ORIGINAL RESEARCH

Comparison of concentration technique with direct microscopic examination for the detection of ova and cyst in stool sample

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ABSTRACT

Introduction: Intestinal parasitic infections pose a significant health challenge globally, particularly in developing countries like India. Neglected tropical diseases, including parasitic infections, account for a substantial health burden. The prevalence of these infections varies regionally, with factors such as poor sanitation, hygiene practices, and immunological status influencing their spread. Protozoa and helminths are the primary agents causing these infections. Children in regions with inadequate sanitation are especially vulnerable, with potential implications for growth, anemia, and cognitive function. Various diagnostic methods are available, with direct wet mounts being common but limited by low sensitivity, particularly in cases of low parasite density. **Materials and Methods:** A prospective study was conducted on 100 stool samples over a 6-month period. Stool samples were processed using direct microscopy, including saline and iodine wet mounts, alongside concentration techniques (salt floatation and zinc sulphate floatation). The samples were analyzed for the presence of parasites, and demographic data were recorded. **Results:** Out of 100 samples, 20% tested positive for parasitic infection, with a higher prevalence in males (60%) compared to females (40%). The rural population showed a higher prevalence (70%) than the urban population (30%). Giardia lamblia cyst was the most common parasite (40%), followed by Entamoeba histolytica cyst (30%). The concentration techniques significantly increased the positivity rate compared to direct microscopy alone. **Discussion:** The study highlights the importance of concentration techniques in enhancing the sensitivity of parasite detection, especially in low-density infections. Factors such as gender, age group, and rural/urban residence influence the prevalence of parasitic infections. The zinc sulphate floatation technique demonstrated higher sensitivity compared to the saturated salt floatation method and direct microscopy. **Conclusion:** Parasitic infections remain a public health concern, particularly in developing nations. The study emphasizes the need for improved diagnostic methods, advocating for the inclusion of concentration techniques in routine stool examinations to enhance sensitivity. The findings underscore the regional variations and demographic factors influencing parasitic infection prevalence. Addressing these concerns requires a comprehensive approach, incorporating health education, sanitation measures, and targeted interventions.

INTRODUCTION

Human intestinal parasitic infections are the most common health problem in the world, mainly in developing countries like India [1]. Parasites are organisms that thrive on another organism known as the host for their nutritional sources. In 2010, according to World Health Organization (WHO) reports parasitic infection diseases are the major neglected tropical diseases worldwide [2]. The burden of intestinal infections requires careful monitoring in developing countries. Several studies have been conducted in different parts of India. In India, the overall spread rate is from 12.5% to 66%. The frequency of individual parasites varies from region to region. These diseases are on the rise and similar studies will add to the existing knowledge of bacterial infections in patients with gastrointestinal disorders[3].The risk factors for these infections in the population are an Increase in tourism and worldwide migration, zoonotic diseases, poor basic sanitation, unhygienic habits, the immunological status of the population, and life cycle along with the adaptation of the parasites to their hosts in adverse environmental conditions[4]. Recent studies suggest that intestinal parasitic infections may have an adverse effect on growth, iron deficiency anaemia, and cognitive

function, practically for children of school age[5,19,20,21]. Based on the agent causing these, infection parasites are divided into two broad groups protozoa (amebae flagellates, ciliates, and coccidia) and helminths (nematode, cestode, and trematode).Entamoeba histolytica / E. dispar, Giardia spp., And Cryptosporidium spp. are among the most common pests reported in kindergarten and school children in developing countries with poor sanitation and hygiene. These protozoa cause high morbidity in dehydrated patients[2]. For the detection of intestinal parasites, many methods are available but various factors such as its effectiveness, level of knowledge in identification, its affordability and ease to carry out are considered for the choice of a particular technique[6]. Because of its simplicity and affordability, direct wet mounts, iodine wet mount and lactophenol cotton blue wet mount are the gold standard diagnostic methods for parasite identification. However, if the parasite density in the feces is low, direct smear methods are not an ideal choice for parasite detection. So in the faecal sample, the detection of the parasites is enhanced by the concentration procedure[7]. In addition to the use of concentration, techniques can detect parasites that are present in small numbers which can be missed easily by using direct wet mounts[3].

