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Study of Prevalence of Intestinal parasitic infection in symptomatic children at Tertiary Care Hospital.

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Abstract

Background

Intestinal parasitic infections cause severe diarrhoea specially in childrens and immunocompromised people amongst which *Cryptosporidium* and *Cyclospora* are emerging protozoan parasites that causes diarrhea worldwide, particularly in young children.

Aims-To know the prevalence of intestinal parasitic infection amongst the symptomatic children at a tertiary care centre.

Methods-This study is conducted at tertiary care hospital from January 2015 to December 2015 for one year in which 100 children were included who came with complains of pain in abdomen and diarrhea. Single stool sample was collected aseptically in wide mouth container following all aseptic precautions. Stool microscopy was performed which included saline mount, iodine mount and modified acid fast stain for the detection of *Cryptosporidium parvum*, *Isospora* and *Cyclospora spp* in stool.

Results-Out of all 100 childrens in the study, 64 were suffering from infection out of which of 7 (10.94%) were positive for *Cryptosporidium* alone, 2 (3.13%) were positive for *Cyclospora* species alone while 34 (53.13%) were positive for both *Cryptosporidium* and *Cyclospora* together resulting into diarrhoea. In 16 (25%) children *Entamoeba histolytica* was detected while in 5 (7.80%) children *Giardia* was found. Among all infected children male cases were more as compared to female cases. Most of the cases were in the 6-10 yrs of the age group followed by 1-5 yrs and 11-15 yrs. The numbers of cases were more in warm season as compared to cooler season.

Conclusions- *C. parvum* and *Cyc. cayetanensis* are major causes of diarrhea, followed by *E.histolytica* and *Giardia species*. So, these pathogens should be taken into account in the differential diagnosis of diarrhea in children particularly from rural areas. And also there is a need of periodic deworming particularly in childrens along with maintenance of hygienic conditions to prevent this parasitic infection as these infections are the leading cause of death among children.

Keywords: Intestinal parasitic infections, *Cryptosporidium parvum*, *Cyclospora species*, *Entamoeba histolytica*, *Giardia species*, deworming.

1. Introduction

Intestinal parasitic infections (IPIs) constitute a global health burden causing clinical morbidity in 450 million people; many of them are children in developing countries¹. Intestinal parasitic infections are among the most common infections worldwide. It is estimated that some 3.5 billion people around the world are affected as a result of these infections, the majority being children¹. The distribution and prevalence of the various intestinal parasites species depend on social, geographical and economical conditions, as well as customs of inhabitants². Poverty, illiteracy, poor hygiene, lack of access to potable water, and hot and humid tropical climate are the factors associated with IPIs³. The main clinical manifestation of the disease caused by these parasites is diarrhoea². Diarrhea is defined as an increased loss of stool with a frequency and fluidity greater than the usual habit for each individual³. Persistent diarrhea is the leading cause of death in children younger than five years of age in developing countries, where it accounts for 30 to 50% of childhood mortality⁴.

It has a devastating effect particularly on infants; annually killing at least 4 to 5 million of those aged less than five years in Africa, Asia and Latin America⁵. The World Health Organization (WHO) suspects that there are more than 700 million episodes of diarrhea annually in children less than five years of age in developing countries⁶.

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Thus, diarrhoea caused by opportunistic intestinal parasites could be life threatening particularly for infants during the course of acute diseases. Although only bacteria and viruses were previously assumed to be the causes of diarrhoea, parasite-caused diarrhoea has also been found in 30- 80% of patients⁷. For example, in 90% of paediatric diarrhoeal patients studied in the Gambia, the etiological agents isolated were opportunistic parasites⁸. The parasites that emerged in recent years, thus, include members of the protozoan genera *Cryptosporidium*, *Cyclospora*, *Isospora* and *Microsporidia*^{9,10}. These opportunistic parasites cause diarrhoeal diseases in children and are also considered as important AIDS-associated pathogens worldwide¹¹.

