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Prevalence of intestinal parasitic infections and attributable factors among patients presenting with gastro-intestinal symptoms at a tertiary care center in Northern India: a prospective cohort study

Authors
Dr Neelima Singh[1], Dr Richa Sinha[2], Dr Randhir Kumar[3], Dr Sweta Muni[4], Dr Kumar Saurabh[5], Dr Namrata Kumari[6]
[1]Senior Resident, Indira Gandhi Institute of Medical Sciences
[2]Assistant Professor, Sanjay Gandhi Postgraduate Institute of Medical Sciences
[3,4,5]Assistant Professor, Indira Gandhi Institute of Medical Sciences
[6]Prof and Head, Indira Gandhi Institute of Medical Sciences
*Corresponding Author
Dr. Richa Sinha
Assistant Professor, Sanjay Gandhi Postgraduate Institute of Medical Sciences

Abstract
Introduction: *Intestinal parasitic infection is prevalent globally and is a major cause of morbidity especially in developing countries. This study was undertaken to assess the magnitude of parasitic infection among patients presenting with abdominal complains.*
Material and Method: *All non-repeat stool samples were collected from suspected patients, were processed and evaluated microscopically over a period of two years (June 2018 - May 2020). Demographic details, stool characteristics and possible attributing factors were analysed using appropriate statistical tests.*
Results: *Of the 1773 stool samples, fifty-six (3.15%) tested positive for intestinal parasites, most common organisms being E.histolytica (n=18,32.14%) and Giardia lamblia (n=15,26.79%). Source of drinking water, use of sanitary latrines, type of occupation, type of residence (rural/urban) or literacy status did not affect the positivity rates. However, male gender, consistency of stool, presence of mucus, presence of foul smell and occult blood positivity was significantly associated with detection of intestinal parasites.*
Conclusion: *Young males (21-40 years) presenting with diarrhoea with presence of occult blood and offensive odour are more likely to be affected by parasitic infection. Improvement in personal hygiene and availability of safe drinking water with government initiatives can help in adequate control.*
Keywords: *Intestinal parasitic infection, neglected tropical disease, hemospot test.*

Introduction

Intestinal parasitic infections (IPI) caused by protozoa and helminths residing in the intestine present a tremendous burden on the health care system contributing significantly to morbidity and mortality[1]. They are considered among Neglected Tropical Diseases (NTDs) and are endemic in developing countries.[2] Nearly one-fourth of the world's population is chronically infected with intestinal parasites. In 2004, parasitic disease resulted in 3.5 billion cases[3]. Attributable factors include poverty, illiteracy,

tropical hot and humid weather conditions, contaminated drinking water resources, conducive environmental conditions for parasites and inadequate medical care[4]. The impact of parasitic infections on public health is often under-reported. WHO estimates that the disease burden due to intestinal helminths in India is considerable (21%) and only a small proportion(undocumented) actually receive anti-helminthic drugs[5]. Lack of data on geographical distribution of infection and demographic variables that influence the prevalence of infection are a deterrent to effective control of parasitic infection[6]. However, recent technical advancements and growing knowledge have made accurate detection of the causative agent possible which plays a critical role in successful management of the disease. In India, regional variation of intestinal parasitic infections is high [7,8]. It may be attributed to the difference in the socioeconomic pattern, variation in climate, use of different diagnostic techniques and general awareness.

Common parasitic forms identified in stool samples include trophozoites, cysts of protozoans (*Entamoeba histolytica* and *Giardia lamblia*), and eggs of helminth (*Ascaris lumbricoides*, *Ancylostoma duodenale* and *Enterobius vermicularis*)[7]. Complete worms or segments of *Taenia* and ova or larvae of *Strongyloides stercoralis* can also be seen. Among the protozoans, *Entamoeba histolytica* infects 500 million individuals annually, causing disease in ~10 percent and ultimately resulting in about 100,000 deaths[9]. *Giardia intestinalis* results in malabsorption and growth retardation[10]. Soil transmitted helminths (STH) are common intestinal parasites in tropical and subtropical areas. Species responsible for human infection include roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), hookworm (*Ancylostoma duodenale)* and *Necator americanus*[11,12]. They are transmitted directly through ingestion of contaminated food or water containing eggs in their infective stage or indirectly by inappropriate personal or social hygiene. Soil-transmitted helminth infection can also infect by direct skin penetrationon contact with infected/contaminated soil. These adversely affect the nutrition, physical growth, and cognitive development, especially in children.

Knowledge regarding the prevalence of different intestinal parasitic infections will be helpful in formulating necessary control measures by government policy makers.

