

Spectrum of Enteric Parasitic Infections in Immunocompromised and Immunocompetent Patients in a Tertiary Care Hospital, New Delhi- A Retrospective Study

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ABSTRACT

Introduction: Parasitic intestinal diseases caused by protozoans and helminths are important causes of gastrointestinal disturbances which are responsible for a high burden of morbidity and mortality as they are detrimental not only to paediatric and immunocompromised patients but also to adults and immunocompetent patients. Hence, it is important to know their burden in a geographical area to develop adequate control measures.

Aim: To estimate the prevalence of various intestinal parasitic infections in symptomatic patients based on age, gender and immune status in a tertiary care hospital, New Delhi, India.

Materials and Methods: A retrospective analysis of 1431 stool samples which were received in the Department of Microbiology VMMC and Safdarjung Hospital, New Delhi, from April 2019 to March 2020 was done. All samples were subjected to macroscopic and microscopic examinations and underwent, wet mount (saline and iodine) examination and Modified Ziehl-Neelson staining. Data was analysed using Statistical Package

for Social Sciences (SPSS) software version 21.0 with two-tailed Chi-square test wherever applicable.

Results: Males (61.36%) outnumbered females. Prevalence of parasites was more in adults (7.76%) than in children. Higher positivity (7.6%) was observed in immunocompetent individuals as compared to immunocompromised. Overall predominance of protozoans (98.26%) over helminths was noted. *Entamoeba histolytica/dispar* group (43.60%) was found to be the most prevalent parasite. *Entamoeba histolytica/dispar* group (5.64%) and *Cryptosporidium* spp. (5.94%) were most common amongst immunocompetent and immunocompromised patients respectively.

Conclusion: A higher prevalence of intestinal parasitic infections in immunocompetent symptomatic patients was observed, requiring attention of clinicians as they are no longer limited majorly to immunocompromised patients. As no effective vaccine is available for these infections, so appropriate laboratory methods, microbiological expertise, proper sanitation measures, availability of potable water supply and properly cooked food are essential to control parasitic intestinal diseases.

Keywords: Helminths, Parasitic intestinal diseases, Protozoan parasites

INTRODUCTION

Parasitic intestinal diseases are important causes of diarrhoea which is a serious health problem worldwide carrying a high burden of morbidity and mortality [1]. About 25% of the world population are infected with intestinal parasites, with high prevalence in developing countries [2]. In India, the overall prevalence rate varies from 11% to 90% [3]. Enteric parasitic infections is a major health challenge being faced by developing countries due to various risk factors like poverty, malnutrition, poor personal and community hygiene, high population density, unavailability of potable drinking water, consumption of contaminated food, poor sanitary facilities, hot and humid tropical climate and exposure to animal excreta [3,4]. Now-a-days enteric parasitic infections are on the rise in developed nations also which could be due to increased immigration and immunocompromised patients [3].

Intestinal parasitic infections can be caused by both protozoans and helminths. The most common protozoans causing the disease are *Entamoeba histolytica/dispar* group, *Giardia* spp. and *Cryptosporidium* spp. Other less common protozoans are *Blastocystis* spp., *Balantidium coli*, *Cyclospora cayetanensis*, *Cystoisospora belli* and *Microsporidia* spp. The most common helminths observed are *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Necator americanus* and *Trichuris trichura* while *Enterobius vermicularis*, *Hymenolepis nana* and *Strongyloides stercoralis* are less commonly reported helminths [2,3].

Intestinal parasitic infections are more common in children due to their habits related to playing with soil and also low immunity [4]. There is good evidence that repeated enteric infections and diarrhoea can lead to malnutrition which is detrimental to the child's growth and development leading to nutritional deficiency, anaemia, growth retardation and impaired learning ability [5].

In recent years, the enteric pathogens have been increasingly reported in immunocompromised individuals like Human Immunodeficiency Virus (HIV) infected/ Acquired Immunodeficiency Syndrome (AIDS), malignancy, organ transplantation, patients on corticosteroid treatment, patients on radiotherapy and chemotherapy. The coccidian parasites, *Cryptosporidium* spp., *Cytoisospora belli* and *Cyclospora* spp. are mostly observed in these patients and can cause severe and lethal diarrhoea. On the other hand, these parasites cause a mild and self-limiting gastrointestinal infection in immunocompetent individuals. The coccidian parasites are often overlooked due to the requirement of microbiological and technical expertise for detection. Hence, their prevalence rates are underestimated in immunocompromised as well as in immunocompetent age group [6].

