

## PREVALENCE OF *CRYPTOSPORIDIUM* INFECTION AMONG CHILDREN IN KASHAN-IRAN

S.A. TALARI (Ph.D.), N. MOMTAZMANESH, A. TALEBIAN, M. ARBABI,  
A. TAGHAVIARDEKANI, M.R. TALARI AND M. GHASEMZADAE

DEPARTMENT OF PARASITOLOGY, KASHAN UNIVERSITY OF MEDICAL SCIENCES,  
KASHAN, IRAN.

---

Cryptosporidiosis is one of the pathogenic parasites of human with different prevalence in each region of the world including our country. This is a self limited parasitic infection which may produce severe fatal disease in immunodeficient patients. Due to lack of information about the rate of human infections by this parasite in Kashan, this study was conducted to determine the rate of infection by the mentioned parasite in the children with diarrhoea referred to central laboratory of Kashan, Iran during 2000-2002. A cross sectional descriptive study was performed on 240 stool samples taken by random sampling from children suffering from intestinal discomforts. Formalin-ether concentration of fecal samples and warm Ziehl-Neelsen stain method was used and the smears were studied under light microscope. The highest rate of prevalence was found in rural areas and children over 5-yrs of age. The prevalence of infection in the hot seasons (*i.e.* spring and summer) of the year was also more than the cold seasons (*i.e.* autumn and winter). It was also observed the prevalence of infection in those people who were in contact with cattle was more than others. Keeping in view the 3.75% prevalence of cryptosporidium infection among children under research, it is hereby recommended that the diagnostic centres pay more attention to this parasite particularly in those cases of diarrhoea where no other pathogenic agent is found in stool.

### INTRODUCTION

Cryptosporidiosis is one of the parasite infectious diseases caused by a Sporozoa of Coccidia from Cryptosporididae. The human type of parasite is known as *Cryptosporidium parvum*, which is the common enteric factor in both human and animals. It is scattered all over the world (Beaver, 1984; Casemor, 1990; Alonso, 1995; Donoghue, 1995). *Cryptosporidium* is reported and identified in more than 170 different kinds of hosts including mammals, birds, fishes and even amphibious (Donoghue, 1995). *Cryptosporidium* was identified from the stomach of a laboratory rat for the first time in 1907 by Tyzzer (Current, 1983) and its human prevalence was found and reported for the first time in 1976 (Tzipori, 1988) in a 3.5 years old girl who was suffering from enterocolic.

Tzipori (1980) and his co-workers could observe the parasite oocysts in the stool of an adult with normal immunity system (Tzipori, 1988). By 1981, only seven of this disease were diagnosed and reported in the patients suffering from abnormality in immunity system. This parasite is an important factor in children diarrhoea and in children under two year of age in developing countries (Current & Garcia, 1991; Macgovant, 1993).

The prevalence of this parasite in the developing countries is between 0.6-20% and 4-32% in the third world (Kenneth *et al.*, 1990; Das *et al.*, 1993). The studies conducted in Iran show that the prevalence of this disease in the child population of different area, Tehran to be 5.2%, Mashhad 3%, Zanjan 2.6% and Kermanshah city is 3.3% (Hanilo, 1995; Hamzavi, 1996; Fata, 1997; Moharam *et al.*, 1997).

At present no particular step has been taken in diagnosis of the prevalence of this disease in most of the diagnostic laboratories in Iran. Therefore, with regard to the importance of this

organism among the pathogenic parasites and also the lack of previous studies in this regard in Kashan city, this research was conducted to identify the prevalence of this parasite in children suffering from diarrhoea, who were brought to the central diagnostic center of Kashan during 2000-2002.

### MATERIALS AND METHODS

This cross sectional descriptive study was conducted in children under 12 years age group who were brought to the central laboratory of Kashan due to their diarrhoea. Samples were prepared in different seasons and the required data was registered on different questionnaires through interviews with parents. Then, 3 morning samples were collected from each patient and 'formalin-ether concentration method' was used (Ritchie, 1948). The slides were prepared from the precipitation and after drying with methanol, they were fixed and stained by Ziehl-Neelsen method to be studied under light microscope by the concerned specialist (Garcia *et al.*, 1983). In this type of staining, the oocysts appeared as red bodies of 5-6 microns over a blue background (Hamzavi, 1996). The rate of contamination in the samples was measured as 95% positive and 0.03% error. The cases were studied on their age, sex, family, different seasons and etc basis.