MATERIAL AND METHODS

The prospective study was carried out on 100 stool samples received in the Department of Microbiology MMIMSR, Mullana (Haryana) for a period of 6 months (August 2021 to January 2022). All Stool samples received for detection of ova and cyst were included in the study.The stool samples which were contaminated with the patient's urine were rejected.
The study was started after takin Ethical Clearance from Institutional ethical committee.

METHODOLOGY

The patients were provided wide mouthed clean, dry, properly labelled plastic containers for collection of samples. The stool samples were examined within 1-2 hours of collection. Both the formed and the unformed stools were examined freshly. After the macroscopic examination of the stool including colour, consistency, presence of blood and mucus were noted. The stool specimens were examined for the presence of worms like segments of Taenia, adult Hookworm, roundworm either with the naked eye or with the aid of a hand lens.[4] Each stool specimen was processed by direct microscopy including Saline wet mount, Iodine wet mount, and concentration technique using simple saturated salt floatation method and zinc sulphate floatation method.

RESULT

A total of 100 stool samples were examined.
From these 100 samples, 20 samples were positive for parasitic infection showing 20% positivity rate as shown in table number 1.

TABLE 1: Rate of positivity of parasite infection

Total Samples	Positivity	Percentage
100	20	20%

The prevalence rate was higher in males 12(60%) as compared to that of females 8(40%) as shown table number 2.

Table 2: Gender Wise Prevalence Of Parasitic Infection

Total Male Patients	Positivity (%)	Total Female Patients	Positivity (%)
58	12(20.68%)	42	08(19.04%)

Out of 100 samples, a maximum of 32 samples were received from the age group between 31-45 years, and in this group, 15 samples were from male patients and 17 were from female patients followed by 29 samples, which were from the age group 15-30 years and out of these 29 samples 22 samples were from males patients and 07 were from the females' patients followed by 32 samples which were from the age group of 46-60 years and above 60 years, followed by 7 samples which were from the age of 0-5 years and 6- 14 years and out of these 7 samples 3 samples received from male patients and remaining 4 samples were from the female patients as shown in table number 3.

Table 3: Gender Wise Distribution of Patients Among Different Age Groups

Age Group	Male	Female
0-5	02	01
6-14	01	03
15-30	22	07
31-45	15	17
46-60	11	06
>60	07	08
Total	58	42

The positivity rate was higher in the rural area 14(70%) as compared to that of urban 6(30%) as shown in table number 4