Due to the availability of various rapid antigen tests and lateral flow assays along with the examination of stool for ova, parasites and cyst under the microscope with special staining methods, the accurate detection of parasitic pathogen can be done even at Primary Health Centre level (PHC). Hence, the need of the hour is that enough information about the epidemiology and prevalence of gastrointestinal parasitic infections in a particular geographical area to know their

burden in order to develop adequate control measures along with an accurate laboratory diagnosis should be available. Keeping these facts in mind, the present study was aimed to estimate the prevalence of various intestinal parasitic infections in the symptomatic patients based on their age, gender and immune status.

MATERIALS AND METHODS

This retrospective study was conducted in Department of Microbiology, VMMC and Safdarjung Hospital, New Delhi. A total of 1431 stool samples were received in the Department of Microbiology from April 2019 to March 2020 from both Outpatient Department (OPD) and Inpatient Department (IPD) of various specialties were tested and data was obtained from the laboratory registers as well as from the laboratory computer (in the form of soft copy). The study protocol was reviewed by scientific advisory committee and approved by Institutional Ethical Committee (IEC/VMMC/SJH/PROJECT/2019-11/66). Convenient sampling method was used wherein all the stool samples from symptomatic patients received in the Department of Microbiology during above mentioned duration were included.

Inclusion criteria: The study included symptomatic patients suffering from any of the mentioned symptoms in the Test Requisition Forms (TRF)- diarrhoea, constipation, abdominal pain, fever, vomiting and abdominal distention belonging to both adult and paediatric age group.

The immunocompromised and immunocompetent patients were segregated on the basis of the ward/clinic from where the sample was received and diagnosis mentioned in TRF. Hence, the samples received from Anti-Retroviral Therapy (ART) centre, leukaemia patients from haematology, critically ill patients from Intensive Care Unit (ICU), cancer patients from oncology wards, kidney failure patients from haemodialysis unit and cancer patients on radiotherapy were classified under immunocompromised category while the samples received from general outpatient departments and general wards of various specialties like medicine, paediatrics, gynaecology, orthopaedics, endocrinology, surgery, gastroenterology with diagnosis other than those mentioned above were classified as immunocompetent.

Exclusion criteria: Patients on any antibiotic or anti-parasitic agents and asymptomatic patients (For e.g., routine examination of kitchen staff) were excluded from the study. Second sample received from the same patient was also excluded from the study.

Study Procedure

The demographic data of each case which included age, sex, outpatient or inpatient, along with stool examination findings were recorded.

a) Collection and processing of the specimen: Stool samples were collected in wide mouth, leak proof, screw capped sterile universal containers and transported to the laboratory within two hours of collection. All positive samples were immediately confirmed by the two laboratory supervisors. To ensure the quality, 10% of negative samples were also cross-checked.

b) Macroscopic examination: The colour and consistency of the stool samples, presence or absence of mucus, blood, adult worms, and body segments of the parasites were recorded.

c) Microscopic examination: Stool samples were further examined microscopically, in a wet mount preparation of normal saline and Lugol's iodine for the presence of ova, larvae, cysts, and trophozoites of various parasites as well as for other intestinal worms. The wet mounts were scanned under low power first (10X) then doubtful structures were confirmed under high power (40X) magnification. A smear of the stool sample was also prepared and air dried for one minute followed by fixation with methanol. Modified Ziehl Neelson staining was performed for all the samples and observed under oil immersion (100X) to look for the presence of coccidian parasites [7]. The stained slides, positive for *Cryptosporidium* spp. were compared and confirmed with the positive control slides.

STATISTICAL ANALYSIS

The data was analysed using SPSS software version 21.0. Two tailed Chi-square test was applied wherever applicable. The p-value <0.05 was considered to be significant.

RESULTS

A total of 1431 samples were obtained from patients attending the hospital inclusive of both OPD and IPD. Adults (70.93%) predominated children. Out of 416 samples obtained from children, 61 (4.26%) were positive for enteric parasites, while 111 (7.76%) samples were positive amongst the total 1015 samples received from adult patients. There was significant association between age and parasitic infections ($p < 0.0489$) [Table/Fig-1].