Real magnitude of this problem in the North Indian state of Bihar is still unknown. Present study was designed to identify the common parasitic infections prevalent in this region along with attributing risk factors (socioeconomic and environmental).

Materials and Methods

This prospective cohort study was conducted over a period of 2 years (June 2018 to May 2020) in the Department of Microbiology, at a tertiary care center in Northern India after obtaining ethical clearance from Institute review board (1910/IEC/IGIMS/2020).Our institute caters to a population of around 59 lakhs.

Non-repeat stool samples received from patients presenting with complaints of diarrhea and other gastrointestinal symptoms such as nausea, vomiting, flatulence, and pain abdomen were included in our study. Samples were collected in sterile leak-proof plastic containers, carefully labeled and transported to the Microbiology laboratory. They were processed and evaluated for color, consistency, presence of mucous and blood, and adult worms. Direct microscopic examination using normal saline and iodine was done to detect the common parasites like *Entamoeba histolytica/dispare*, *Giardia lamblia*, *Ascaris lumbricoides*, Hook-worm, *Trichuris trichiura*, *Hymenolepis nana*, *Enterobius vermicularis*, *Taenia* species, *Strongy loidstercularis* and other intestinal parasites based on their characteristic morphological feature. Presence of fecal occult blood (FOB) was evaluated using Hemospot test, a rapid card-based test (Lot no. HSP1345B).

Results of routine stool examination and demographic details prospectively maintained in the laboratory registration notebook were analyzed using SPSS-20 software. Chi-square test was used to compare the frequencies. P-value of < 0.05 was considered statistically significant.

Results

During the study period, a total of 1773 stool samples (1159, 56.36% belonging to males)were analysed for the presence of intestinal parasites [Table 1]. Median age of patients was 36 (range 18 – 90) years. Fifty-six (3.15%) samples tested positive for intestinal parasites, the most common organism being *E.histolytica* (n=18, 32.14%), followed by *Giardia lamblia* (n=15, 26.79%), *H.nana* (n=8, 14.29%), *Strongyloides stercoralis* (n=5, 8.92%), *Ancylostoma duodenale* (n=4, 7.14%) *and A. lumbricoides* (n=4, 7.14%) [Figure 1].

Intestinal parasite detection in stool samples were significantly more common among male patients (3.80% vs 1.96%, p - 0.035). However, literacy status, source of drinking water, use of sanitary latrines, type of occupation and type of residence (rural/urban) did not affect the positivity rates. [Table 1]

On univariate analysis of stool characteristics: loose/watery consistency, presence of mucus, presence of foul smell and occult blood were significantly associated with detection of intestinal parasites in stool samples. However, color of the stool did not influence the positivity rate. [Table 2]

On multivariate analysis: male gender, loose stool, presence of offensive smell and occult blood were the factors found to be significantly associated with detection of intestinal parasites in stool. [Table 3]

Table 1: Association between stool positivity for Common Intestinal parasite and Socio-demographic & Environmental Factors of study participants [N=1773]

Socio-demographic & Environmental Factors	***Stool Positivity N= 56 (3.15%) n(%)***	***Stool negativity N= 1717 (96.84) n (%)***	***Pearson's Chi square (df)***	***P- value***
Gender				
Male (n=1159)	44 (3.79)	1115 (96.20)	4.466 (1)	**0.035**
Female (n=614)	12 (1.95)	602 (98.05)		
Occupation				
Student (n=535)	20 (3.73)	515 (96.26)	1.30 (4)	0.862
Private Job (n=392)	13 (3.31)	379 (96.68)		
Government Job (n=325)	9 (2.76)	316 (97.23)		
Farmer (n=233)	7 (3.00)	226(97.00)		
Homemaker (n=231)	3 (1.29)	228 (98.70)		
Unemployed (n=57)	4 (7.01)	53 (92.98)		
Residence				
Rural (n=491)	20 (4.07)	471 (95.92)	1.85 (1)	0.174
Urban (n=1282)	36 (2.80)	1246 (97.19)		
Literacy				
Literate (n=1371)	40 (2.92)	1331 (97.08)	1.15 (1)	0.284
Illiterate (n=402)	16 (3.98)	386 (96.02)		
Latrine Usage(n=1518)	48 (3.16)	1464 (96.44)	0.002(1)	0.958
Open defecation(n=255)	8 (3.14)	249 (97.65)		
Drinking water source				
Tap water/Well (n=1214)	42 (3.46)	1172 (96.54)	1.317 (1)	0.251
RO/Candle filter (n=559)	14 (2.50)	545 (97.50)		
Presence of animals in the vicinity(n=530)	19 (3.58)	511 (96.41)	0.434 (1)	0.510

