

ORIGINAL ARTICLE

CRYPTOSPORIDIOSIS AMONG CHILDREN OF DISTRICT SKARDU, PAKISTAN

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Background: Cryptosporidiosis is a common cause of persistent diarrhoea in children in developing countries. Previously it was thought to be a major pathogenic organism in immuno-compromised children, but recent studies have shown it to be a common infectious agent in immune-competent children as well. **Methods:** This descriptive case-series was carried out at Combined Military Hospital Skardu, Pakistan from August 2014 to June 2015. Fifty-three children aged 12 months to 12 years with history of persistent watery diarrhoea for more than 2 weeks, with no blood or mucous in it and no other systemic findings, were included in the study. **Results:** We examined 53 stool specimens from children with persistent diarrhoea for the presence of cryptosporidium as well other pathogenic organisms. There were 22 (41.5%) females and 31 (58.5%) males. Out of 53 samples, 11 (20.8%) samples were found to be positive for cryptosporidium. Twenty-seven (50.9%) samples were found to be negative for any intestinal pathogen. **Conclusion:** It is concluded from this study that cryptosporidium is a very common infectious organism of persistent diarrhoea in this part of the country.

Keywords: Persistent diarrhoea; cryptosporidium; ascariasis

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INTRODUCTION

Diarrhoea is one of the leading causes of childhood mortality and morbidity especially in the developing world causing more than half a million deaths per year.¹ It may be caused by a variety of conditions like viruses, bacteria, protozoa, drug intake and various systemic diseases.^{2,3} Among the infectious causes of diarrhoea, Rotavirus, *Giardia lamblia* and *Cryptosporidium parvum* are the most common offending agents.^{4,5}

Cryptosporidium is a zoonotic intracellular spore forming protozoan parasite of the intestinal tract of humans as well as a number of domestic animals.⁶ Potential sources of cryptosporidium are contaminated food and water, pet as well as farm animals. The transmission is by direct person to person contact, contact with infected animals or ingestion of contaminated food or water.⁷ It is highly infectious and one single oocyst is enough to infect an individual.⁸

It causes a chronic or severe life threatening infection in immunocompromised individuals such as those with acquired immunodeficiency syndrome or children who are severely malnourished. Infection in the immunocompetent individuals is mostly asymptomatic or mild and self-limiting but can be severe and prolonged leading to severe malnutrition.^{9,10}

The prevalence of cryptosporidium infection in developing countries ranges from 5% to more than 10% while in the developed world the prevalence ranges from less than 1-3%.¹¹⁻¹⁴ In Pakistan, very few studies have been conducted on the prevalence of cryptosporidium as the causative organism of diarrhoea in immunocompetent children.^{15,16} We carried out this

study in order to document the presence of cryptosporidium infection in children with persistent diarrhoea in district Skardu of Pakistan. It is the first study from northern areas of Pakistan, to document cryptosporidium as a causative organism of persistent diarrhoea in children. This will highlight the disease burden in this area so that proper preventive and therapeutic modalities are adopted in order to reduce the burden of diarrheal disease in this area in an effective manner.

MATERIAL AND METHODS

This descriptive case-series was carried out at Combined Military Hospital Skardu, Pakistan from August 2014 to June 2015. Children aged 12 months to 12 years who reported to Out-Patient Department (OPD) of CMH Skardu with history of persistent watery diarrhoea for more than 2 weeks, with no blood or mucous in it and no other systemic findings suggestive of any immunodeficiency, were included in the study through consecutive non-probability sampling technique. Diarrhoea was defined as the passage of liquid or semi formed stools at a frequency more than the usual for the child previous bowel habits. Children with blood in faeces, suspected of having other systemic disease, immunodeficiency and on any prolonged drug treatment were excluded from the study.

The study was reviewed by the hospital ethical committee and permission was granted. Consent was obtained from parents of all participating children after verbally explaining the purpose of study to them. All the relevant technical staff was guided regarding the collection and processing of the faecal specimen.

Faecal samples were collected from the affected children (n=53) in a sterilized plastic bottle in the laboratory of CMH Skardu and analysed within 24 hours. A modified acid fast staining method was used to identify the oocysts of cryptosporidium. A methanol fixed thin smear of faeces was stained in cold carbol fuchsin for ten minutes. Differentiation was done with 1% hydrochloric acid and ethanol. For counterstaining we utilized 0.25% methylene blue. With this technique, cryptosporidium oocysts appear as bright rose-pink spheres.¹⁷

All the samples were analysed by our laboratory technician who was specially trained in the technique from Armed Forces Institute of Pathology, Rawalpindi. The sample was analysed macroscopically for colour, consistency, presence of blood and mucus, undigested food particles and parasites. Microscopically the specimen were examined for ova, cysts, eggs and trophozoites after making a direct wet mount with

normal saline. Data was analysed using SPSS version 17.0.