Table 4: Area Wise Prevalence Of Parasitic Infection

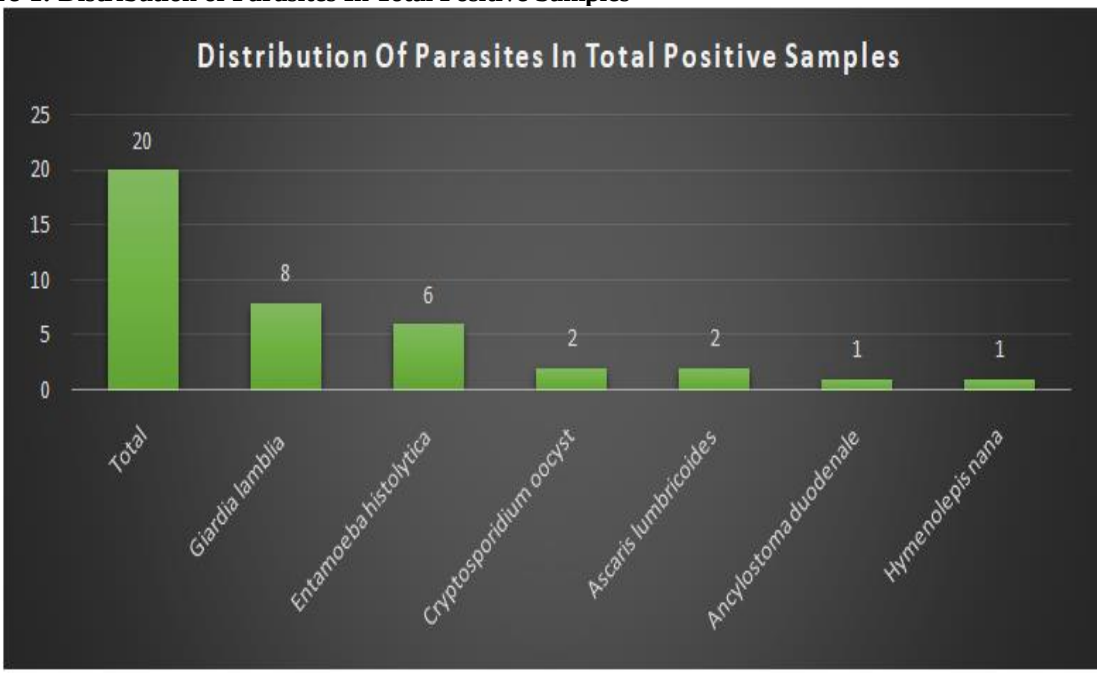
Total Rural	Positivity (%)	Total Urban	Positivity (%)
67	14(20.89%)	33	06(18.18%)

Giardia lamblia cyst was the most prevalent intestinal parasite showing 40% positivity followed by 30% Entamoeba histolytica cyst followed by 10% Cryptosporidium oocyst and Ascaris lumbricoides egg and followed by Ancylostoma duodenale and Hymenolepis nana showed 5% positivity as shown in table number 4.

Table 5: Distribution Of Parasites In Total Positive Samples.

Parasite	Number (%)
Giardia lamblia Cyst	08(40%)
Entamoeba histolytica Cyst	06(30%)
Cryptosporidium oocyst	20(10%)
Ascaris lumbricoides Eggs (Fertilized and Unfertilized)	20(10%)
Ancylostoma duodenale Egg	01(5%)
Hymenolepis nana Egg	01(5%)
Total	20(100%)

Figure-1: Distribution of Parasites In Total Positive Samples



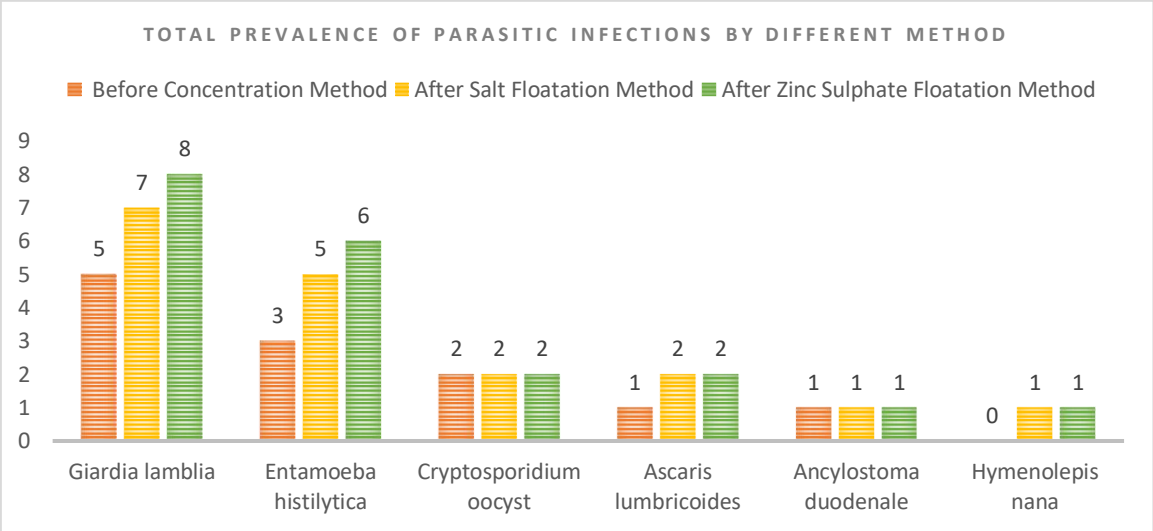
The identification of various parasites before the concentration method and after the concentration methods. Giardia lamblia was the most common parasite identified and maximum positivity was seen with both concentration techniques. Giardia lamblia, Ascaris lumbricoides, and Hymenolepis nana showed differences in the identification by different methods. Hymenolepis nana egg was identified by concentration methods not identified before the concentration method as shown in table number 6