Age group (in years)	Positive samples n (%)	Negative samples n (%)	Total N (%)	p-value
<18	61 (4.26%)	355 (24.81%)	416 (29.1%)	<0.0489
≥18	111 (7.76%)	904 (63.17%)	1015 (70.9%)	
Total	172 (12.02%)	1259 (87.98%)	1431 (100%)	

[Table/Fig-1]: Distribution of positive and negative samples on the basis of age group (N=1431).
The p-value <0.05 was considered to be significant

Overall samples received from males (61.36%) outnumbered the samples received from female (38.64%) patients, with higher positivity being reported in the samples from the males. However, there was no significant association between gender and positivity of parasitic infections (p -value=0.852) [Table/Fig-2].

Gender	Positive samples n (%)	Negative samples n (%)	Total n (%)	p-value
Male	100 (7%)	778 (54.36%)	878 (61.36%)	0.852
Female	72 (5.03%)	481 (33.61%)	553 (38.64%)	
Total	172 (12.03%)	1259 (87.97%)	1431 (100%)	

[Table/Fig-2]: Distribution of positive and negative samples on the basis of gender (N=1431).
The p-value <0.05 was considered to be significant

On comparing to enteric pathogen prevalence amongst adults and children, it was observed that majority of samples from children were received from IPD settings (19.01%) while samples from adults mainly belonged to OPD settings as shown in [Table/Fig-3].

Location	<18 years n (%)	≥18 years n (%)	Total N (%)
OPD	144 (10.06%)	679 (47.45%)	823 (57.51%)
IPD	272 (19.01%)	336 (23.48%)	608 (42.49%)
Total	416 (29.07%)	1015 (70.93%)	1431 (100%)

[Table/Fig-3]: Distribution of patients attending OPD and IPD based on age group (N=1431).

Out of 337 immunocompromised patients, 61 (4.26%) were positive for enteric parasites. While amongst 1094 immunocompetent patients, 111 (7.76%) were positive for enteric parasites which was found to be significantly high (p -value <0.000086) [Table/Fig-4].

Immune status	Positive samples n (%)	Negative samples n (%)	Total N (%)	p-value
Immunocompromised	61 (4.26%)	276 (19.29%)	337 (23.55%)	<0.000086
Immunocompetent	111 (7.76%)	983 (68.69%)	1094 (76.45%)	
Total	172 (12.02%)	1259 (87.98%)	1431 (100%)	

[Table/Fig-4]: Distribution of positive and negative samples on the basis of immune status (N=1431).
The p-value <0.05 was considered to be significant

Out of total 1431 samples, 172 (12.02%) were positive for various parasites. *E.histolytica/dispar* group (43.6%) was found to be most prevalent protozoan parasite followed by *Cryptosporidium*

spp. (23.26%), *Giardia* spp. (20.93%), *Cytoisospora* spp. (6.4%). However, *H.nana* (1.16%), *A.lumbricoides* (0.58%) were the only helminths detected [Table/Fig-5].

Parasite	Positive sample (n)	Percentage (%)
<i>E.histolytica/dispar</i> group	75	43.60%
<i>Cryptosporidium</i> spp.	40	23.26%
<i>Giardia</i> spp.	36	20.93%
<i>Cytoisospora</i> spp.	11	6.40%
Co-Infections	7	4.07%
<i>H.nana</i>	2	1.16%
<i>A.lumbricoides</i>	1	0.58%
Total	172	100.00%

[Table/Fig-5]: Distribution of various enteric parasites found in faecal samples (N=172).

The distribution of various enteric parasites amongst males and females has been depicted [Table/Fig-6].

Parasites	Male n (%)	Female n (%)	Total N (%)
<i>E.histolytica/dispar</i> group	56 (32.5)	19 (11.1)	75 (43.6)
<i>Cryptosporidium</i> spp.	28 (16.28)	12 (6.98)	40 (23.26)
<i>Giardia</i> spp.	26 (15.12)	10 (5.81)	36 (20.93)
<i>Cytoisospora</i> spp.	8 (4.7)	3 (1.7)	11 (6.4)
Co-infections	5* (2.91)	2# (1.16)	7 (4.07)
<i>H.nana</i>	2 (1.16)	0	2 (1.16)
<i>A.lumbricoides</i>	1 (0.581)	0	1 (0.58)
Total	100 (73.25)	72 (26.75)	172 (100)

[Table/Fig-6]: Distribution of enteric parasites based on gender (N=172).

