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Symptomatic and Asymptomatic Cryptosporidiosis in Young Children in Iran

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Abstract: The present study was conducted during the period May 2003 to October 2003 in 616 children less than three years of age with and without diarrheal disease presenting at the pediatric clinic of teaching hospitals in Kermanshah, Iran. Single stool specimens were collected from 515 diarrheic and 99 non-diarrheic children. Two smears were made from each stool samples and were stained by a modified Ziehl Neelsen technique. *Cryptosporidium parvum* (*C. parvum*) were detected in 10.4% of children. The *C. parvum* infection rate was significantly higher in diarrheic children (11.6%) than in non-diarrheic children (4.0%). *C. parvum* was observed more frequently in stool samples of children who lived in rural areas (15.2%) than those who lived in urban areas (7.2%). In regard to the presence of animals, the infection rate was 18.5% among children who lived in association with animals in comparison with 8.2% among those who lived in compounds with no animals. The majority of *C. parvum* cases occurred in children between the ages of 0-12 months (11.9%), followed by in children between the ages of 13-24 months (9.2%) and in children between the ages of 25-36 months (3.0%). The data suggest that *C. parvum* is relatively endemic in young children and that Cryptosporidium may be an important pathogen associated with diarrhea in young children.

Key words: *Cryptosporidium parvum*, Cryptosporidiosis, Symptomatic, Asymptomatic

INTRODUCTION

Over the past decade, there has been a dramatic rise in the number of reports of *C. parvum* infections and outbreaks from all over the world (Smith and Rose, 1998). Estimates from United States public health records suggest that, 2% of all stools tested by health care providers are positive for *C. parvum* (Mead *et al*., 1999). Estimating, 15 million annual visits for diarrhea, infection with *C. parvum* might be expected in 300000 persons annually (Mead *et al*., 1999).

After ingesting *C. parvum*, immunocompetent humans can experience asymptomatic infection or self-limited diarrhea (DuPont *et al*., 1995). However, those with defects in innate (Kelly *et al*., 2000), humoral (Levy *et al*., 1997) or cellular immunity (Gomez Morales *et al*., 1996) can experience severe or prolonged illness. The life-threatening potential of *C. parvum* in immunocompromised and immunosuppressed individuals has increased the importance of Cryptosporidiosis as a global public health problem. Although *C. parvum* infections are usually of short duration and self- limiting in individuals with an intact immune system the lack of effective anticryptosporidial drugs (Reynoldson, 1999) means the very young and elderly may be at risk of severe disease as a result of *C. parvum* infection.

Though AIDS, immunosuppressive infectious diseases, diarrhea and enteric diseases are not uncommon in Iran, the present study was conducted to determine the presence and probably prevalence of *C. parvum* among young children in Iran.

MATERIALS AND METHODS

Study population: The study was conducted during the period May to October 2003 in 515 children less than three years of age with diarrhea diseases presenting at the pediatric clinic of teaching hospitals in Kermanshah, Iran. The control group included 99 comparable children presenting at the same hospitals with complaints other than gastrointestinal symptoms. These hospitals cover a large area of population living in urban as well as in the rural areas. The parents of the children were interviewed to complete a questionnaire on demographic information: age, sex, area of residence, contact with animals and health status of the child.

Stool collection and examination: Single fecal samples were collected in plastic cups within two days of hospital admission or when the child presented at the hospital. All stool specimens were concentrated using the formalin-ethyl acetate method by centrifugation at 800 g in a fecal parasite concentrator (Zierdt, 1984) and two smears were made from the resulting pellet. The slides prepared with fecal pellet were used to detect *Cryptosporidium parvum* oocysts by a modified Ziehl Neelsen technique (Henriksen and Pohlemz, 1981). Briefly, slides were stained for 2 min with carbol fucshin and were destained for 30-60 sec with a 10% solution of sulfuric acid. After being washed, the slides were counterstained with methylene blue for 1 min. Red-stained *Cryptosporidium parvum* oocysts were observed microscopically using a 40x objective.

Statistical analyses: Data were analyzed using Chi-square and Fisher exact tests. Results were considered significant when the p-value was less than 0.05.

