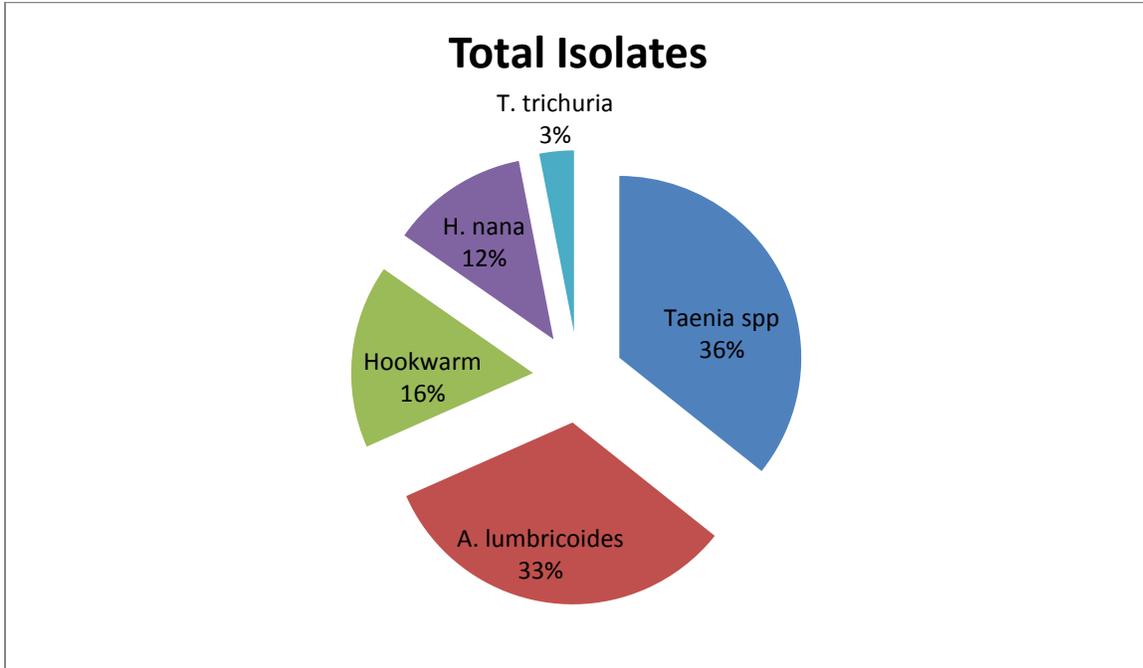


Fig 2:- Total no of isolates

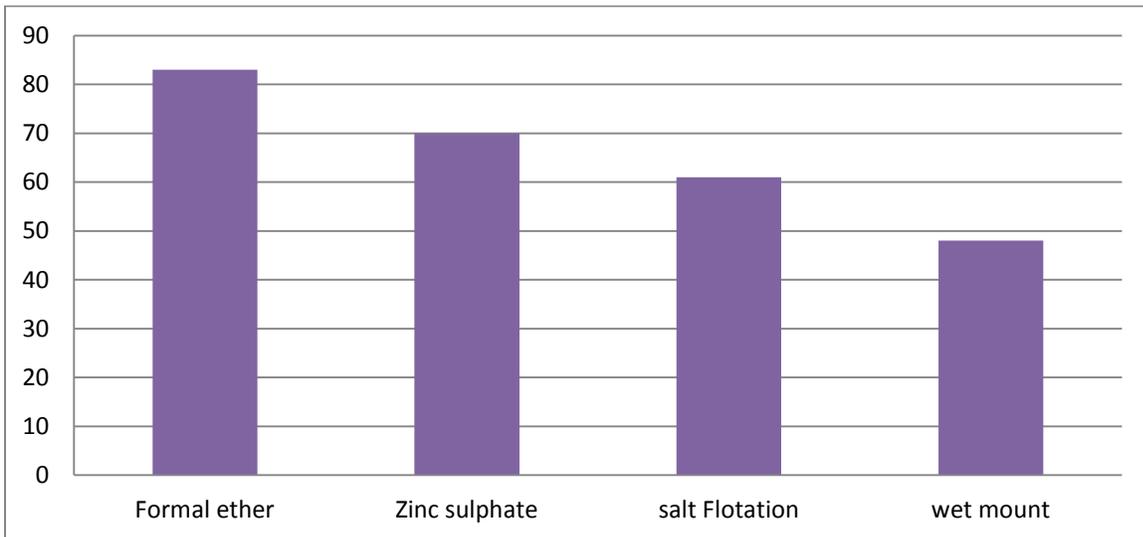


Fig 3:- Total no. of technique use to isolate in parasite

Discussion

Parasitic infestations are the major causes of morbidity and mortality in developing countries like India. The data on their prevalence and the sensitivity of various diagnostic methods help the clinicians and the microbiologists in the diagnosis and the management of the patients. Various studies have shown different prevalence rates of the parasitic infestations in different parts of India. But most of the studies had less sample sizes. In this study, 154 samples were included, both from the rural and the urban areas to know the prevalence of the disease.

The prevalence rate in this study was 20.1%. This study is in accordance with study by sujatha et al (15.5%)[8] Various studies from rural and urban regions have shown different prevalence rates ranging from 5.56% to 46.7% [6,7]. This varied differences could be due to seasonal variations, geographical areas, health education and awareness, sanitation measures and socioeconomic conditions of the

different communities. The prevalence of parasitic infestations in this study was more common in males (64.5%) as compared to that in females (35%) similar to study by pareshwarappa et al and in contrast to Marothi Y et al., [10] showed that the infestations had a female preponderance, difference between male and female patients in these study, may be due to equal involvement of females in outdoor activities as compared to males nowadays. In various studies, the most common age group involved was 6-20 years as compared to this study, where it is 20-30 years. In a previous study also,

the trend of parasitic infection in recent years has shown to predominate among adults as compared to children [11-14]. The most common parasites identified in this study were *Taenia*(35%), *A.lumbricoides*(32%), *Ancylostoma duodenale*(16%), *H.nana*(16%) and *T.trichura*(3%) species are similar to other studies[11] other studies showed prevalence of 9.8% and 13.4% by Kumar et. al. and Choubisa et al[14,15], as it is an important public health problem, data on the prevalence of parasites in a given region, is necessary since variations in the parasite prevalence may be accounted to the difference in the geographic conditions and cultural practices and food habits in different regions, which future strengthens the need for such type of studies.