**E.histolytica/dispar* group+*Cryptosporidium* spp. (1) and *E.histolytica/dispar* group+*Cryptosporidium* spp. (1) and *E.histolytica/dispar* group+*Giardia* spp.+*H.nana* (1) and *Giardia* spp.+*Cryptosporidium* spp. (2)
#*Giardia* spp.+*Cryptosporidium* spp. (1) and *E.histolytica/dispar* group+*Giardia* spp. (1)

The spectrum of various enteric parasites according to the age group has been shown [Table/Fig-7].

Parasites	<18 years n (%)	≥18 years n (%)	Total N (%)
<i>E.histolytica/dispar</i> group	21 (12.2)	54 (31.4)	75 (43.6)
<i>Cryptosporidium</i> spp.	14 (8.14)	26 (15.12)	40 (23.26)
<i>Giardia</i> spp.	16 (9.30)	20 (11.63)	36 (20.93)
<i>Cytoisospora</i> spp.	6 (3.4)	5 (3.0)	11 (6.4)
Co-Infections	3# (1.74)	4* (2.33)	7 (4.07)
<i>H.nana</i>	1 (0.58)	1 (0.58)	2 (1.16)
<i>A.lumbricoides</i>	0	1 (0.58)	1 (0.58)
Total	61 (35.36)	111 (64.64)	172 (100)

[Table/Fig-7]: Distribution of enteric parasites based on age group (N=172)

**E.histolytica/dispar* group+*Cryptosporidium* spp. (1) and *Giardia* spp.+*Cryptosporidium* spp. (3)
#*E.histolytica/dispar* group+*Cryptosporidium* spp.(1) and *E.histolytica/dispar* group+*Giardia* spp.+*H.nana* (1) and *E.histolytica/dispar* group+*Giardia* spp. (1)

Departments	Samples n (%)	Positive samples n (%)	<i>Cryptosporidium</i> spp. n (%)	<i>Cytoisospora</i> spp. n (%)	<i>Giardia</i> spp. n (%)	<i>E.histolytica/dispar</i> group n (%)	Helminths n (%)	Co-infections n (%)
ARTC	181 (53.71%)	18 (5.34%)	3 (0.89%)	5 (1.48%)	3 (0.89%)	7 (2.08%)	-	-
Haematology	60 (17.81%)	16 (4.75%)	7 (2.08%)	3 (0.89%)	-	2 (0.59%)	-	4* (1.19%)
Intensive care unit	33 (9.79%)	6 (1.78%)	2 (0.59%)	-	1 (0.29%)	2 (0.59%)	1 (0.29%) (<i>Ascaris</i> spp.)	-
Oncology	31 (9.20%)	10 (2.97%)	3 (0.89%)	-	2 (0.59%)	5 (1.48%)	-	-
Haemodialysis unit	24 (7.12%)	6 (1.78%)	3 (0.89%)	-	2 (0.59%)	1 (0.29%)	-	-
Radiotherapy	8 (2.37%)	5 (1.48%)	2 (0.59%)	1 (0.29%)	-	2 (0.59%)	-	-
Total	337 (100%)	61 (18.10%)	20 (5.94%)	9 (2.67%)	8 (2.37%)	19 (5.64%)	1 (0.29%)	4 (1.19%)

[Table/Fig-8]: Department wise distribution of enteric parasitic infections in immunocompromised patients (N=337).

ARTC: Anti-retroviral therapy centre;

**E.histolytica/dispar* group+*Cryptosporidium* spp. and *Giardia* spp.+*Cryptosporidium* spp.

Amongst 337 immunocompromised patients, maximum number of samples were received from ART center (53.71%) followed by haematology (17.81%), ICU (9.79%), oncology (9.2%), haemodialysis unit (7.12%) and radiotherapy (2.37%) [Table/Fig-8].

