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Full Length Research Paper

Frequency of *cryptosporidium* infection in children under five years of age having diarrhea in the North West of Pakistan

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Cryptosporidium **species are minute, coccidian protozoan parasites that have been associated with enterocolitis. It has worldwide distribution and has emerged as an important cause of diarrhea, particularly in children less than 5 years of age and in immunocompromised individuals. Waterborne transmission is particularly troublesome because *Cryptosporidium parvum* oocysts are not eliminated by chlorination or domestic disinfectants. In the present study, single stool specimens from young children (< 5 years) presented with diarrhea were collected in Khyber teaching hospital, Peshawar, Pakistan. Wet mount preparation and modified Ziehl-Neelsen staining were used for identification of oocysts in stool specimens. *Cryptosporidium* oocysts were found in 18 (9.0%) out of 200 children suffering from diarrhea. Infection was common in children between 1 - 24 months of age and associated with abdominal cramps (50%), vomiting (61.1%) and prolonged duration of diarrhea (88.9%). Direct and indirect contact with animals was present in most of *C. parvum* infected children (83.3%). Most of *C. parvum* infected children were consumers of well water (77.8%). *Cryptosporidium* spp. are highly infectious causes of diarrheal illness around the world. It is an important cause of diarrhea in children. Clinician and laboratories should be encouraged to include *C. parvum* diagnostic techniques while dealing with diarrheal stool samples of young children.**

Key words: *Cryptosporidium parvum,* cryptosporidiosis, modified Ziehl-Neelsen staining, immunocompromised, immunocompetent, diarrhea, Pakistan.

INTRODUCTION

Cryptosporidium spp. are tiny microscopic parasites that cause cryptosporidiosis. It is classified as an emerging pathogen by the Center for Disease Control and prevention (CDC) (Guerrant, 1997). Using genotyping tools, five species of *Cryptosporidium*, that is, *Cryptosporidium hominis, Cryptosporidium parvum, Cryptosporidium meleagridis, Cryptosporidium felis* and *Cryptosporidium canis* have been shown to be responsible for most human infections. Of these five species, *C. hominis* and *C. parvum* are the most common species involved in clinical infections (Sulaiman et al., 2005).

C. parvum is an obligate intracellular parasite that infects the epithelial lining of luminal surfaces of gastrointestinal and respiratory tract in a wide variety of hosts. *C. parvum* ingested as an oocyst, undergoes excystation and the sporozoites parasitizes the host. Infection can occur in areas of esophagus and any portion of gastrointestinal tract can be involved. It usually starts in the lower small intestine. Other areas include the gall bladder, bile ducts, pancreas and respiratory tract. Infection induces symptoms of diarrhoea, abdominal cramps, vomiting, low grade fever, generalized malaise, weakness, fatigue, loss of appetite and nausea (Armson et al., 2003).

In immunocompetent individuals, the organism is primarily localized in the distal small intestine and proximal colon, whereas in immunocompromised hosts, the parasite has been identified throughout the gut, biliary and respiratory tracts. After the ingestion of oocyst of *C. parvum*, immuno-competent persons can either experience

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asymptomatic infection or self limiting diarrhoea. Those patients, who have defect in innate, humoral or cellular immunity, can experience severe or prolonged illness. The life threatening potential of *C. parvum* in immunocompromised and immunosuppressed individual has increased the importance of cryptosporidiosis as a global public health problem. The infection by *C. parvum* is usually of short duration and is self-limiting in immunocompetent individual (Moghaddam, 2007).

Cryptosporidium is transmitted through multiple routes. The infection may be transmitted by direct person to person, contact with infected animal or by ingestion of contaminated food or water (Khan et al., 2004). The oocyts are highly resistant to common household disinfectants and survive for long periods in the environment. It has major public health implication because infection can result from exposure to low doses of *Cryptosporidium* oocysts (Gatei et al., 2006).

According to Ajjampur et al. (2007), cryptosporidiosis is a major cause of diarrhoea in children with or without human immunodeficiency virus (HIV) in developing countries. AIDS and protein energy malnutrition (PEM) severely impair immune system (Ajjampur et al., 2007). According to study by Banwat et al. (2004), there is a high prevalence rate of *Cryptosporidium* in young children suffering from protein energy malnutrition (PEM) (Banwat et al., 2004).