RESULTS

As shown in Table 1, 10.4% of stool samples were positive for Cryptosporidium infection. Cryptosporidium infection rates in diarrheic children (11.6%) were more than in non-diarrheic children (4.0%; p<0.05). *Cryptosporidium parvum* oocysts was observed more frequently in stool samples of children who lived in rural areas (15.2%) than those who lived in urban areas (7.2%; p<0.05). Shedding of oocysts were significantly higher in both diarrheic (15.1%) and non-diarrheic (16.0%) children who lived in rural areas than diarrheic (9.0%) and non-diarrheic (0.0%) children who lived in urban areas (p<0.05). In regard to the presence of animals, the infection rate of children who lived in association with animals (18.5%) was more than those who lived in compounds with no animals (8.2%; p<0.05). Shedding of oocysts was significantly higher (p<0.05) in diarrheic children (21.0%) who had contact with animals in comparison with those (9.1%) who had not contact with animals, the difference was not significant in non-diarrheic children with (5.0%) and without (3.8%) contact with animals (p>0.05). The infection rate was similar (p>0.05) between boys (10.5%) and girls (10.3%). The difference between diarrheic male (11.6%) and female (11.6%) and non diarrheic male (4.8%) and female (2.9%) was not significant (p>0.05).

As shown in Table 2, the majority of *Cryptosporidium parvum* cases occurred in children between the ages of 0-12 months (11.9%), followed by in children between the ages of 13-24 months (9.2%) and in children between the ages of 25-36 months (3.0%). The difference among children between ages of 0-12 and 13-24 months as well as 13-24 and 25-36 months was not significant (p>0.05), but more oocyst were isolated in age group of 0-12 months (p<0.05) in comparison with age group of 25-60 months. The prevalence rates for cryptosporidium infections in diarrheic children in age groups of 0-12 months (14.2%) and 13-28 months (9.0%) were similar (p>0.05), but in both groups were higher than the age group of 25-60 months (0.0%; p<0.05). In non-diarrheic children, infection rates was similar between age groups of 13-24 months (10.5%) and 25-60 months (20.0%) and in both groups were higher than (p<0.05) the age group of 0-12 months (0.0%).

Table 1: Socio-demographic data of children with and without diarrhea

Variables	Children with diarrhea		Children without diarrhea		Total	
	No.	No. positive (%)	No.	No. positive (%)	No.	No. positive (%)
Locality[1]						
Rural	218	33 (15.1)[a]	25	4 (16.0)[a]	243	37 (15.2)[a]
Urban	299	27 (9.1)[b]	74	0 (0.0)[b]	373	27 (7.2)[b]
Animal at residence[2]						
With	110	23 (21.0)[a]	20	1 (5.0)	130	24 (18.5)[a]
Without	407	37 (9.1)[b]	79	3 (3.8)	486	40 (8.2)[b]
Sex[3]						
Male	310	36 (11.6)	63	3 (4.8)	373	39 (10.5)
Female	207	24 (11.6)	36	1 (2.9)	243	25 (10.3)
Total[4]	517	60 (11.6)[a]	99	4 (4.0)[b]	616	64 (10.4)

[1, 2, 3]: Means within the same column with different letter(s) are significantly different (p< 0.05), [4]: Means within the same row with different letter(s) are significantly different (p<0.05)

Table 2: Age-wise distribution of *Cryptosporidium parvum* in children with and without diarrhea

Age groups (months)[1]	Children with diarrhea		Children without diarrhea		Total	
	No.	No. positive (%)	No.	No. positive (%)	No.	No. Positive (%)
0-12	359	51 (14.2)[a]	70	0 (0.0)[a]	429	51 (11.9)[a]
13-24	101	9 (9.0)[a]	19	2 (10.5)[b]	120	11 (9.2)[ab]
25-36	57	0 (0.0)[b]	10	2 (20.0)[b]	67	2 (3.0)[b]
Total	517	60 (11.6)	99	4 (4.04)	616	64 (10.4)

[1]: Means within the same column with different letter(s) are significantly different (p<0.05)

DISCUSSION

The present study revealed existence of Cryptosporidium infection among children with and without diarrhea in Kermanshah Iran. Current *et al*. (1991) reported that acute infection with *Cryptosporidium* sp. among children and infants is more prevalent in developing countries (5% to >10%) than in developed countries (1%). The worldwide prevalence of Cryptosporidium among asymptomatic children is less than 0.5% (Vuorio *et al*., 1991). The overall percentage of diarrheic individuals positive for cryptosporidium infection in this study (11.6%) was lower than reported figures of 45 and 32% from Peru (Checkley *et al*., 1997) and Guatemala (Laubach *et al*., 2004), respectively, but was higher than corresponding figure of 5.6% reported from India (Pal *et al*., 1989). The proportion of Cryptosporidium infection associated with diarrhea in this population is similar to that reported from Venezuela (Chacin-Bonilla *et al*., 1997), Mexico (Enriquez *et al*., 1997) and Pakistan (Iqbal *et al*., 1999). The relatively high percentage of asymptomatic infection detected in this study (4.0%) is consistent with studies from developing countries (IQbal *et al*., 1999; Palit *et al*., 2005). In this study Cryptosporidium was isolated significantly more frequently from children with diarrhea than from those without diarrhea, suggesting that children with diarrhea are more likely to have Cryptosporidium oocysts identified in their stools; furthermore it appears that Cryptosporidium may be an unrecognized pathogen responsible for diarrhea and other gastrointestinal disorders in the pediatric population. It has been predicted that the proportion of asymptomatic carriers of important diarrheagenic pathogens like *Cryptosporidium* sp. may be high in area of low socio-economic status in developing countries (Chacin-Bonilla *et al*., 1993) which is very much evident in the present study. This is attributed to the unsanitary living condition, paucity of clean drinking water supplies, mixed dwelling habit (i.e., domestic/Pet animals are kept near or inside the houses), improper sewage or waste disposal facilities, intake of contaminated food, etc. However, over-crowding and previously unnoticed diarrhea in the family may also become positive factors, as found in a study in Mexico (Solorzano-Santos *et al*., 2000).