### RESULTS

Out of 240 cases studied, 120 (50%) were males and remaining 50% were females. 143 individuals (59.5%) selected from urban and 97 cases (40.5%) were rural. 74 individuals were (30.8%) in contact with cattle and 162 (64.2%) had no contact with cattle. Studies showed that the rate of prevalence to *Cryptosporidium* in the 'group under study' was 3.75% (9 cases). Fig. 1 shows the relative prevalence in children under 12 on age basis. As it is seen, out of 9 cases, 7 cases (2.9%) were found in children under 5 years old and only 2 cases (0.85%) were reported in children older than 5 years. This difference has a statistic value ( $P < 0.005$ ).

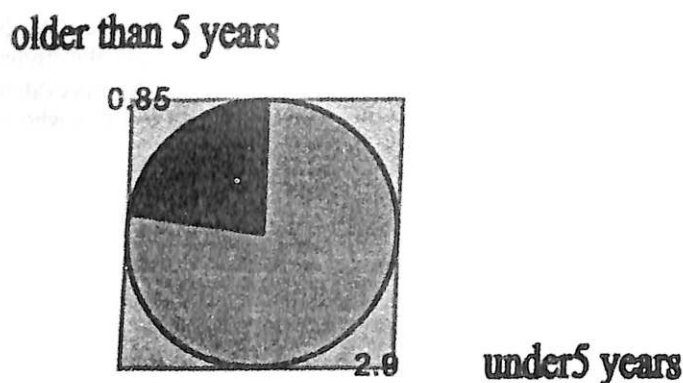


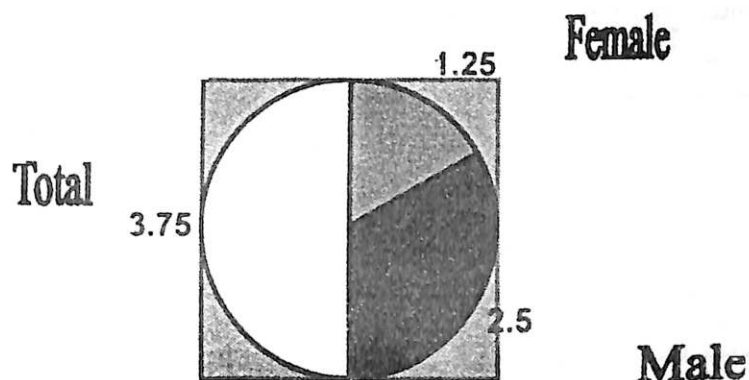
Fig. 1 : The age wise prevalence of *Cryptosporidium* in 9 infected children.

As it is seen, 06 cases (2.42%) were reported in the first half and 03 cases (1.23%) of prevalence were identified in the second half of the year. Therefore the rate of prevalence in hot seasons (autumn, summer) was more than the cold season (spring, winter). This difference had a good statistic value ( $p < 0.005$ ) (Table I).

**Table I :** The seasonal prevalence of *Cryptosporidium* among children.

Cummulated percentage	Percentage	Number	Seasons
22.2	22.2	2	Spring
66.6	44.4	4	Summer
88.8	22.2	2	Autumn
100	11.2	1	Winter

The relative prevalence of *Cryptosporidium* in children under 12 years age on their sex basis were shown in Fig. 2. Out of 240 samples, 120 (50%) were collected from males and remaining (50%) from females, in which 03 (1.25%) and 06 (2.5%) cases of *Cryptosporidium* prevalence were relatively reported.

**Fig. 2 :** A significant statistic relation was observed among children on their sex basis.

The distribution of infested children with *Cryptosporidium* on indigenous basis of continental area is shown in Table I in which the infestation in rural and urban was 08 (2.9%) and 01 (0.85%) respectively. The highest infection in rural area was related to hilly areas ( $p < 0.05$ ).

**Table II :** The distribution of diarrheal infested children with *Cryptosporidium* on the basis of residence referring to Central Laboratory of Kashan (2000-2002).