Table 6: Total Prevalence of Parasitic Infections By Different Method

Parasite	Before Concentration Method		After Salt Floatation Method		After Zinc Sulphate Floatation Method	
	Saline Wet Mount	Iodine Wet Mount	Saline Wet Mount	Iodine Wet Mount	Saline Wet Mount	Iodine Wet Mount
Giardia Lamblia Cyst	05	05	07	07	08	08(40%)
Entamoeba Histolytica Cyst	03	03	05	05	06	06(30%)
Cryptosporidium Oocyst	02	02	02	02	02	02(10%)
Ascaris Lumbricoides Eggs (Fertilized and Unfertilized)	01	01	02	02	02	02(10%)
Ancylostoma Duodenale Egg	01	01	01	01	01	01(05%)

Hymenolepis nana Egg	00	00	01	01	01	01(05%)
Total	12	12	18	18	20	20

Figure 2

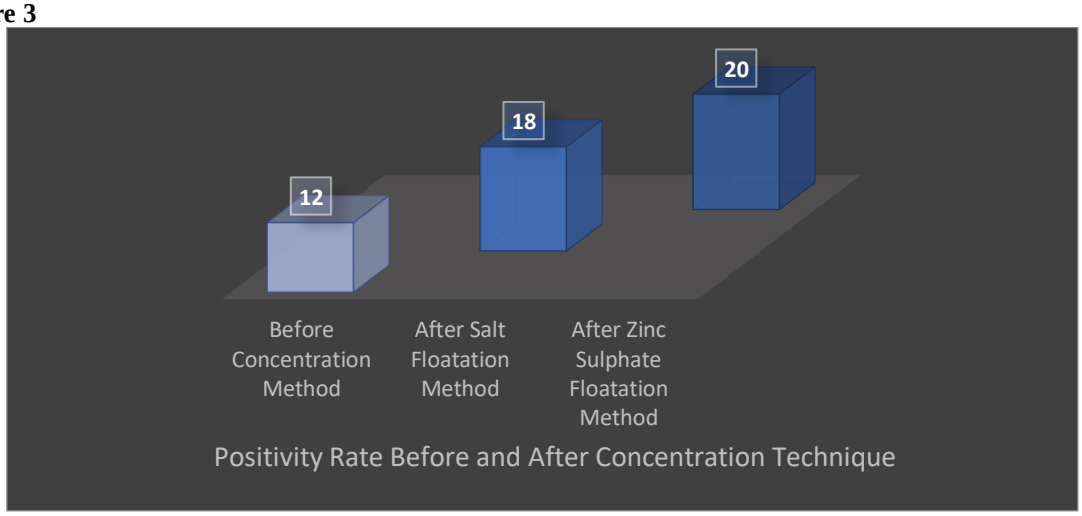


Out of 100 samples 12 samples were positive before concentration technique and 18 samples were positive after salt floatation. Technique and 20 samples were positive with zinc sulphate floatation technique as shown in table number 7

Table 7: Positivity Rate Before and After Concentration Technique

Total Sample	Positivity Before Concentration technique (%)	Positivity After Concentration technique	Concentration Techniques used	
			Positivity after Salt Floatation technique	Positivity After Zinc Sulphate Floatation Technique
100	12(12%)	20(20%)	18(18%)	20(20%)

Figure 3



DISCUSSION

Intestinal parasitic infections are common in the general population of underdeveloped nations, and they can cause a variety of harmful implications such as anaemia, stunted physical growth, mental retardation, abdominal colic, cholestasis, cholecystitis, and pancreatitis [8].