Total of 1094 samples were received from immunocompetent patients, of which maximum samples were received from medicine department (50.09%) followed by paediatrics (34.73%), gynaecology (8.05%), orthopaedics (2.65%), endocrinology (2.29%), surgery (1.37%) and gastroenterology (0.82%) [Table/Fig-9].

DISCUSSION

Microbial health threats are a major source of concern since times immemorial. But in the present times due to an upsurge in the number of immunocompromised patients, the spectrum of the intestinal parasites has changed drastically [8]. Parasitic intestinal diseases are one of the major public health problems in developing countries like India [3]. The prevalence of parasitic infections in our study was 12.02% which is in accordance with findings observed by Rathod PG et al., (17.54%) and Kumar S et al., (16.3%); while a much higher prevalence was observed by Mundhada SG et al., (56.40%) and Jayalakshmi S and Dharanidevi S (77%) and Kumar H et al., (49.38%) [3,5,9-11]. This difference in prevalence of intestinal parasitic infections amongst various study groups could be due to the diversity in the geographical distribution of the parasites, personal hygiene and sanitary habits of the individuals and their immune status [9].

Majority of our patients belonged to the adult age group (70.9%) pertaining to easy accessibility of health care centres to adults. The overall positivity rate was also higher amongst adults (7.76%) than children (4.26%) owing to high turnover of adult patients at the hospital. Rathod PG et al., (29.82%) and Kumar S et al., (21.1%) also found similar results with maximum positivity rate amongst 31-40 years of age [3,5]. Most prevalent parasite amongst children was *Entamoeba histolytica* (15.5%) which is consistent to findings of Gebretsadik D et al., (15.5%) [12]. Authors also observed that higher number of paediatric samples were isolated from IPD settings while samples from adults were mainly received from OPD. This may be due to the fact that children tend to deteriorate faster due to diarrhoea and get admitted in the hospital.

Though the parasitic prevalence does not depend on gender but still we observed higher prevalence amongst males with 100 positive samples out of 878 samples received from males which is consistent with the findings of Kumar S et al., (98/604) and Rehana I et al., (170/463) [5,13]. This could be due to the fact that outdoor activities increase the exposure of males to outside food and other environmental factors like lack of proper sanitation facilities at work place [14].

Predominance of immunocompetent patients was observed (1094/1431) which is in concordance to study done by Rathod PG et al., (47/57) [3]. Out of total 172 positive cases, 111 were immunocompetent patients. These findings are in contrast to other

Departments	Samples n (%)	Positive samples n (%)	<i>Cryptosporidium</i> spp. n (%)	<i>Cytoisospora</i> spp. n (%)	<i>Giardia</i> spp. n (%)	<i>E.histolytica/dispar</i> group n (%)	Helminths n (%)	Co-infections n (%)
Medicine	548 (50.09%)	53 (4.85%)	7 (0.64%)	2 (0.18%)	8 (0.73%)	35 (3.20%)	1 (0.09%) (<i>H.nana</i>)	-
Paediatrics	380 (34.73%)	39 (3.57%)	8 (0.73%)	-	15 (1.37%)	13 (1.19%)	1 (0.09%) (<i>H.nana</i>)	2* (0.18%)
Gynaecology	88 (8.05%)	9 (0.82%)	3 (0.27%)	-	2 (0.18%)	3 (0.28%)	-	1† (0.09%)
Orthopaedics	29 (2.65%)	3 (0.27%)	1 (0.09%)	-	1 (0.09%)	1 (0.09%)	-	-
Endocrinology	25 (2.29%)	3 (0.27%)	-	-	2 (0.18%)	1 (0.09%)	-	-
Surgery	15 (1.37%)	2 (0.18%)	-	-	-	2 (0.18%)	-	-
Gastroenterology	9 (0.82%)	2 (0.18%)	1 (0.09%)	-	-	1 (0.09%)	-	-
Total	1094 (100%)	111 (10.14%)	20 (1.83%)	2 (0.18%)	28 (2.56%)	56 (5.12%)	2 (0.18%)	3 (0.27%)

[Table/Fig-9]: Department wise distribution of enteric parasites in immunocompetent patients (N=1094).