Cummulated Percentage	Percentage	Number	Prevalence place of settlement
11.1 (59.58)	11.1 (53.33)	1 (65.83)	City
33.3 (17.08)	22.2 (16.66)	2 (17.5)	Desert villages
100 (23.34)	66.7 (30.01)	6 (16.64)	Hilly village

**Table III :** The distribution of coinfection with *Cryptosporidium* and *Giardia lamblia* in diarrheal children referring to Central Laboratory of Kashan (2000-2002).

Total	Negative	Positive	Result ( <i>Giardia</i> )
46 (100)	39 (84.8)	7 (15.2)	Positive
194 (100)	192 (99.0)	2 (1.0)	Negative
240 (100)	231 (96.25)	9 (3.75)	Total

In this study, 2.9% of infested children had the history of contact with domestic animals and their products. The parents of this child were farmers or labours. The duration of infection 6 cases (2.5%) was 20 days and 3 cases (1.25%) was 15-20 days, 7 cases (2.9%) were coinfecting with *Cryptosporidium* and *Giardia* (Table II).

The clinical manifestations shown in Table IV is another point found in the research on the patients suffering from *Cryptosporidium* infection. As it is shown, the highest lymphadenopathy and anemia was not observed in any of the patients. Among the children suffering from *Cryptosporidium* infection, 05 (55.5%) suffered from watery diarrhoea, 03 (33.3%) from odorous stethorrea and 01 (11.2%) from mucous diarrhoea.

**Table IV :** The clinical manifestation among 09 *Cryptosporidium* infected children who consulted the Central Laboratory, Kashan city in 2000-20002.

Sr. No.	Clinical signs	Number	Percentage
1.	Diarrhoea	9	100
2.	Nausea	9	100
3.	Abdominal pain	7	77.7
4.	Fever	7	77.7
5.	Anorexia	6	66.6
6.	Vomiting	5	55.5
7.	Dehydration	1	11.2
8.	Lymphadenopathy	0	0
9.	Anaemia	0	0

## DISCUSSION

In this study, the prevalence of *Cryptosporidium* in children under 12 years age is estimated to be 3.75%. The 3.3% epidemic in children under 12 years age of Kermanshah city (Hamzavi, 1996) and research on 200 children in 1994 who consulted Dr. Shaikh and Hasheminejad Hospital of Mashhad showed the above rate to be 3% higher, but compared with the research of Hanibo *et al.* (1993) in Zanjan city (6.3%), Mafi (5.2%) in Pediatric Centre of Tehran and Nouri-Sardari studies (1990) on the common infections of human and cattle in Mashhad (Nouri & Sardari, 1980; Hanilo, 1995; Fata, 1997; Moharam *et al.*, 1997), it is at a lower level, reason being the regional and environmental differences.

According to epidemiologic studies in developed countries, the rate of prevalence of *Cryptosporidium* in patients suffering from diarrhoea in the third world countries is between 0.6 to 0.20, in Latin America 0.4-0.32, in Sudan and Bangladesh 0.6 and in Libia 7.9% relatively (Fata, 1997).

Based on the researches done on children suffering from *Cryptosporidium* infection, the prevalence in Guinea, Nigeria, Kuwait, Malaysia, Rawalpindi (Pakistan) and north-east India is 7.7%, 15.1%, 10%, 0.9%, 10.3% and 7.2% relatively (Lqbal *et al.*, 1999; Nath *et al.*, 1999; Lqbal *et al.*, 2001; Menon *et al.*, 2001; Nwabuisi, 2001; Perch *et al.*, 2001). The researches showed the level of prevalence in boys is 1.93 times that of the girls and the highest rate of prevalence is in children over 5 years of age. So it was on this basis that Dabirzadah *et al.* (1990) reported the 17.25% prevalence in boys and 8.25% (Perch *et al.*, 2001) in girls. According to Fata studies