The parasites exhibit tropism for the jejunum and ileum in immunocompetent persons. When the immune response is weak such as in patients with AIDS, the infection is more widespread. It may involve stomach, duodenum, colon and the biliary tract. In such patients, cell mediated immunity is low. It means that cell mediated immunity is the major component in eliminating the infection. This is evident by correlation between lower CD4 T cells count, risk and severity of cryptosporidiosis. Interferon-α, interleukin-12 (IL-12) and tumor necrosis factor-α (TNF- α) are involved in protection against cryptosporidiosis (Wyatt et al., 1997). HIV infected person with CD4 count <50/cubic mm, when exposed to *Cryptosporidium* are at high risk for biliary symptom and for death within a year after infection (Vakil et al., 1996).

This is the first report of prevalence of *Cryptosporidium* infection in children from Peshawar, North West of Pakistan. The aim of this study was to find out frequency of *Cryptosporidium* infection and risk factors in children under five years of age having diarrhoea with the objective of creating awareness among the population and in caretakers of the diarrheal disease patients, to highlight the importance of detection of this parasite in routine stool examination and to improve and modify the treatment of these patients.

MATERIALS AND METHODS

A total of 200 stool specimens from children (under 05 years of age) suffering from diarrhoea were examined for the presence of *C. parvum* oocyst. This study was carried out prospectively at clinical laboratory of Khyber Teaching Hospital (KTH) Peshawar and in the pathology department of Khyber Medical College (KMC), Peshawar.

This was a simple descriptive study carried out to find the frequency of *C. parvum* infection in young children with diarrhoea less than 5 years of age. The study was conducted from March, 2007 to April, 2008. Random survey was done with non probability convenience sampling. A proforma was designed with inclusion and exclusion criteria to record the information of patients.

Inclusion criteria

1 All children under 5 years of age with diarrhoea, whether immunocompetent or immunocompromised.
2 Duration of diarrhoea greater than 5 days.
3 Children already on anti diarrhoeal treatment but not receiving antibiotics.

Exclusion criteria

4 Patients who refused submission of specimen.
5 Specimen delayed for more than six hours after collection.
6 If pus cells were reported or any other parasitic infection was detected in stool examination.

The parents/guardians were interviewed using designed proforma including demographic and clinical history. Results were entered in computer database and evaluated. All relevant nurses and technical staff were guided regarding collection and processing procedure of the specimen. Inclusion and exclusion criteria were laid down, before collection of stool specimens. Faecal specimens meeting the inclusion criteria were collected in clean, dry, leak proof glass/plastic containers. Each sample was examined for appearance (or by naked eye for consistency), color, presence of mucus or blood. Samples were kept at 4°C and processed in 6 - 8 h.

According to Morgan et al., (1998), microscopy is a reliable diagnostic method than immunologic based methods for detection of *C. parvum* oocysts (Morgan et al., 1998), hence wet mount preparation, modified Z-N staining and microscopy was carried out for the detection of *C. parvum* oocysts in stool specimens. The specimens were examined on the same day of collection, using wet mount preparation and modified Z-N staining technique. Wet mount preparation was carried out along with Lugol's iodine (01%). This method helped in the differentiation of yeasts cells from *C. parvum* oocysts. Yeasts cells appeared deep yellow by accepting iodine while *C. parvum* oocysts do not accept iodine stain and appeared as transparent discs (Mahgoub et al., 2004).

RESULTS

Out of 200 stool specimens collected from indoor patients of two pediatric units of KTH, a total of 18 cases (9.0%) were found positive for the oocysts of *C. parvum* using modified Z-N staining (Figure 1). The gender distribution of the positive cases was 13 (72.2%) male and 5 (27.8%) were female.

Majority of the children with *C. parvum* infection were less than 24 months of age (Table 1). Mean age of the patients with *C. parvum* infection was 20 months with standard deviation of 15.29 months (Min = 1 month and max = 60 months).

Mean duration of diarrhoea was 11 days in positive cases.

