Iranian Journal of Clinical Infectious Diseases
2009;4(3):151-155

ORIGINAL ARTICLE

Frequency of *Cryptosporidium* and risk factors related to cryptosporidiosis in under 5–year old hospitalized children due to diarrhea

Bahman Khalili[1*], Masoud Mardani[2]

[1] *Cellular and Molecular Research Center, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran*

[2] *Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University, M.C., Tehran, Iran*

ABSTRACT

Background: Recently *Cryptosporidium* has gained much attention as a clinically human pathogen in immunocompromised cases and young children. This study investigated frequency and risk factors related to cryptosporidiosis in under 5–year old children.

Patients and methods: Stools were examined by ELISA method to detect *Cryptosporidium* surface antigen (CSA) using Remel Prospect *Cryptosporidium* (monoclonal) Microplate Assay. Stool samples were collected from children at admission and were kept at –70ºC until examination. Data were collected by a standard questionnaire and analysed in Epi info 2002 software.

Results: Of 171 children, 8(5%) were infected with *Cryptosporidium*. Most of the cases (6 cases) aged 2-12 months. Boys were more frequently infected than girls ($p<0.05$). Meanwhile, cryptosporidiosis was associated with less breast-feeding and lower birth weight ($p<0.05$).

Conclusion: Results revealed that the frequency of cryptosporidiosis was similar to other parts of the world. Similarly, lower birth weight, less breast-feeding and male gender were associated with a higher frequency of cryptosporidiosis.

Keywords: *Diarrhea, Children, Cryptosporidium, Iran*.
(Iranian Journal of Clinical Infectious Diseases 2009;4(3):151-155).

INTRODUCTION

Cryptosporidium, a coccidian protozoon, was first described in association with diarrhea in humans in 1976 (1-5). Since then, it has commonly been reported as an enteric pathogen in both immunocompromised and immunocompetent subjects worldwide (6,7). Although self-limiting in healthy individuals, cryptosporidiosis can be fatal in immunocompromised individuals (8). In the early 1980s, the strong association between cryptosporidiosis and immunodeficient individuals brought *Cryptosporidium* to the forefront as a ubiquitous human pathogen. Presently, the increasing population of immunocompromised persons and the various outbreaks of cryptosporidiosis through infection by water-borne *Cryptosporidium* oocysts have placed an even greater emphasis on this pathogen. Based on host occurrence and preference, parasite morphology

Received: 24 January 2009 *Accepted*: 27 June 2009
Reprint or Correspondence: Bahman Khalili, PhD.
Cellular and Molecular Research Center, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran
E-mail:Bahman55_khalili@yahoo.com

and site of infection, at least 22 species of *Cryptosporidium* have been named up to now. However, to date, most human infections are due to genotypes 1 and 2 of *C. parvum* (3,4).

Infection by *Cryptosporidium* has been reported everywhere and identified in patients aged 3 days to 95 years old (4,9,10). Transmission is usually through fecally contaminated food and water, from animal-person contact, and via person-person contact. In addition, immunocompromised cases, infants and younger children, patients in a nosocomial setting, those involved in farming practices, veterinarians, travellers and people with other infected patients or health-care employees are regarded as high risk groups for cryptosporidiosis. The disease was initially diagnosed in intestinal biopsy specimens by electron microscopy or with various stains (10,11). Diagnosis is now largely coprologic, although not all routine techniques have been proven effective. A conventional method of identification is the examination of faecal smears with acid-fast stains (12,13). Recently immunological detection methods have also been developed for clinical and environmental monitoring and the use of immunoassays has proven to be very helpful in providing a more sensitive method for detecting organism in stool. Enzyme immunoassay method also provide excellent specificity and sensitivity for laboratories using this method (5,8,13,14). Polymerase chain reaction (PCR) methods have also been developed recently, but are used mainly in research fields (12-14). Many studies investigated cryptosporidiosis by acid-fast stains in Iran and these studies have reported an infection rate of 2-27% in young children (9,15). However, in Chaharmahal-Bakhtiari province no comprehensive study has investigated infection with *Cryptosporidium* and risk factors related to cryptosporidiosis. This study has made an attempt to carry out a survey to understand the frequency of the parasite and related risk factors.