This study shows Formal ether concentration technique(83%), has a better sensitivity compared to Zinc sulphate floatation technique(70%), Simple salt floatation(61%), wet mount

(48%), which is in contrast to study by Hersh ahmed et al [16] where Zinc sulphate floatation technique(49.3%), followed by Formal ether concentration technique(43.3%), an wet mount (22.6%). This may be due to Flotation technique permits the separation of protozoan cysts and certain helminthes eggs from excess debris through the use of a liquid with a high specific gravity, where the parasitic elements are recovered from the surface film and the debris remains in the bottom of the tube. This technique yields a cleaner preparation and morphology of the parasites than the sedimentation procedure. A study by (Parameshwarappa *et al.*[9]) showed that the formol-ether concentration technique was more sensitive as compared to the other methods and found that 64.5% of the cases were detected by the formol-ether method and 55% by Zinc sulphate floatation. This may be due to that Sedimentation procedure leads to the recovery of all protozoa, eggs and larvae present. However, the preparation contains more debris than the flotation procedure, though Ethyl acetate is used as an extractor of debris and fat from the faeces and leaves the parasites at the bottom of the suspension. Concentration by formalin-ethyl acetate sedimentation is recommended because it is the easiest to perform, allows recovery of the broadest range of organisms and is the least subject to technical error. In our study the direct wet mount showed the lowest sensitivity from the concentration methods, but the advantage of this method is to provide a quick diagnosis of a heavily infected specimen, to check organism motility and to diagnose parasites that may be lost in concentration techniques [16,17]

Conclusion- In this study, formal-ether concentration technique has showed a high sensitivity and specificity. Its implementation along with routine method, will improve the diagnostic accuracy of stool examination to detect the intestinal parasites.

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