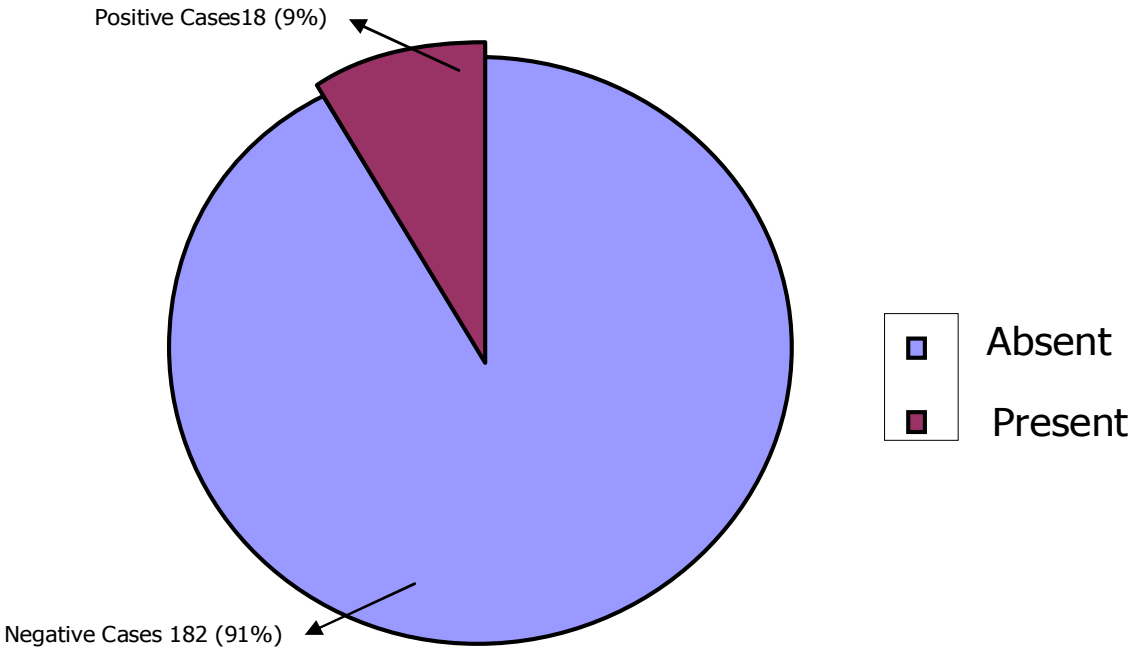

Figure 1. Frequency of *C. parvum* in diarrhoeal Stool (n = 200).

According to the onset of illness, (diarrhoea) 16 cases (88.9%) presented with persistent diarrhoea and the remaining 2 cases (11.1%) are of children that had acute diarrhoea (Table 1).

Abdominal pain was presented in 9 (50%) patients (Table 1). Fever was present in 15 (83.3 %) children with *C. parvum* associated diarrhoea. Only 1 child (5.6%) had blood in his stool (Table 1).

Moderate dehydration was present in 15 (83.3%) and only 3 (16.7%) children had mild dehydration at the time of presentation (Table 1). Watery diarrhoea mixed with mucus was present in 11 (61.1%) children (Table 1). Watery diarrhoea with abdominal pain was the most frequent presenting symptom in all cases.

Majority of infected children, that is, 17 (94.4%) were from poor socioeconomic group and only one case (5.6%) came from satisfactory group.

The frequency of cryptosporidiosis has strong association with large family size 15 (83.3%) as compared to small family size 3 (16.7% p value < 0.03). The history of animal contact was present in 15 (83.3%) children. The source of drinking water was well water in 14 (77.8%) children with *C. parvum* infection and only 4 (22.2%) children were using municipal water supply.

DISCUSSION

C. parvum is an endemic, zoonotic parasite that is highly prevalent in developing countries. Cryptosporidiosis is a serious disease in these countries, because it increases morbidity and mortality associated with poverty and malnutrition (Shoaib et al., 2003; Laubach et al., 2004). In developing countries, it is a leading cause of persistent diarrhoea especially in children. Microscopic analysis of Z-N stained faecal smear is the most commonly used method for screening stool specimens for *C. parvum* oocysts. Although microcopy is considered to be a reliable diagnostic tool for the detection of cryptosporidial infection, it is however, not practiced routinely in Pakistan.

Different epidemiologic studies have demonstrated the prevalence rate of *C. parvum* varies in different parts of Asia. In this study, it was found that 18 (9.0%) out of 200 children under five years of age had *C. parvum* oocysts in their faeces. They had watery diarrhea (61.1%) and few complained of abdominal colic (50%) as well. They were clinically dehydrated (83.3%) while other associated symptoms were fever (83%) and vomiting (61.1%). The incidence of *C. parvum* infection in the present study is consistent with a previous study from Rawalpindi, Pakistan by Iqbal et al. (1999). This study showed similar rate of cryptosporidial infection in children with diarrhea (10.3%) than in children without diarrhoea (3.3%) (Iqbal et al., 1999). The prevalence rate in China is 3.6% (Wang et al., 2002). The low infection rate in China is likely due to better living and sanitary conditions. However, an earlier study from Karachi, Pakistan by Shoaib et al. (2003) showed significantly low rate of *C. parvum* infection (1.7%) in children presented with persistent diarrhoea. Failure to detect *Cryptosporidium* oocysts in the stool of infected person may be due to seasonal variation, while intermittent nature of excretion of this parasite in stool, can also lead to low detection rate (Nimri and

Table 1. Frequeny of *C. parvum* in children <05 years of age having diarrhoea (n = 200).