**E.histolytica/dispar* group+*Cryptosporidium* spp. and *E.histolytica/dispar* group+*Giardia* spp.+*H.nana*

†*E.histolytica/dispar* group+*Giardia* spp.

study by Rathod PG et al., wherein they reported higher positivity in immunocompromised cases [3]. On the contrary, current study recorded a significant association between immune status and parasitic prevalence. The higher positivity rate in immunocompetent patients could be due to the predominance of those patients in our study. These findings indicate that, the identification of parasitic aetiology should be an essential part of investigation for all symptomatic patients irrespective of their immune status as the clinicians do not suspect parasitic causes as a predominant cause of diarrhoea in immunocompetent patient population.

This study shows the overall high isolation rate of *E.histolytica/dispar* group (43.6%) which is consistent with the study done by Kumar S et al., (37.57%) and Rehana I et al., (8.1%) [5,13]; while Rathod PG et al., and Mundhada SG et al., found divergent results with *Cryptosporidium* spp. to be most prevalent [3,9]. *Entamoeba histolytica* are commonly transmitted by drinking contaminated water and food. A higher infection rate with these parasites may be attributed to the poor sewage system in the community and faecal contamination of water supply as most of the patients coming to a government hospital belong to lower social strata. *Cryptosporidium* spp. oocysts resist chlorination of water and are able to pass through various water treatment processes thus attributing to high infection rate [6].

Hereby, authors observed very low prevalence of helminths with two cases of *H.nana* and one case of *A.lumbricoides*. This could be due to ongoing community-based deworming programs and awareness. On analysing the positivity of the enteric parasites amongst the various immunocompromised cases, it was observed that *E.histolytica/dispar* group was the most common parasite detected amongst HIV/AIDS patients from ARTC followed by *Cytoisospora* spp., *Cryptosporidium* spp. and *Giardia* spp. On the contrary, Rao RP had reported a higher prevalence of *Cryptosporidium* spp. [8]. In our research, the lower prevalence of *Cryptosporidium* spp. could be attributed to irregular shedding of oocysts and administration of cotrimoxazole prophylaxis for *Pneumocystis Carinii* Pneumonia (PCP) in HIV/AIDS patients, which inhibits the multiplication of *Cryptosporidium* spp. also. Unavailability of molecular methods like polymerase chain reaction could be another reason for lower prevalence of *Cryptosporidium* spp. amongst HIV/AIDS individuals in this study [3]. Overall, amongst all the immunocompromised patients, *Cryptosporidium* spp. (5.94%) was the most common parasite detected similar to other studies like Rathod PG et al., and Mundhada SG et al., [3,9]. These findings emphasise that the clinical microbiologists should not overlook the presence of coccidian parasites in the stool samples while investigating cases of diarrhoeal diseases, in spite of the fact that it has not been requested by the treating physician.

Co-infections with two or more parasites were observed in around 1% of patients. However, a higher rate of co-infections had been reported by other researchers like Choubisa SL et al., (13.4%) [14,15].

Mixed infections are more common amongst patients with severely compromised immune status.

After the implementation of deworming programs, there has been a decline in the prevalence of the gastrointestinal parasites and very few studies have been conducted to estimate the prevalence of enteric parasites both in adults and children, in recent years. This study exhibits the spectrum of parasites according to age, gender and immune status of the symptomatic patients as well as highlights the changing epidemiology of enteric parasitic infections.

Limitation(s)

Lack of availability of stool antigen detection tests and molecular tests for *Cryptosporidium*, *Giardia* and *Entamoeba histolytica* was one of the major limitations of the study. Nevertheless, this study emphasises that diligent microscopy can still generate important epidemiological data in resource limited settings where expensive antigen detection kits are unavailable.

CONCLUSION(S)

Our study highlights the higher prevalence of enteric parasitic infections amongst immunocompetent patients as compared to immunocompromised patients, thereby indicating the clinicians to look for these parasites with equal importance in both immunocompromised and immunocompetent patients. Overall, *E.histolytica/dispar* group and *Cryptosporidium* spp. were found to be most prevalent parasites amongst immunocompetent and immunocompromised patients respectively. Since these parasites can affect anyone irrespective of their age, gender or immune status which warrants their identification by quick, easy and reliable methods like direct microscopy, rapid antigen test by immunochromatography, modified acid-fast staining which can be performed even at PHC level.

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