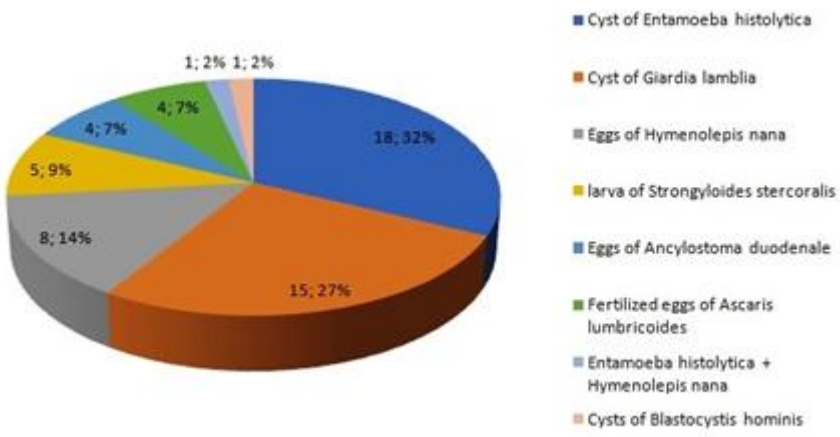

Figure 1: Prevalence of common intestinal parasitic infections among study participants.

Table 2: Association between stool positivity for Common Intestinal parasite and characteristics of stool of study participants [N= 1773]

Characteristics of Stool	**Stool Positivity N=56 n (%)**	**Stool negativity N=1717 n (%)**	**Pearson's Chi square (df)**	**P- value**
Colour of the stool				
Yellow (n=898)	34 (3.78)	864 (96.21)	4.82 (4)	**0.160***
Brown (n=837)	20 (2.39)	817 (97.61)		
Black (n=25)	01 (4.00)	24 (96.00)		
Clay- colored (n=9)	01 (4.00)	08 (96.00)		
Dirty green(n=4)	0 (0.00)	04 (100.00)		
Consistency of stool				
Semi formed (n=1065)	29 (2.72)	1036 (97.28)	73.97 (3)	**<0.001**
Formed (n=602)	09 (1.50)	593 (98.50)		
Loose (n=58)	11 (18.96)	47 (81.03)		
Watery (n=48)	07 (14.58)	41 (85.42)		
Presence of Mucus(n=45)	08 (17.78)	37 (82.22)	32.26 (1)	**<0.001**
Offensive odour of stool(n=03)	02 (66.67)	01 (33.33)	39.62 (1)	**0.003**
Presence of Occult Blood in stool(n=21)	14 (66.67)	07 (33.33)	280.25 (2)	**<0.001**
Presence of Charcot laden crystal in stool(n=1)	0 (00.00)	01 (100.00)		

Table 3 Multivariate analysis of predictors of Stool Positivity among the patients [N= 1773]

Predictors	**Adjusted Odd's Ratio**	**95% Confidence Interval**		**P- value**
Gender	0.40	0.18	0.86	**0.02**
Colour of the stool				
Yellow	0.56	0.30	1.08	0.09
Brown	2.08	0.26	16.66	0.49
Black	4.27	0.50	36.43	0.19
Consistency of stool				
Formed	2.33	0.99	5.44	**0.05**
Semi-formed	15.05	5.11	44.34	**0.001**
Loose	4.75	1.21	18.63	**0.03**
Watery				
Presence of mucus in stool	2.22	0.73	6.79	0.16
Presence of occult blood in stool	81.42	28.65	231.40	**0.001**
Presence of offensive odour in stool	82.13	2.84	2379.46	**0.01**

Discussion

Intestinal parasitic infection is an important overlooked issue, especially in developing countries. It is commonly related to poor sanitation, lack of access to clean drinking water and improper hygiene. It negatively impacts the nutritional status, resulting in weight loss, anemia (due to gastrointestinal bleed) and growth retardation in children. It can also be a cause for various medical complications of worm infestation like cholecystitis, intestinal/biliary obstruction or intestinal perforation[13]. The present study was conducted to explore the frequency of parasitic disease by stool examination and evaluate the impact of attributing factors. Out of 1773 stool samples examined during the study period, 56 were found to be positive for common intestinal parasites with an overall prevalence rate of 3.15%. Reports from other countries exhibited higher prevalence rate. Studies from Egypt, Turkey, Gaza have shown prevalence rates of 57.3%, 53%, 27.6% respectively among school going children[14,15,16]. Studies conducted in different regions of the country have reported diverse prevalence ranging from 6.6% [17,18,19] to 87%[20,21,22]. The wide variation can be attributed to several socio-economical and ecological factors (source of drinking water, sanitation facilities and environmental conditions), population group studied (very high prevalence in immune-compromised individuals – chronic liver disease, retroviral infection), method of stool examination, age of the study group (higher prevalence among children) and varying health services (availability of diagnostic facilities and accessibility to health facilities).