Characteristics		Cases	%Age	Controls	% Age	O.R.	95% C.I.	P-Value
	Present	11	61.1	98	53.8			
Age	1 - 36 Months	15	83.3	105	57.7	3.67	0.95 - 16.55	0.06
	37 - 60 Months	03	16.7	77	42.3			
Sex	Male	13	72.2	100	54.9	2.13	0.67 - 7.18	0.05
	Female	05	27.8	82	45.1			
Socio-Economic Status	Poor	17	94.4	112	61.5	218.88	0.01	0.05
	Satisfactory	01	5.6	70	83.5			
Hydration	Moderate	15	83.3	81	44.5	6.23	1.62 - 28.14	0.001
	Mild	03	16.7	101	54.5			
Family Size	Large	15	83.3	100	54.9	4.1	1.08 - 18.50	0.03
	Small	03	16.7	82	45.1			
Source of Drinking Water	Well	14	77.8	88	48.9	3.7	1.08 - 13.89	0.03
	Municipal	04	22.2	93	51.1			
Duration of Diarrhoea	11 - 20 days	16	88.9	100	54.9	6.56	1.39 - 42.58	0.01
	5 - 10 days	02	11.1	82	45.1			
Abdominal Cramps	Present	09	50	115	63.2	0.84	0.29 - 2.43	0.38
	Absent	09	50	67	36.8			
Animal Contacts	Yes	15	83.3	89	48.9	5.22	1.34 - 23.8	0.01
	No	03	16.7	93	51.1			
Blood	Absent	17	94.4	170	93.4	1.2	0.15 - 26.17	0.74
	Present	01	5.6	12	6.6			
Fever	Present	15	83.3	11	60.9	3.2	0.83 - 14.45	0.1
	Absent	03	16.7	71	39.1			
Mucus	Absent	07	38.9	84	46.2	0.74	0.25 - 2.19	0.73

Batchoun, 1994). Other studies have reported prevalence rate of 17% in Egypt, 9 - 13% in Tanzania, 5.9% in Uganda and 14.2% in Gaza (Gatei et al., 2006).

Despite its wide distribution in the environment and obvious relevance to public health, there is very little awareness about this protozoan parasite in Pakistan. In this study, *C. parvum* oocysts were more frequently detected in children between 13 - 24 months of age, the mean age group being 20 months (SD = 15.29). Children in this age group were found to be more vulnerable when exposed to contaminated environment, food and water. Immunity is less than optimal at both ends of life, that is, in new born and in the elderly. New born babies appeared to have less T-cell function and antibodies are provided primarily by the transfer of maternal IgG crossing the placenta. Maternal antibodies decay over time (little remains by 3 - 6 months of age) and the risk of infection in children is high after the age of six months (Levinson and Jawetz, 2003).

In this study, many patients were Afghan refugees with large families. They were mostly uneducated and lived in poor hygienic conditions. These children were not properly looked after and hand washing was not practiced. The crawling children who are more exposed to dirty surfaces are therefore more vulnerable to these oro-faecal infections. Similar observations were made in the study conducted by Nagamani et al. (2007) in Indonesia and Ghana (Nagamani et al., 2007). The increased infection rate in this age group could be explained by their immunologic immaturity and other factors as mentioned above. Results from study by Nagamani et al. (2007) suggested that infections were more common in young children. Children in this age group were found to be more vulnerable because of weaning. Contaminated food and water could be the source of infection in these children.

In the present study, it was found that only one case of *C. parvum* oocysts in diarrheal stools of children below 6 months of age was presented. The *p* value (< 0.05) was marginally significant and this may be due to small sample size. In the present set up, this age group was usually breast fed. Colostrum contains antibodies especially secretary IgA, which protects newborn babies against various respiratory and gastrointestinal infections. Studies have shown that persistent diarrhoea is uncommon in breast fed babies (Meremikwu et al., 1997; Mihrshahi et al., 2007). In developing countries including Bangladesh, cryptosporidiosis has been reported in non breast fed babies (Khan et al., 2004).

In this study, the frequency of *Cryptosporidium* infection

in male and female was statistically significant (p value < 0.05). The high prevalence rate of this intestinal parasite observed among males could be attributed to the fact that males are more active, mobile and integrated to the environment. Moreover, it may involve gender specific immunological differences (Agnew et al., 1998).

