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Full Length Research Paper

Frequency of *Cryptosporidium* infection and related factors under five year's old children hospitalized with gastroenteritis

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In recent years, *Cryptosporidium* has been considered, especially in children, as one of the causative agents in acute and/or chronic diarrhea. Studies had indicated that the prevalence of this protozoa is more common in children under five years old than other age groups. Therefore, determining the prevalence of *Cryptosporidium* in this age group is the main step to assist in designing and implementing health programs. In this cross-sectional study of 374 children admitted to 17- Shahrivar Hospital in Borazjan City, stool samples were obtained throughout the whole year of 2010. Stool samples were examined by ELISA method to detect *Cryptosporidium* surface antigen (CSA). Of the 374 stool samples, 49 (13.1%) were confirmed as positive for *Cryptosporidium* surface antigen. The highest prevalence was observed in children around 1-2 years old. Although infection rate in boys (29 cases, 7.8%) was higher than girls (20 cases, 5.3%); however, no significant association was observed between gender and infection. Significant relationship was observed between *Cryptosporidium* infection and diarrhea, but variables such as vomiting, breast feeding, fever and seizures did not show a significant relationship with *Cryptosporidium* infection. The highest seasonal frequency of infection was observed in autumn. The relative high prevalence of infection in 1 to 5 years old children, the characteristics of the tropical area, the status of poor hygiene among the people due to absence of health infrastructure, and lack of adequate health information demonstrates the fact that proper plan for prevention, accurate diagnosis and treatment of cryptosporidiosis is an essential matter.

Key words: Gastroenteritis, children, *Cryptosporidium*, enzyme-linked immunosorbent assay (ELISA).

INTRODUCTION

Cryptosporidiosis is a zoonotic gastrointestinal infection and its causative agent is a small apicomplexan protozoan called *Cryptosporidium* (Dillingham et al., 2002). Until 1976, this protozoan were reported as a digestive tract parasite in vertebrates, including reptiles, birds and mammals, but now is considered as an important intestinal pathogens in humans (Chai et al., 1996). *Cryptosporidium* is an obligatory intracellular parasite which is colonized in the margins of the intestinal microvilli (outside the cell cytoplasm), particularly in the area of epithelial cells in the lungs and jejunum, biliary system, pancreas and lymph nodules (Chai et al., 1996; Maleki et al., 2005). *Cryptosporidium* infection can be transmitted via the fecal-oral route and through ingestion of contaminated water and food with oocysts excreted by human and animals, and even mothers may infect their babies during childbirth (Maleki et al., 2005). Clinical symptoms of Cryptosporidiosis appear as acute and self-limited watery diarrhea, associated with nausea, vomiting, dehydration, abdominal pain and mild fever (Carey et al., 2004). But in immuno-compromised

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individuals and children it can cause life threatening severe gastroenteritis (Brandonisio et al., 1993; McGowan et al., 1993). Although, *Cryptosporidium* infections are usually limited to small intestine, also it can be colonized in the gallbladder and pancreatic ducts (Franklin and Harold, 1996). This infection has been reported in all age groups (Maleki et al., 2005), but according to many studies, it is more prevalent in children under 5 years (Omar, 2007). Cryptosporidiosis has a worldwide distribution and has been reported in different countries (Omar, 2007; Gatei et al., 2006; García-Rodríguez et al., 1990; Ballal and Shivananda, 2002), and its prevalence in developing countries varies between 4 and 30%, while in developed countries ranges from 0.6 to 20% (Zu et al., 1992; Das et al., 1993). In some studies, factors such as age, sex, nutrition and underlying infectious diseases have been reported to be the predisposing factors (Dillingham et al., 2002; Tzipori and Ward, 2002). *Cryptosporidium* also can cause diarrhea in 10 to 20% of AIDS patients in industrialized countries, but in developing countries can reach around 50% (Akbari, 2004). Studies conducted in Iran, the prevalence rate of Cryptosporidiosis has been reported to vary between 2 to 27 percent (Khalili et al., 2006; Hamedi et al., 2005). Sero-epidemiological studies have shown that the *Cryptosporidium* infection is more prevalence than the reported results based on parasitological detection (Akbari, 2004; Flanigan et al., 1993; Clark, 1999). Determining the prevalence of *Cryptosporidium,* especially in children under 5 years, with the evaluation of its related factors has provided health policy makers in utilizing the basic information to design appropriate health program to reduce the incidence of this infection.