Coccidian parasite are been increasingly recognized as important causes of diarrhea in children. Species of *Cryptosporidium* are ubiquitous in their distribution¹². In recent years, *Cryptosporidium parvum* occurs in up to 7% of children with diarrhoea in developed countries and up to 12% of children with diarrhoea in developing countries¹³. In temperate climate it accounts for only 1%-4% of the cases of self-limiting childhood diarrhea while in case of tropical climate cryptosporidiosis is much prevalent accounts for 4%-20% cases of childhood diarrhea and is associated with high morbidity and mortality in children¹². Clinically immunocompetent patients usually suffer from watery diarrhea occasionally accompanied by nausea, vomiting, weight loss, anorexia, and dehydration. The duration of symptoms and excretion of oocysts might last from 1 day to 8 weeks^{14,15}. In developed countries various modes of transmission have been identified, among which are consuming contaminated water and food, through recreational water activities, close person-to-person contact⁴. Intestinal parasites would be expected to be highly prevalent in tertiary care centre because of the shortage of clean water, problems in the sewage system and other unhygienic factors that increase the probability of infection by these parasites. Therefore, this study was aimed at determining the extent and association of opportunistic intestinal parasites in paediatric diarrhea cases in Tertiary care centre.

Coccidian protozoa of genus *Cyclospora* are obligate intracellular apicomplexan parasites that infect the mucosal epithelium of the small intestine¹⁶. *Cyc. cayetanensis* was first described in 1986 as a pathogen and is now recognised as a worldwide cause of diarrhoea in humans¹⁷. *Cyclospora cayetanensis*—originally referred to as “cyanobacterium-like bodies”—has recently been recognized as a waterborne pathogen and reclassified^{18,19}. It has been associated with several waterborne outbreaks worldwide. *Cyclospora* was first identified as a human pathogen in three patients from Papua, New Guinea but it was thought to be a coccidian, *Cyclospora* is an important emerging cause of diarrhea worldwide that leads to significant morbidity and mortality. The clinical presentation of *C. cayetanensis* also includes gastrointestinal (GI) symptoms such as loose or watery diarrhea, nausea, vomiting, abdominal cramps and loss of appetite; or constitutional symptoms such as unintentional weight loss, fever, chills, muscle aches, joint aches, generalized body aches, headache, or fatigue. *Cyclospora* oocysts can be transmitted in humans through exposure to fecally contaminated environmental water, food or soil¹⁷.

Entamoeba histolytica is the third leading parasitic cause of death in the developing countries, infecting more than 10% of world's population²⁰. It is endemic in India and affects all age groups. Humans are affected through food and water

contaminated with the cysts of *E. histolytica* due to feces, flies or unwashed hands of food handlers²¹.

Giardia lamblia is a flagellate of worldwide distribution. It is the most common flagellate of intestinal tract, causing Giardiasis. Most common symptom of Giardiasis is diarrhea²². It is well documented that in developing countries, infections are associated with poor sanitary conditions, poor water quality and overcrowding²³. In developing countries, there is a very high prevalence and incidence of infection and data suggest that long-term growth retardation can result from chronic giardiasis²⁴.

This study was carried out to know the prevalence of intestinal parasites among the symptomatic childrens.

2. Material and methods

The present study was conducted in Department of Microbiology from January 2015 to December 2015 at tertiary care hospital, Solapur. Any case suffering from a chronic disease or receiving treatment leading to immune-suppression was excluded. Stool was collected in a clean container. Patient name, date, & time of collection were written on the container. The specimens were transported to the Laboratory as soon as possible.

Stool examination

- Direct smear examination (saline and iodine smear).
- Staining using modified Ziehl–Neelson stains.

Saline mount and Iodine mount: Iodine and saline wet mount preparation of stool samples were made and microscopically examined under a magnification of 400x to detect ova and/or parasites. The cyst of *Entamoeba histolytica* were detected which were round in shape, 12 µm x 20 µm in size²⁰ (Figure 1) and also the cysts of *Giardia lamblia* were observed in saline and iodine mount which were about 8µmX 12µm in size oval in shape. It has two pairs of nuclei which were seen on both the sides of axostyle (Figure 2)²⁴.