In the present study, protozoal infection was more common than helminthic infections, most dominant parasite being *E.histolytica* (32%) followed by *G.lamblia* (27%).Protozoa can easily spread from person to person by direct contact or through contaminated food and water, while majority of the helminthes follow a middle path of hatching outside the human body to attain an infective stage. Takalkar et al., Bisht et al. and Sahai et al. also reported higher positivity rate (13.7%, 55.3% and 9.3% respectively) for *E.histolytica* [23,20,24]. Stool samples positive for E. histolytica demonstrated significant association with occult blood positivity (14 of 18 samples, 77.77%) ($p<0.001$). High positivity for occult blood among patients with entamoeba infection was also reported in a study from Japan[25]. Other parasites detected were *H.nana* (0.50%), *Strongyloides stercoralis* (0.22%), *Ancylostoma duodenale* (0.22%) and *A. lumbricoides*(0.22%). One stool sample was positive for *Blastocystis hominis* similar to a report by Sanjeev et.al. from Lucknow [24]. This organism was earlier considered as a commensal but now there is ample evidence to call it a potential pathogen. Babb et al. demonstrated a strong link between *Blastocystishominis* and gastrointestinal disorders[26]. No stool sample was found positive for *Enterobius vermicularis* or T*richuris trichura* in the present study. Increasing use of safe drinking water, foot-wears and sanitary latrines, even in villages may have cut down the transmission of these helminths. However, a study from South Indiadid demonstrate the two organisms in less than 0.5% samples[27].

On univariate as well as multivariate analysis, stool samples from male patients were significantly more likely to show the presence of a parasite as compared to female patients (3.38% Vs 1.95%, p – 0.03). Higher positivity rate among young males (21-40 years) was observed in other studies as well: Sahai Sanjeev (10%) Saraswathi R (60%), Salem Belkessa (52%) and Emrah Guler(54%)[24,28,29,30]. This can be explained by the fact that young males have higher occupational exposure (field works, sanitation works), and they engage in more outdoor activities.

Other factors which were associated with higher positivity rates included use of tap or well water as compared to filtered water (3.46% Vs 2.50%) and rural population versus urban population (4.07% Vs 2.80%), although the difference was

not statistically different. This was in concordance with the facts reported by Fernandez et al[31] where villagers were more affected by parasite infestation. This can be attributed to more field work, lack of safe drinking water supply and residual activities of open field defecation in rural areas.

Stool characteristics which directly correlated with detection of parasites on multivariate analysis were: loose or semi-formed stools (odd's ratio 4.75 to 15.05), presence of offensive odour (odd's ratio 82.13) and presence of occult blood in the stool (odd's ratio 81.42). Patients presenting with these stool characteristics are more likely to show the presence of parasites. Presence of blood is often associated with amoebiasis, while diarrhoea is a symptom of protozoan infection.

The overall prevalence of IPIs reported in this study is considerably low. This can be explained by the significant initiatives by World Health Organization in North India including mass de-worming program for population at risk especially the school age children as a part of Millennium Development Goal, availability and accessibility of efficient antiparasitic drugs and awareness programs on personal hygiene including hand wash and use of slippers among general population, a key factor in curtailing the soil transmitted helminthic infection. According to a report, 26.68 crore children in India have been administered Albendazole till February 2018, and more than 114 crore doses of Albendazole were administered to children 1-19 years since 2015[32].

The present study has few limitations. A single stool sample was analysed in each case whereas, screening three consecutive samples in a single patient may have increased the yield as the parasites are not shed in the stool continuously. Modified Ziehl-Neelson staining and modified trichrome staining was not performed which would have given added prevalence of opportunistic parasites (*Cryptosporidium* spp., *Isospora belli*).

The outcomes of this study will definitely help the healthcare professionals to better understand the burden of intestinal parasitic infections in North Bihar and surrounding regions. Prevalence of intestinal parasitic infections is directly linked with the overall well-being of the individual as well as the community. High burden of IPIs was found in the age group of 21-40 years can be as this age group is neglected in deworming programs. Mass deworming measures by WHO has definitely helped to curb the menace by helminthic infection to some extent, however increasing protozoal infection needs further attention.

Conclusion

Young males (21-40 years) presenting with diarrhoea with presence of occult blood and offensive odour are more likely to be affected by parasitic infection. Improvement in personal hygiene and availability of safe drinking water with government initiatives can help in better control.

Ethical Approval and/or Institutional Review Board (IRB): Included as attachment

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