Socioeconomic status (SES) plays a major role in health. This, not only affects the level of health care but also affects diet, housing and environmental conditions that can affect health. Intestinal parasitic infections are associated with poor socioeconomic group of people, unsanitary environments and inadequate personal hygiene. In this study, there was high incidence of cryptosporidial infection among low socioeconomic group, p value was highly significant (< 0.01).

C. parvum is one of the most common waterborne human parasitic protozoa (Casemore and Roberts, 1993). There have been eleven outbreaks of crypto-sporidiosis in the United States during 1991 - 2000 due to contamination of drinking water (Roy et al., 2004). Contaminated drinking and recreational water are associated with outbreaks of cryptosporidiosis. In this study, many patients belonged to rural areas having low socioeconomic conditions. There was no awareness regarding cleanliness, sterilization and disinfection. These children were more at risk from *C. parvum* infection. Source of drinking was well water in 14 (77.8%) infected children and only 4 (22.2%) children were using municipal water supply. Most of human infections are caused by contaminated water supply. Because of small size of *C. parvum* oocysts and its ability to survive chlorination, it can contaminate municipal water supply with ease.

The results showed that the mean duration of diarrhoea was 11 days in children infected with *C. parvum*. Sixteen out of 18 (89%) children presented with persistent diarrhoea at the time of admission to the hospital. In developing countries, persistent diarrhoea is an important child health problem but is given less attention regarding its control and prevention. Cryptosporidial infection usually leads to chronic watery diarrhea associated with dehydration. Moderate dehydration occurred in 15 (83.3%) children and only 3 (16.7%) children had mild dehydration at the time of presentation, p-value was highly significant (< 0.001).

Epidemiology of cryptosporidiosis in human is not completely understood because of multiple transmission routes such as person to person, animal to person, water and possible air borne transmission (Ajjampur et al., 2007). Many outbreaks of gastroenteritis by *C. parvum* are caused by contaminated water and food (Verweij et al., 2004). A similar association at risk by acquiring infection of *C. parvum* due to consumption of contaminated water and *C. parvum* infection was observed in various studies from Pakistan, Bangladesh, Kuwait and India (Iqbal et al., 1999; Sulaiman et al., 2005).

According to Arrowood (2002), cryptosporidiosis has worldwide distribution and affects a wide range of reptiles, avian, fishes and mammals (Arrowood, 2002). In Kuwait and UK, genotyping of *Cryptosporidium* isolates from human cases revealed zoonotic transmission involving livestock (Nagamani et al., 2007). In this study, majority of children (83.3%) had history of contact with animals either within the house or in close neighborhood. These animals could have been the reservoir of transmission of infection. This parasite is not host specific and infection can spread from infected animals to humans (Laubach et al., 2004).

Highly variable clinical manifestations of cryptosporidiosis in healthy population and in disease endemic areas were observed. These clinical manifestations can be attributed in part to the different species of *Cryptosporidium*. Results showed that molecular mechanisms may play an important role with clinical manifestations of human cryptosporidiosis (Cama et al., 2008). Clinical disease and morbidity is determined by combination of host related factors such as nutritional status, local and systemic immunity.

Diarrhoea due to *C. parvum* was recognized to have seasonal variation showing increased incidence during wet and hot months of the year (Gatei et al., 2006). In this study, most of the cases occurred during rainy season of July and August. The weather during these months in Peshawar is usually hot and humid.

Although only 9.0% of patients in the present study were shown to be excreting *C. parvum* oocysts, it is significant from the public health point of view, as specific diagnostic methods are not routinely used in stool examination (especially from patients suffering from chronic gastroenteritis). Therefore, it seems reasonable to test apparently healthy people with undiagnosed chronic diarrhea, particularly the animal handlers, travelers to endemic areas, hospital workers, household contacts of infected persons and children in day care centers. The present study signifies that *C. parvum* is associated with early childhood diarrhoea. The focus should be to reduce the infectious period, length of illness, risk of dehydration and to prevent complication and death. Health education, personal hygiene and awareness of clinician about importance of cryptosporidiosis testing is useful. This is due to the fact that *C. parvum* will not be detected unless specific diagnostic tests are use by clinicians. Facilities for the detection of *C. parvum* oocysts should be made available as an investigation of diarrheal stool specimens that come to the laboratory. Routine testing of stool will help to explore the gravity of the situation even further. Vigilant surveillance and control measures will help to improve overall health of children.

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