(1997) in Mashhad city, the prevalence in boys and girls is 2:1 relatively (Fata, 1997). Mosayyebi (Nwabuisi, 2001) research in Arak city proved the prevalence level in boys to be 1.5 times higher than girls under 5 years of age (Dabirzadah *et al.*, 1990). Hence, aside from the techniques and ways of research in each of the studies on parasite, there are other factors and conditions *i.e.* : The climate, ecology, behavioural changes, personal and environmental conditions, the quality and purification of drinking water, raw food study, contact with cattles, age and sex which can play an and sex which can play an important role in prevalence of this organism (Nouri & Toroghi, 1991). The study on seasonal distribution also shows the meaningful difference in the level of prevalence in cold and hot seasons. Their results completely match the findings of other research (Nouri & Toroghi, 1980; Kenneth, 1990; Addis *et al.*, 1991; Das & Sengupta, 1993; Sardi & Bava, 1998). It seems that the sensi of the oocysts to cold and hot, the reduction in contamination in human (17). Among the colic parasites, *Giardia lamblia* was the only parasite seen with *Cryptosporidium*, so that only 09 cases, 07 (15.2%) were also contaminated with *Giardia*. Certainly this relation is a proven to the common source not to the point that prevalence of one of these parasites can enhance the presence of the second one.

It is one of the findings of this study that the highest rate of prevalence (2.9%) was among children in rural areas *i.e.* in children who were in direct contact with cattles and domestic animals (Nouri & Toroghi, 1991; Hanilo, 1995).

Nouri & Sardari (1980) and Nouri & Toroghi (1991) studies show that the number of cattles in an area cannot be the only factor in prevalence of this parasite. Possibly there are other factors playing their role. Also there are reports from other countries according to which, the highest rate of infection is in rural areas (Nouri & Toroghi, 1991; Hanilo, 1995).

Another finding of the research showed the presence of diarrhoea and nausea in 100% and dehydration in 11.3% of the children infected with *Cryptosporidium*, but the other manifestations such as lymphadenopathy and anaemia were not found in any patient. These findings matched the conclusions reported from different part of the world (Casemor, 1990; Fata, 1997; Froutani, 2000; Haller, 1994; Mandell, 1995).

Based on different reports from all over world, it seems that the rate of prevalence is more related to the geographical and environmental significances and the world 'urban and rural' cannot be considered as a 'unique symbol' for epidemiologic studies in all countries, therefore, the different 'ways of transfer' are to be considered. The diagnostic centres are here by suggested at least prepare a slide from the sample in conforming ' the presence of *Cryptosporidium* oocysts' in cases of diarrhoea in hot seasons, particularly when the microscopic and cultural examination results have been negative.

#### ACKNOWLEDGEMENTS

The Deputy Director, of Research, Kashan Medical University, Iran for his financial support of this research, to Mr. Akbar Masjedi and Mrs. Mahzad Erami for their great task and support in laboratory part of this research.

#### REFERENCES

- ADDIS, D.G. 1991. *Giardia lamblia* and *Cryptosporidium* infection in child day-care centers in Fulton Country, Georgia. *Pediatr. Infect. Dis. J.* **10**(12) : 907-911.
- ALONSO, S.M. 1995. Intestinal parasite in the person population in the Madrid area. *Inferm. Infec. Microbiol. Clin.* **13**(2) : 295-281.