The most prevalent and widespread chronic human infections worldwide are intestinal infections. The most prevalent intestinal protozoan parasites are Giardia and Entamoeba histolytica[9] These intestinal protozoan parasites cause giardiasis and amoebiasis, which are both linked with diarrhoea[7].

The diagnosis of parasitic infection in humans is difficult, and it needs the ability to detect and distinguish parasites from one another. Concentration techniques are used to detect organisms that are present in extremely minute quantities and may be undetected using a direct wet mount[7]. The wet mount which is the routine diagnostic method for the

identification of parasites lacks sensitivity. For the identification of intestinal parasites in the stool, concentration methods should be used to increase the sensitivity[10]. In the present study the lactophenol cotton blue wet mount was included which help demonstrate the morphology of eggs and cysts of parasites and along this comparison is made between the different wet mounts before the salt floatation method and after the salt floatation method and was seen that addition of simple salt floatation method help to increase the sensitivity. Helminth eggs and larva, Entamoeba histolytica cysts of Giardia lamblia are the organisms that are generally identified using concentration technique[11].

Studies from different parts of India and outside India have reported a parasite prevalence rate of 25% to 70 %. [45] Sanitation measures, seasonal variations, geographical areas, health education awareness and socioeconomic conditions of the different communities could be the reason for these differences[12,9].

In the present study, the Positivity rate was 20 (20%). This is in accordance showing in the study done by Nitasha Kumari, Ritu Garg, et al, in 2019 examined 100 stool samples out of which 47(47%) samples were positive for intestinal parasite [13] and also related study done by Hamad and Ramz et al, 2012 positivity was (30%)[14]. Such differences could be due to health education, seasonal variation, geographical areas, sanitation measure, awareness, and socioeconomic conditions of different communities.

In the present study, of the 100 samples, 58 samples were from male patients and 42 were from females showing positivity of 20.68% & 19.04% respectively. This is in accordance with the study done by Parameshwarappa KD et al observed infection was higher in males at 33.29% as compared to females at 21.29% [15]. The reason behind this more prevalence in males maybe, in addition to farm workmen are more exposed to contaminated soil and water and due to handling of livestock and fieldwork than women. A similar study was done by Jonathan Wrights et al, which examined 36 stool samples out of which 26 were positive and the prevalence of parasitic infection was more common in males (52.8%) as compared to that in females (47.2%) [16].

In the present study of parasitic infection out of a total of 100 samples, 67 samples were received from the rural area and 33 samples were from the urban area, and from these 67 samples total of 14(20.58%) samples were positive for a parasitic infection and out of 33 samples from the urban area 06(18.75%) (Table 5) were positive showing a high rate of positive in the rural area as compared to the urban area. This finding was compared with the findings of Parameshwarappa KD et al, showing positivity in rural areas (28%) and in urban areas (26.4%). This is because maybe in rural areas people are don't aware of health education, poor sanitation, and lack of proper hygiene compare to people who live in developed areas [15].

In the present study, of the 100 stool samples, maximum positivity shown in the age group of 6-14 years (75%) followed by age group above 60 and 1-5 years showing positivity 40% and 33.33% respectively while age group of 46-60 years showed positivity of 29.41% and least positivity was shown in the age group of 15-30 years and 31-45 years (10.34% and 06.25%). A similar study was done by Showkat Ahmad Wani et al, which observed, 75.28% of stool samples were positive for the parasitic infection in children[12]. and another same study was done by Allavarapu Ramya Sree et al 41.18 % positivity[17]. Factors for higher positivity of parasitic infection in these groups may be due to lack of personal hygiene, awareness, and education [16].