MATERIALS AND METHODS

In this cross-sectional study, we assume the prevalence of *Cryptosporidium* to be 6 to 10% in neighboring provinces and a margin of error of 5 and 95% confidence. A sample size of 350 was calculated for determining the prevalence of *Cryptosporidium,* but for more confidence 374 specimens were obtained.

During the year 2010, stool specimens were obtained from a total of 374 children under 5 year olds hospitalized in 17-Shahrivar hospital of Borazjan city (Bushehr province, Iran) and were kept at –70°C until examination, an informed patient consent was received from all the parents and a questionnaire was completed with information from the parents. Collected samples were studied using the *Cryptosporidium* Antigen ELISA Kit (Diagnostic Automation-Inc. USA) to detecting the *Cryptosporidium* surface antigen (CSA) based on manufacturer instructions. Briefly, homogenous suspension of stool samples were prepared with diluents buffer, and 100 µl of each suspension was added to each well of 96-wells micro plate (Diagnostic Automation- USA) .After incubation and washing, No.1 and 2 kit reagents were added to each well and finally substrate was added to all wells, after stopping the reaction, micro plate wells were read at the absorption wavelength of 450 nm and background wavelength of 630 nm. Samples with OD above 0.15 were considered as positive and equal or below 0.15 as negative. Statistical analyses were performed using the non-parametric Chi-square and Logistic Regression tests. Test results and questionnaires information were analyzed by SPSS version 15 (Chicago, IL Inc. SPSS).

RESULTS

In this study 374 stool samples were tested for detecting *Cryptosporidium* surface antigen and a total of 49 cases (13.1%) were positive. Although the frequency of *Cryptosporidium* infection in boys was higher than girls (Table 1); however there was no significant relationship between the type of sex and *Cryptosporidium* infection. Although the highest frequency of *Cryptosporidium* infection was observed in one and two years old age groups, 4.5 and 4.8 % respectively, nevertheless, there was no significant relationship between age and *Cryptosporidium* infection (Table 2).

Symptoms of diarrhea were observed in 47 (12.6%) of *Cryptosporidium* infection positive cases, but statistical analysis did not show significant relationship between diarrhea symptoms and *Cryptosporidium* infection (Table 3). In this study there was no significant correlation between *Cryptosporidium* infection and clinical symptoms such as dehydration, vomiting, breastfeeding, fever and abdominal cramps (Table 3). Although, most *Cryptosporidium* infection cases were seen in autumn there was no significant correlation between *Cryptosporidium* infection and season.

DISCUSSION

Different studies have shown that *Cryptosporidium* infection is more prevalent in children below 5 years of age than other age groups. However, in some countries, it is considered as the third or fourth causative agent of diarrhea in children under 5 years old (Maleki et al., 2005; Moseyebi et al., 2001). In this study 13.1% of the samples that were tested revealed positive for *Cryptosporidium* surface antigen, this infection rate is higher than any most studies that have been carried out in our country till nowadays. For example, prevalence of *Cryptospridium* infection have been reported by (Dabirzadeh et al., 2003; Mohammadi.et al., 2006; Moseyebi et al., 2001; Khalili and Mardani, 2009; Maleki et al., 2005; Moghaddam, 2007), 4.7, 4.4, 7.7, 5, 4.75 and 10.4%, respectively.

Studies that were conducted in the United States of America, Spain, Kenyia and some parts of India showed that the recorded prevalence in their studies is lower than that is obtained in our study (Omar, 2007; Gatei et al., 2006; García-Rodríguez et al, 1990; Ballal and Shivananda, 2002; Akbari, 2004). In most studies the prevalence rate of *Cryptosporidium* in boys and girls have been reported to be the same (Khalili and Mardani, 2009), but results of some studies have shown that the prevalence rate of *Cryptosporidium* sp. in boys is higher than that in girls (Dabirzadeh et al., 2003; Logar et al., 1996; Lee et al., 2005). The results in our study showed

Table 1. Frequency distribution of *Cryptosporidium* infection in relation to sex.