RESULTS

A total of 53 stool samples from children who met the inclusion criteria were analysed. The age range was 12 months to 12 year. There were 22 (41.5%) females and 31 (58.5%) males. Majority of children (n=25) were between 12 and 24 months of age. Twenty-six (49.1%) stool samples were found to be infected with different pathogenic organisms. Out of 53 samples, 11(20.8%) samples were found to be positive for cryptosporidium. *Ascaris lumbricoide*s was found in 9 (17%) samples while *H. nana* and *Giardia lamblia* were found in 5 (9.4%) samples and 1 (1.9%) sample respectively. Twenty-seven (50.9%) samples were found to be negative for any intestinal pathogen. Table-1 and 2 show the distribution of cryptosporidiosis and other intestinal parasites by gender and age respectively.

Table-1: Gender-wise distribution of intestinal parasites

Gender	Parasite Present					Total
	Ascaris	Cryptosporidium	Giardia	H.nana	No Abnormality Detected	
Female	5	3	1	2	11	22
Male	4	8	0	3	16	31
Total	9	11	1	5	27	53

Table-2: Age wise distribution of cryptosporidium and other intestinal parasites

Age in years	Parasite present						Total
	Ascaris	Crypto	Giardia	H.nana	NAD		
1	2	2	1	3	17	25	
2	3	4	0	1	5	13	
3	1	1	0	0	2	4	
4	1	1	0	0	0	2	
7	0	0	0	0	2	2	
8	0	3	0	0	0	3	
9	0	0	0	0	1	1	
10	0	0	0	1	0	1	
12	2	0	0	0	0	2	
Total	9	11	1	5	27	53	

NAD= no abnormality detected

DISCUSSION

Intestinal parasites have long been recognized to be responsible for diarrheal diseases worldwide. *Giardia lamblia* and *Cryptosporidium parvum* are the two well recognized pathogens.^{2,3} Previously *Cryptosporidium* was thought to cause infection in immunocompromised persons only, but recent studies have shown it to be a major pathogen responsible for diarrhoea in immunocompetent children as well.¹⁸

This study has shown cryptosporidium infection in district Skardu, Pakistan in children with persistent diarrhoea. Faecal samples of 11 (20.8%) children were positive for cryptosporidium, which is a high percentage. This shows that cryptosporidium is the leading infectious cause of persistent diarrhoea in children of this area. No such studies on the presence of cryptosporidium in children of this area have been conducted previously; however, studies from other areas of Pakistan showed that the rate of cryptosporidium

infection was low as compared to our study. These studies revealed that 9% and 10.3 % of faecal samples from children were positive for cryptosporidium.^{15,16} The factors responsible for the high percentage of cryptosporidium infection in this area may be attributed to the lack of safe drinking water, poor personal hygiene, lack of facilities for proper disposal of human and animal wastes and non-availability of the drainage system in this area. Our study also revealed that cryptosporidiosis was more common in male as compared to female children. Out of 11 children who tested positive for cryptosporidium, 3 were females and 8 were males. Also most of the infection (54.54%) occurred in children less than 2 years of age. Among male children, half of the infection occurred in children who were less than two years of age, while 2 out of 3 female children were less than 2 years of age. Male children were more likely to be infected with cryptosporidium because they are more active and exposed to the outside environment as compared to the

female children. These results are consistent with the findings of other studies from Pakistan^{15,16} as well from India and Iran. The high prevalence of cryptosporidium in children less than 2 years of age may be related to the low immunity of children in this age group. Also most of the children still crawl at this age and hence more exposed to the dirty environment.

There are certain limitations of our study. First, this study had a small sample size, therefore, more studies need to be conducted with larger sample size in order to determine the exact prevalence of disease burden in this region. Second, we used the modified acid fast staining technique to identify cryptosporidium in faecal specimen which, although is sensitive and specific, the ELISA and immune fluorescence techniques are more sensitive in this regard. We did not have such facilities available in our set up. We also did not screen for other causes of persistent diarrhoea, like bacteria and viruses, in our study subjects.

CONCLUSION

We conclude from our study that cryptosporidium, is highly prevalent in young children less than 12 years of age in this part of the country. Although it is highly prevalent in other parts of the country as well, but the awareness among paediatricians as well as family physicians regarding its epidemiology is very low. Most of the children who present with persistent diarrhoea do undergo a faecal routine examination but the detection of cryptosporidium requires specific mention in the clinical notes on request form for the faecal examination.

RECOMMENDATION

On the basis of our study, we recommend that family physicians and paediatricians must keep in mind the possibility of cryptosporidium infection as a causative organism of persistent diarrhoea while doing workup for persistent diarrhoea in children. Facilities for the detection of cryptosporidium must be provided at least in the district headquarters hospitals, to detect and promptly manage the disease.

AUTHOR'S CONTRIBUTION

AK conceived the idea, planned and wrote the manuscript of the study. FM assisted in the collection and interpretation of data and gave input in the write-up of the manuscript. MNC wrote the final manuscript after collecting and analysing the data and giving final approval of the manuscript. All the authors contributed

significantly to the research that resulted in the submitted manuscript.

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