PATIENTS and METHODS

This cross-sectional study was achieved between August 2005 and May 2006. Children aged <5 years were recruited from Hajar hospital in Shahrekord, Iran. Stools were examined by ELISA method to detect *Cryptosporidium* surface antigen (CSA). For this purpose, Remel Prospect *Cryptosporidium* (monoclonal) Microplate Assay (product and reference numbers, 487410 and 2445096, respectively, United Kingdom) was used. Since optical density (OD) of negative control was set at 0.023, any sample with OD more than 0.023 was determined as positive sample, however, all positive samples had OD greater than 0.0800.

The study aimed to describe the frequency of *Cryptosporidium* infection in this age group and possible risk factors associated with cryptosporidiosis.

During the study period, 175 children were enrolled. Children's parents gave informed consent. Stool samples were collected from children at admission and were kept at –70ºC until examination. Characteristics of children including their socio-economical status, history of previous illness and clinical symptoms were all gathered from their medical files.

Diarrhea was defined in any child who had watery diarrhea or if the child had 3 or more than 3 times stool per day.

Data were analysed by Epi-info 2002 software and chi-squire and Fisher's exact test were applied, when appropriate.

RESULTS

Totally, 175 children were enrolled, however, finally 171 stools were examined. Of 171 children, 74 (43.3%) were girls. Mean age (±standard deviation) of children was 14.0±10.4 months (a range, 2-57 months). Children age distribution was as follow; 99 cases (57.8%) less than 12 months, 49 (28.7%) 13-24 months, and 23 cases (13.5%)

over 2 years. Children's birth weight was 3009±4950gr.

A total of 8 (4.6%) stool samples revealed positive for *Cryptosporidium* surface antigen, among which 7 cases (87.5%) were boys and 6 (75%) aged 2-12 months while the other 2 cases aged 13-24 months.

Twenty-two (12.9%) children had low birth weight (<2500gr). Interestingly, the frequency of cryptosporidiosis was significantly higher in this group when compared to others. Indeed, half of the infected children (4 of 8) had low birth weight (OR=7.9, p<0.01).

Close animal contact was reported in 54 children, however, the difference with non-contacted children did not reach a statistically significant level.

Our findings revealed a negative relationship between *Cryptosporidium* infection and duration of breast-feeding. Children breast-fed one month or less were more commonly infected when compared with children breast-fed more than one month. Of 7 children breast-fed for less than 1 month, 2 (28.6%) had positive *Cryptosporidium* surface antigen (OR= 7.33, p<0.04).

In our setting, the youngest mother aged 17 years old while the oldest was 41 years, however, no statistically significant relationship was found between maternal age and cryptosporidiosis.

Children's dehydration status was studied. Thirty-two (18.7%) had no clear dehydration signs, 89 (52%) had mild while 17 (9.9%) had severe dehydration. Of 17 severely-dehydrated children, 2 were infected with *Cryptosporidium*, however, the difference was not statistically significant. Although the frequency of *Cryptosporidium* in children with chronic diarrhea was higher when compared to children with acute diarrhea (16% vs. 80%), the difference was not statistically significant.

No statistically significant relationship was found between parent's job and education, previous hospitalisation or illness, access to unsafe water, living with people with diarrhea, using oral rehydration therapy, kind of diarrhea and cryptosporidiosis. Table 1 summarizes some of the studied characteristics. None revealed statistically significant association with cryptosporidiosis.

Table 1. Selected characteristics of under 5-year old hospitalized children

	Positive subjects (%)	**Negative subjects (%)**
Stool consistency 24 hours before admission		
Watery	159 (93)	12 (7)
Bloody	10 (6)	161(94)
Semi liquid	92 (53)	79 (47)
Previous hospitalization	56 (33)	115 (67)
Diarrhea during the last year	94 (55)	77 (45)
Breast feeding (>1month)	164 (96)	7 (4)
Use of ORS before admission	67 (39)	104 (61)
Keeping animal at home	54 (31)	117 (69)
Access to safe water at home	164 (96)	7 (4)
Using untreated water	13 (8)	158 (92)
Vomiting at admission	115 (67)	56 (33)

DISCUSSION

Studies addressing *Cryptosporidium* in human mainly focused on immunocompromised cases and few studies have investigated prevalence and risk factors related to *Cryptosporidium* in non-immunodeficient subjects worldwide. This study has investigated the frequency of *Cryptosporidium* and related risk factors in under 5-year old hospitalised children due to diarrhea.