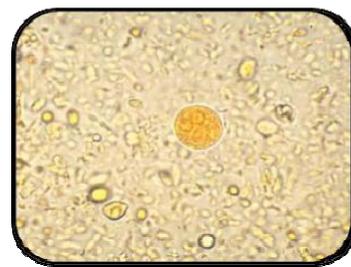


Fig 1: Cyst of *E. histolytica* in Iodine mount

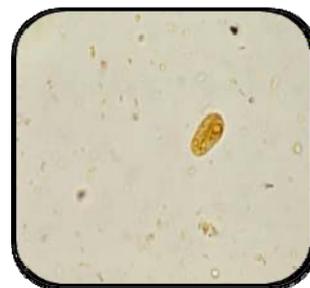


Fig 2: Cyst of *G. lamblia* in saline mount

Modified Ziehl-neelson staining method was performed for detection of Coccidian parasites. Modified acid fast stain positive oval to round structures with size varying from 2-6µm, with or without the presence of retracted cytoplasm were identified as *cryptosporidium* oocyst²⁵ (Figure 3).

Cyclospora oocysts were identified as acid-fast round structures with crumpled celophane appearance and size of approximate 8-10µm²⁵ (Figure 4).

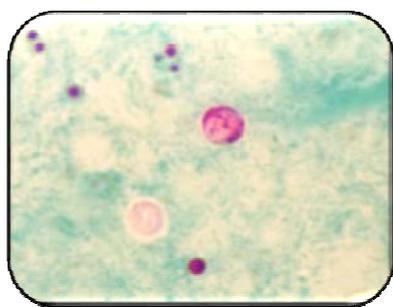


Fig 3: *Cryptosporidium* oocyst in Mod. ZN Stain

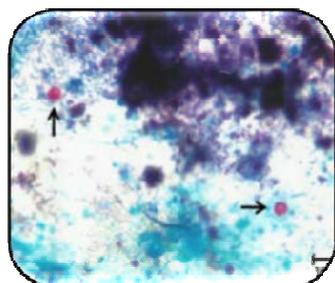


Fig 4: *Cyclospora* oocyst in Mod. ZN Stain

3. Results

Table 1: Age and sex distribution of the cases.

Age distribution	In the year 2015		
	Male	Female	Total
1-5 yrs	6	9	15
6-10 yrs	25	14	39
11-15 yrs	7	3	10
Total	38	26	64

Out of total 64 positive patients, most of the cases were observed in the age group of 6-10 years followed by 1-5 years in both sexes. Male cases were slightly more as compared to female cases.

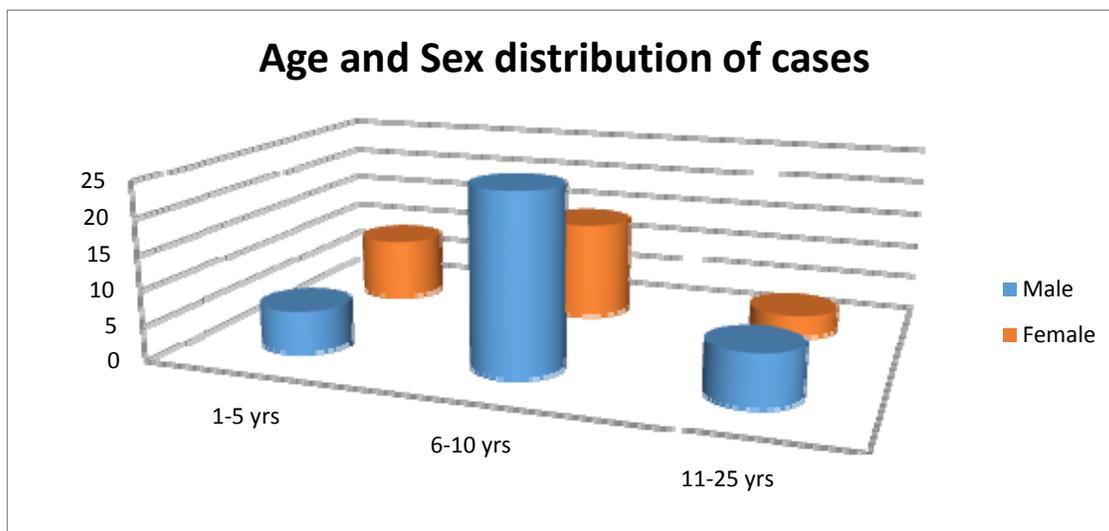


Fig 5: Age and sex distribution of cases

Table 2: Seasonal variation of cases.