Sex	Positive No.	%	Negative No.	%	Total No.	%
Female	20	5.3	137	36.6	157	42
Male	29	7.8	188	50.3	217	58
Total	49	13.1	325	86.9	374	100

Table 2. Frequency distribution of *Cryptosporidium* infection in relation to age.

Age	Positive No.	%	Negative No.	%	Total No.	%
1	17	4.5	139	37.2	156	41.7
2	18	4.8	93	24.9	111	29.7
3	6	1.6	26	7	32	8.6
4	1	0.3	28	7.5	29	7.8
5	7	1.8	39	10.4	46	12.3
Total	49	13.1	325	86.9	374	100

Table 3. Frequency distribution of *Cryptosporidium* infection in relation to clinical symptoms, season and nutrition.

Variable	Result				P value
	Positive		Negative		
	%	n	%	n	
Dehydration					
Severe	4	15	26.5	99	0.162
Moderate	8	30	44.8	167	
Mild	1.1	4	15.5	58	
Disgorge					
Yes	6.4	24	43.4	162	0.508
No	6.7	25	43.4	162	
Diarrhea					
Yes	12.6	47	70	261	0.05
No	0.5	2	16.9	63	
Feeding					
Brest feeding	7.2	27	169	45.3	0.656
Brut milk	2.4	9	63	16.9	
Brest feeding and brut milk	0.5	2	7	1.9	
General feeding	2.9	11	22.8	85	
Fever					
Yes	5.4	20	42.9	160	0.167
No	7.8	29	44	164	
Season					
Spring	4	15	22.2	83	0.402
Summer	0.3	1	5.6	21	
Autumn	7.5	28	46.5	174	
Winter	1.3	5	12.6	47	
Convulsion					
Yes	0.5	2	2.1	8	0.387
No	47	12.6	84.7	316	
Cramp					
Yes	5.1	19	28.4	106	
No	8	30	58.4	218	0.248

no significant relationship between *Cryptosporidium* infection and gender. Some studies have reported the highest prevalence of *Cryptosporidium* infection in children under 5 years old (Casemore, 1988; Casemore, 1990; Crawford and Vermund, 1988). Although, in our study the frequency of infection was higher in 1 and 2 year-old children and this result is in accordance with other studies (Maleki et al., 2005; Moseyebi et al., 2001; Khalili et al., 2006; Dabirzadeh et al., 2003; Mohammadi et al., 2006; Assadamongkol et al., 1992). However, our results showed no significant relationship between *Cryptosporidium* infection and age, and this can be partly related to the equal risks of infection under 5 year's old children. Studies have shown that the sporolation of *Cryptosporidium* oocysts occurs faster in warm and humid weather (Moseyebi et al., 2001). And some studies have reported a significant relationship between *Cryptosporidium* infection and the summer season (Maleki et al., 2005; Moseyebi et al., 2001). On the other hand, in some studies significant relationship has not been established between *Cryptosporidium* infection and different seasons (Dabirzadeh et al., 2003). Although, we could not find a significant relationship between *Cryptosporidium* infection and season , but the highest frequency of *Cryptosporidium* infection was observed in autumn, this can partly be explained that in southern Iran, like Bushehr province, in autumn the temperature and humidity is appropriate for sporolation and transmission of *Cryptosporidium* oocyst, and also in this same season of the year cattle are brought out of the stables; therefore, this event can increase the chance of transmission of Cryptosporidium infection, which is in accordance with some other studies (Haniloo, 1994). Although breast feeding has been reported as an important factor to prevent *Cryptosporidium* infection (Dabirzadeh et al., 2003; Khalili and Mardani, 2009), however,in our study there was no significant relationship between *Cryptosporidium* infection and breast feeding in accordance with Khalili et al. (2006). Furthermore, although there was no significant relationship between *Cryptosporidium* infection and dehydration, vomiting, fever, convulsion and cramps, nonetheless, our results showed that there was a significant relationship between *Cryptosporidium* infection and diarrhea symptoms. This result also is in accordance with Moseyebi et al. (2001). It seems that the prevalence of *Cryptosporidium* infection in our country, especially in Bushehr province, is higher than it is routinely reported. Therefore, educating people about the methods of infection transmission and training laboratory staff in high sensitive laboratory about the diagnostic methods are the basic subjects to be used in design appropriate health program to reduce the incidence of this infection.

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