Our study showed that the frequency of cryptosporidiosis (5%) was more or less the same as other studies conducted in Iran or other countries (9,12-16).

We have found a statistically significant relationship between *Cryptosporidium* and gender. Boys were found more susceptible to cryptosporidiosis. Although gender differences were not reported in some studies, others demonstrated male predominance (16-18).

Children with lower birth weight were shown to be more frequently infected with *Cryptosporidium*. This fact was investigated in few studies, however, epidemiological studies generally have reported that immunocompromised subjects are more susceptible to infectious diseases and in particular to cryptosporidiosis. Similarly, a significant association between *Cryptosporidium* infection and birth underweight status was reported in Hamedi study (15). Additionally, among factors associated with immunodeficiency, it seems that low birth weight and malnourished status are the main causes (19- 21).

Infection with *Cryptosporidium* was not influenced by the use of unsafe water and animal contact. In contrary to ours, many studies have reported untreated water and farm visit or animal contact as risk factors for *Cryptosporidium* (22-24), however others have documented no relationship between cryptosporidiosis and animal contact or drinking water (25). This controversy may be in part explained by the age distribution of our cases and less animal contact in younger children or few positive cases in our setting.

Most of the infected cases (75%) aged less than one year. In agreement with ours, many studies have reported higher prevalence of cryptosporidiosis in young children, especially those aged less than 2 years (17,19,21). Furthermore, there is a statistically significant relationship between cryptosporidiosis and breast feeding duration. Frequency of infection with *Cryptosporidium* was higher in children breast-fed less than one month. Similarly, some studies have reported higher frequency of cryptosporidiosis in malnourished children when compared to non-malnourished children (19-21). Nevertheless, a large scale study is required to draw a firm conclusion.

In conclusion, cryptosporidiosis should be considered in children with gastrointestinal complaints while being hospitalized for diarrhea, especially for those aged less than 5 years.

ACKNOWLEDGEMENT

We wish to thank vice-chancellor for research of Shahrekord University of Medical Sciences for financial support and our colleague Dr Shahhai for his help in this regard.

REFERENCES

1. Nime FA, Burek JD, Page DL, Holscher MA, Yardley JH. Acute enterocolitis in a human being infected with the protozoan Cryptosporidium. Gastroenterology 1976; 70(4):592-8.

2. Meisel JL, Perera DR, Meligro C, Rubin CE. Overwhelming watery diarrhea associated with a Cryptosporidium in an immunosuppressed patient. Gastroenterology 1976;70(6):1156-60.

3. Garcia LS, editor. Diagnostic medical Parasitology. 4th edition. Washington, WB Saunders. 2001;p:60-76.

4. Flanigan TP, Soave R. Cryptosporidiosis. Prog Clin Parasitol 1993;1-20.

5. Kaushik K, Khurana S, Wanchu A, Malla N. Evaluation of staining techniques, antigen detection and nested PCR for the diagnosis of Cryptosporidiosisin HIV seropositive and seronegative patients. Acta Trop 2008;107:1-7.

6. Casemore DP, Miller KB. Epidemiologic aspects of human Cryptosporidiosis and the role of waterborne transmission. Epidemiol Rev 1996;18(2):118-36.

7. Ungar BL, Kao TC, Burris JA, Finkelman FD. Cryptosporidium infection in an adult mouse model. Independent roles for IFN-gamma and CD4+ T lymphocytes in protective immunity. J Immunol 1991;147(3):1014-22.

8. Chen XM, LaRusso NF. Cryptosporidiosis and the pathogenesis of AIDS-cholangiopathy. Semin Liver Dis 2002; 22(3):277-89.