Month	Cases in 2015
Jan	5
February	9
March	7
April	6
May	6
June	5
July	3
August	2
September	2

October	4
November	7
December	8
Total	64

In present study most of the cases were observed in the warm season (February to July) as compared to cooler season (August to January).

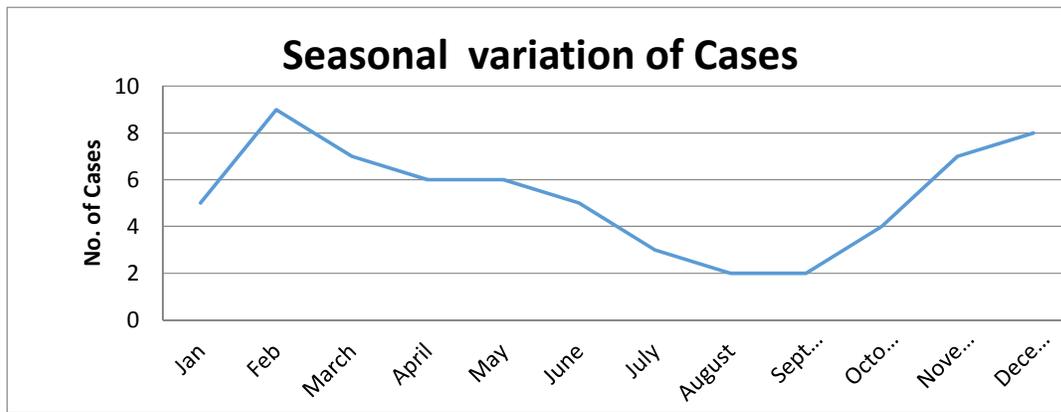


Fig 6: Seasonal variation of cases

Table 3: The prevalence of diarrhea causing intestinal parasites detected among cases.

Name parasite	No of cases
<i>Cryptosporidium parvum</i>	7 (10.94%)
<i>Cyclospora Cyatenensis</i>	2 (3.13)
Both <i>C.parvum</i> an <i>Cyc Cyatenensis</i>	34 (53.13%)
<i>Entomoeba hystolytica</i>	16 (25%)
<i>Giardia lamblia</i>	5 (7.80%)

Out of total 64 cases, 34 (53.13%) cases were positive for both *C. parvum* and *Cyclospora Cyatenensis* infection. *C. parvum* alone was detected in only 7 (10.94%) cases, *Cycl. Cyatenensis* in 2 (3.13%) cases, *E.histolytica* in 16 (25%) cases and *G. lamblia* was detected in 8(7.80%) cases.