In the present study, 12(12%) samples were positive by direct wet mount without applying any concentration technique. And after applying concentration techniques positivity rate was 20% i.e after applying the salt floatation technique 18(18%) samples were positive and zinc sulphate floatation technique showed 20(20%) positivity for parasitic infection. This is in accordance with the study done by Hersh Ahmad Amin et al, the results showed that the Zinc sulphate floatation technique was the highest sensitivity rate 148(49%), followed by without applying concentration technique which showed the lowest rate 68(22.6%) [14]. Also compare with the study of Dr Shailendra Singh Thakur et al, the study was found that 50.80% of the cases were detected by the zinc sulphate centrifugal technique and 45.95% cases were detected by salt floatation technique and 40.77% cases were detected without concentration technique applying[18]. and another similar study done by Parameshwarappa KD et al, in this study zinc sulphate centrifugal floatation technique with 55% more sensitive technique compare to saturated salt (42%) and direct microscopy (38%) [15].

This study showed that the zinc sulphate floatation technique is better than the saturated salt floatation technique and direct microscopy wet mount (like iodine and normal saline). The reason for more positivity after the zinc sulphate floatation technique is because of the low sensitivity of the saturated salt technique and direct microscopy. Zinc sulphate floatation technique is helpful when a number of parasites is low in stool samples and these can be missed by direct microscopic examination.

In the present study out of positive samples, Giardia lamblia cyst has maximum positivity of 40%. out of 100 samples total of 20 samples were positive for parasitic infection. 8 samples were positive for Giardia lamblia cyst followed by Entamoeba histolytica cyst 06(30%) and cryptosporidium oocyst and Ascaris lumbricoides eggs (10%) and the least positive was for Ancylostoma egg and Hymenolepis nana eggs (05%). This study compared with a study done by Rajender Singh et al, In his study, Giardia lamblia was found the most common intestinal parasite in 20 number of stool 37.74 % [10]. and another

study done by Rajvir Singh et al, the parasites detected were Giardia lamblia (58.5%) maximum positivity and followed by Entamoeba histolytica (32%)[8]. The wide variation in the magnitude of intestinal parasites may be due to variations in factors like sanitation, quality of drinking water supply, and various climatic factors. In our study, Giardia and Entamoeba were the common isolates, which imitate the habit of defecation in open places and insufficiency of knowledge of sanitation among people [10].

The present study shows the identification of various parasites by different techniques before & after the concentration techniques,Giardia lamblia was the most common parasite identified with both the concentration techniques i.e. zinc sulphate floatation technique & salt floatation technique followed by Entamoeba histolytica, Cryptosporidium oocyst, Ascaris lumbricoides egg Ancylostoma Duodenale, Hymenolepis nana egg. A similar study done by Bhavna Kumari et al, showed off the total of 16 Giardia lamblia cyst 6 were identified without applying the concentration technique and 10 more were identified after applying the zinc sulphate method followed by a total of 6 Entamoeba histolytica cyst 2 were identified without applying the concentration technique and 4 more were identified after applying zinc sulphate [12].

CONCLUSION

The prevalence of parasitic infections remains high because of malnutrition, unhygienic conditions, the improper disposal of sewage, and the non-availability of potable water supplies in the rural areas. The high prevalence of parasitic infections is the cause of concern in developing countries like India. So, to deal with this public health problem, clinical microbiology laboratories should give importance to the problem of concern by using different methods of detection of ova/cyst. The conventional methods which are used for the recovery of parasites from the stool include direct saline wet mount and iodine mount. The conventional methods lack sensitivity to detect parasites in a stool sample. The detection of parasites in faecal specimens is improved by the use of the concentration technique of stool samples.

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Authors Contribution:

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Sample Collection & Methodology: Mr. Veerendra Sen

Analysis of Data: Dr. Ritu Garg &Mr. Veerendra Sen

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Writing- Review & editing- Dr. Rajdeep Paul& Dr. Saurabh G Agarwal

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