9. Khalili B, Shahabi GH, Besharat M, Mardani M, Hart LA. Determining the prevalence of Cryptosporidium and measuring of micronutrients in Cryptosporidiosis among children under 5 years in shahrekord. Journal of Shaheed Beheshti University of Medical Sciences and Health Services. 2006;3(30):5. (Abstract) (Article in Persian)

10. Tzipori S, Ward H. Cryptosporidiosis: biology, pathogenesis and disease. Microbes Infect 2002; 4(10):1047-58.

11. Janoff EN, Reller LB. Cryptosporidium species, a protean protozoan. J Clin Microbiol 1987;25(6):967-75.

12. Morgan UM, Pallant L, Dwyer BW, Forbes DA, Thompson RG. Comparison of PCR and microscopy for detection of Cryptosporidium parvum in human fecal specimens: clinical trial. J Clin Microbiol 1998; 36(4):995-8.

13. Magi B, Canocchi V, Tordini G, Cellesi C, Barberi A. Cryptosporidium infection: diagnostic techniques. Parasitol Res 2006;98(2):150-2.

14. Sharp SE, Suarez CA, Duran Y, Poppit I. Evaluation of the Triage Micro Parasite Panel for detection of Giardia lamblia, Entamoeba histolytica/Entamoeba dispar, and Cryptosporidium parvum in patient stool specimens. J Clin Microbiol 2001;39(1):332-4.

15. Hamedi Y, Safa O, Haidari M. Cryptosporidium infection in diarrheic children in southeastern Iran. Pediatr Infect Dis J 2005;24(1):86-7.

16. Park JH, Kim HJ, Guk SM, Shin EH, Kim JL, Rim HJ, et al. A survey of Cryptosporidiosis among 2,541 residents of 25 coastal islands in Jeollanam-Do (Province), Republic of Korea. Korean J Parasitol 2006; 44(4):367-72.

17. Lee JK, Song HJ. Prevalence of diarrhea caused by Cryptosporidium parvum in non-HIV patients in Jeollanam-do, Korea. Korean J Parasitol 2005; 43(3):111-4.

18. Laupland KB, Church DL. Population-based laboratory surveillance for Giardia spp. and Cryptosporidium spp infections in a large Canadian health region. BMC Infect Dis 2005;5:165-72.

19. Newman RD, Sears CL, Moore SR, Nataro JP, Wuhib T, Agnew DA, et al. Longitudinal study of Cryptosporidium infection in children in northeastern Brazil. J Infect Dis 1999;180(1):167-75.

20. Kirkpatrick BD, Daniels MM, Jean SS, Pape JW, Karp C, Littenberg B, et al. Cryptosporidiosis stimulates an inflammatory intestinal response in malnourished Haitian children. J Infect Dis 2002;186(1):94-101.

21. Mukhopadhyay C, Wilson G, Pradhan D, Shivananda PG. Infestation profile in persistent diarrhea in children below age 5 years in western Nepal. Southeast Asian J Trop Med Public Health 2007; 38(1):13-9.

22. Stantic-Pavlinic M, Xiao L, Glaberman S, Lal AA, Orazen T, Rataj-Verglez A, et al. Cryptosporidiosis associated with animal contacts. Wien Klin Wochenschr 2003;115(3-4):125-7.

23. Hunter PR, Thompson RC. The zoonotic transmission of Giardia and Cryptosporidium. Int J Parasitol 2005;35(11-12):1181-90.

24. Roy SL, DeLong SM, Stenzel SA, Shiferaw B, Roberts JM, Khalakdina A. Risk factors for sporadic Cryptosporidiosis among immunocompetent persons in the United States from 1999 to 2001. J Clin Microbiol 2004;42(7):2944-51.

25. Khalakdina A, Vugia DJ, Nadle J, Rothrock GA, Colford JM Jr. Is drinking water a risk factor for endemic Cryptosporidiosis? A case-control study in the immunocompetent general population of the San Francisco Bay Area. BMC Public Health 2003;1:3-11.