Our results confirm the fact that diarrheic children even symptomless individuals can have been colonised by Cryptosporidium, may be in very early in life, as has also been reported from a study in southern India (Mathan *et al*., 1985). This fact may be more alarming because it has already been shown (Checkley *et al*., 1998) that even asymptomatic cryptosporidial infection are associated with significant growth shortfalls in children in developing countries. In Africa, cryptosporidial diarrhea in the first year of life was associated with diminished linear growth effect with blunting of catch-up growth in young children (Molbak *et al*., 1997). Thus, cryptosporidial infection even without overt diarrhea may be an emerging cause of malnutrion in the developing world.

In the present study; the prevalence of Cryptosporidium infection in males and females were not statistically significant (p>0.05, Table 1), a finding that supports observation made in studies from Peru (Xiao *et al*., 2001), Korea (Jae-Ran *et al*., 2004) and Jordan (Mahgoub *et al*., 2004). Other studies showed higher percentage of Cryptosporidium infection amongst females as compared to that in males (Smith *et al*., 1989; Laubach *et al*., 2004). Smith *et al*. (1989) have stated that the reason for this higher proportion of females than males were unknown. However Laubach *et al*. (2004) have suggested that the male's exposure to Cryptosporidium was affected by life style risk factors that increased the exposure of the females to untreated water.

In the present study, the infection rate in children who lived in rural areas was significantly (p<0.05) higher than those lived in urban areas, a finding that supports observation made in studies from Korea (Chai *et al*., 1996) and Jordan (Mahgoub *et al*., 2004). In this study the positive rate in rural areas was much lower than the figures of 43.5 and 32% reported from Jordan (Mahgoub *et al*., 2004) and Guatemala (Laubach *et al*., 2004), respectively, but was higher than corresponding figure of 3.3% reported by Jae-Ran *et al*. (2004) from Korea. The cryptosporidium infection rate in urban areas in this study is similar to figure of 8.64% reported from Pakistan (Iqbal *et al*., 1999) and lower than the corresponding figures of 32.1 and 24.67% reported from Jordan (Mahgoub *et al*., 2004) and Ghana (Adjei *et al*., 2004), respectively. An epidemiological study has indicated a remarkable difference in positive rates between rural area and urban area, from 10.6 to 0.5% (Chai *et al*., 1996). Most people in rural areas depend on wells and stored water (tanks) as a source of drinking water. Transmission of *Cryptosporidium* sp. by consumption of contaminated surface or ground water is well documented and waterborne outbreaks have been reported (D'Antonio *et al*., 1986; Rush *et al*., 1987). However, in spite of the low socioeconomic status of pediatric population in rural areas in this study, there was no significant difference between cryptosporidiosis and living condition such as presence of domestic animal at house. Although the positive rate in children with contact with animal was higher than those without contact; but the difference was not significant (p>0.05). In the present study, the majority of *Cryptosporidium parvum* cases occurred in children below two year of age (Table 2). This result is consistent with those of previous reports (Iqbal *et al*., 1999; Adjei *et al*., 2004; Palit *et al*., 2005).

In conclusion, the data presented here demonstrate the frequent occurrence of Cryptosporidium among children with and without diarrhea. Since Cryptosporidiosis appears to be more prevalent in developing countries than in the developed world and asymptomatic and symptomatic Cryptosporidial infections are associated with significant growth shortfalls in children (Molbak *et al*., 1997; Checkley *et al*., 1998), it would be logical to emphasize that the inclusion of a search for Cryptosporidium oocysts be considered part of the routine clinical microbiological examination of cases of diarrhea. Additionally, with the continuous rise in the number of HIV/AIDS-infected persons in Iran and the designation of Cryptosporidiosis as an AIDS-defining opportunistic infection, the routine examination for oocyst of cryptosporidium in diarrhea stools may be used as a first-line screening measure for clinically suspected AIDS patients in Iran.

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