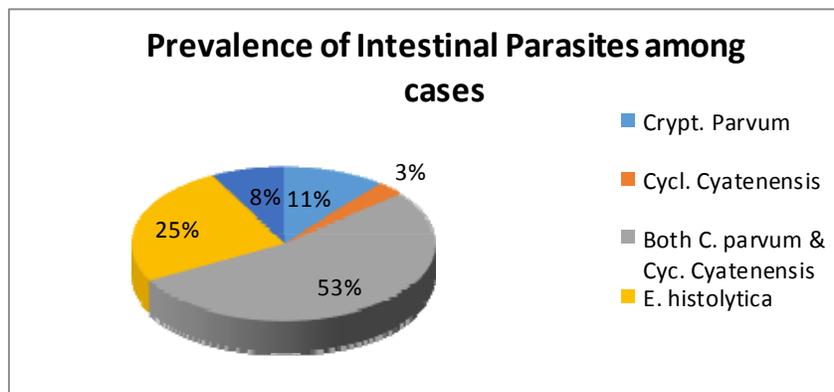


Fig 7: Prevalence of Intestinal parasites among cases

4. Discussion

It is estimated that diarrhoeal diseases are responsible for more than 2 million deaths in children less 5 years of age, most of those deaths being in developing countries²⁶. Rotavirus is the commonest cause of acute watery diarrhea in children throughout the world. *Cryptosporidium spp.* are usually the third or fourth commonest cause of diarrhoeal diseases¹². *Cryptosporidium* infection continues to be a significant health problem in both developed and developing countries, where it is recognized as an important cause of diarrhoea in both immunocompromised and immunocompetent people²⁷. The members of the protozoan genera- *Cryptosporidium*, *Cyclospora*, *Isospora* and *Microsporidia* are the opportunistic parasites which causes diarrhoeal diseases in children¹. Malnutrition, immunosuppression, young age and an increase in the preceding diarrhoea burdens are all risk factors for the development of persistent diarrhea. Persistent diarrhoea seriously affects nutritional status, growth, and intellectual function²⁷. In present study most commonly affected age group is 6-10 yrs, followed by 1-5 years and lastly 11-15 years. Naguib *et al* included the children less than 5 years of

age group and they observed that infection is most commonly seen in the age group is less than 2 years as compared to more than 2 years¹⁷. The numbers of male cases were more as compared to female cases in present study. Adamu *et al* performed a study at Ethiopia found that there were almost equal numbers infected cases among both sexes¹. The slight male preponderance in our study may be because of cultural environment. In this study most of the cases were seen in warm season as compared to cold season which is also seen in the study conducted by Snelling *et al*⁴. Among total 64 cases, both *C. parvum* and *Cyclospora Cyatenensis* infection was observed in 34 (53.13%) cases. *C. parvum* alone was detected in only 7 (10.94%) cases, *Cycl. Cyatenensis* in 2 (3.13%) cases, *E.histolytica* in 16 (25%) cases and *G. lamblia* was detected in 8 (7.80%) cases. The infection prevalence of *Cryptosporidiosis* in Middle East is 1.6% - 10%¹². In Saudi Arabia the prevalence was 1% very low as compared to present study¹². In Tanzania, 1.8% of children with acute diarrhea were found to have *C. cayetanensis* while 5.6% of the children under 8 years of age in Peru 2.3% of children in Guatemala were found to be infected with the same¹. The difference in prevalence of

infection may be because of the geographical distribution. By this study it is confirmed that there is the existence of various opportunistic parasitic infections in children with diarrhea.

The high prevalence of organisms, such as *Cryptosporidium*, and *cyclospora*, among the young people is of concern and deserves careful consideration in the development of health policies in the region. This is even more important because of the lasting detrimental effects of enteric infections that occur during early childhood and later on physical and cognitive development². Emerging pathogens, such as *Cryptosporidium* and *C. cayetanensis*, which are resistant to chlorine disinfection, were found in the hospital patients and school children. All together, these pathogens can be transmitted through contaminated water or food, or poor hygiene. Factors that might be associated with the transmission of these pathogens may include low socioeconomic status, low level of education, use of unchlorinated well or river-water, and a low level of personal hygiene. These factors reflect the living conditions, lifestyle, and environmental conditions of the local population. The level of gastrointestinal disease associated with the faecal-oral route of transmission could be decreased significantly by implementing relatively simple strategies, such as better wastewater treatment and hygiene education. Studies in Indonesia suggested that a systematic and sustained effort to teach children to (a) avoid certain types of behaviour that favours infection and (b) practice good personal hygiene are the best approaches to significant and enduring reduction of the scourge of intestinal parasitism²⁸. This can help for the proper care of pediatric diarrheal patients and for controlling the disease.

5. Conclusion

The prevalence of *Cryptosporidium* in humans in both developed and developing countries demonstrates the magnitude of this parasite in public health. This study confirms the presence of different opportunistic parasites in children with diarrhea. *C. parvum* and *Cyc cayetanensis* are major causes of diarrhea, followed by *E.histolytica* and *Giardia species*. So, these pathogens should be taken into account in the differential diagnosis of diarrhea in children particularly from rural areas. As there is no effective and specific treatment to these diseases, prevention and control of these infections is very important. These parasitic infections can be avoided by increasing the awareness about personal hygiene in public as well as by providing and monitoring the quality of drinking water. Better education and increased awareness of cryptosporidiosis among the general public could potentially reduce case numbers. Along with all these measures there is a need of periodic deworming particularly in children along with maintenance of hygienic conditions to prevent this parasitic infection as these are the leading cause of death among children.

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