- BEAVER, P.C. 1984. Coccidia, Microsporidia and pneumocystis. *Clinical Parasitology*. **13** : 149-173. Lea & Fabiger.
- CASEMOR, D.P. 1990. Epidemiological aspects of human Cryptosporidiosis. *J. Epidemiol. Infect.* **104** : 1-28.
- CURRENT, W.L. & GARCIA, L.S. 1991. Cryptosporidiosis. *Clin. Microbiol. Rev.* **4**(3) : 325-358.
- CURRENT, W.L. 1991. Human cryptosporidiosis in immunocompetent and immunodeficient persons, studies often outbreak and experimental transmission. *N. Engl. J. Med.* **396** : 1252-1257.
- DABIRZADAH, M., BAGHAEL, M., BOKAIAN, M. & NOURI, N. (1990). Cryptosporidiosis in Zahedan seasonality and endemicity in children under 5 years old. *3<sup>rd</sup> Intern. Congr. Parasitol.*, Iran, Zahedan, University of Medical Sciences.
- DAS, P. & SENGUPTA, K. 1993. Significance of Cryptosporidium as an aetiological agent of acute diarrhea. *Calcutta J. Trop. Med. Hyg.* **96**(2) : 124-127.
- DONOGHUE, P.J. 1995. Cryptosporidium and Cryptosporidiosis in man and animals. *J. Parasitol.* **25**(2) : 139-195.
- FATA, A.M. 1997. Prospective study of the incidence of cryptosporidiosis among children suffering from gastroenteritis in Mashhad. *Med. J.* **40** : 106-111.
- FROUTANI, M.R. 2000. Prevalence of Cryptosporidium infection in children with diarrhea in Lar, Iran. *1<sup>st</sup> Intern. Congr. Parasitol.*, Iran, Mazenderan.
- GARCIA, L.S., BRUCKNER, D.A., BREWER, T.C. & SHIMIZU, R.Y. 1983. Technique for the recovery and identification of Cryptosporidium oocysts from stool specimens. *J. Clin. Microbiol.* **18** : 185-190.
- HALLER, J.O. 1994. Gastrointestinal manifestation of AIDS in children, *AJR. Am. J. Roentgenol.* **162**(2) : 387-393.
- HAMZAVI, Y. 1996. Cryptosporidial infection in the children under 12 years old, referred to Shahid Fahmideh Hospital, Iran, Kermanshah. **4** : 8-13.
- HANILO, A. 1995. An epidemiological study of determination of effective factors in the transmission pattern of cryptosporidium. **9** : 8-13. (Title of Journ.)
- KENNETH, S., ADEL, A. & WARREN, F. 1990. *Tropical and Geographical Medicine*. 2<sup>nd</sup> edn., McGraw-Hill. London.
- LQBAL, J., HIRA, P.R., AL-ALI, F. & PHILIPS, R. 2001. Cryptosporidiosis in Kuwaiti children : Seasonality and endemicity. *Clin. Microbiol. Infec.* **7** : 261-266.
- LQBAL, J., MUNIR, M.A. & KHAN, M.A. 1999. Cryptosporidium infection in young children with diarrhea in Rawalpindi, Pakistan. *Am. J. Trop. Med. Hyg.* **60** : 668-670.
- MACGOVANT, I. 1993. The natural history of Cryptosporidial diarrhea in HIV-infected patients. *AIDS*. **7**(3) : 349-540.
- MANDELL, 1995. Principals and practice of infectious disease. 4<sup>th</sup> edn. Churchill-Livingston, pp. 2503-2504.
- MEHRABI, M. & ESLAMIRAD, Z. Cryptosporidiosis in under 5 years children in Arak. *1<sup>st</sup> Intern. Congr. Parasitol.* Iran, Zahedan, University of Medical Sciences.
- MENON, B.S., ABDULLAH, S. & MAHAMUD, F. 2001. Low prevalence of *Cryptosporidium parvum* in hospitalized children in Koto Bharu, Malaysia. *Southeast Asian J. Trop. Publ. Health.* **32** : 319-322.
- MOHARAM, M., MOHAMMAREZAI, G. & REZAEIAN, M. 1997. Cryptosporidiosis in children with gastrointestinal. *J. Kosar. Med.* **2** : 179-185.
- NATH, G., CHOUDHURY, A. & SHUKLA, B.N. 1999. Significance of Cryptosporidium in acute diarrhoea in North-Eastern India. *J. Med. Microbiol.* **48** : 523-526.
- NOURI, M. & SARDARI, K. 1980. Prevalent of different clinical infection of cryptosporidiosis in Mashhad. *Vet. Parasitol. Med. Veterinaria Tehran.* **20** : 1-10.
- NOURI, M. & TOROGHI, R. 1991. Asymptomatic Cryptosporidiosis in cattle and human in Iran. *The Veterinary Record.* **128** : 358-359.
- NWABUISI, C. 2001. Childhood cryptosporidiosis and intestinal parasitosis in association with diarrhoea in Kwara State, Nigeria. *West Afr. J. Med.* **20** : 165-168.
- PERCH, M., SODEMANN, M. & JAKOBSEN, M.S. 2001. Seven years experience with *Cryptosporidium parvum* in Guinea-Bissau, West Africa. *Ann. Trop. Paediatr.* **21** : 313-318.
- RITCHIE, L.S. 1948. An ether sedimentation technique for routine stool examination. *Bull. US Army Med. Dept.* **8** : 326.
- SARDI, N. & BAVA, J. 1998. Cryptosporidiosis in pediatric patients. *Rev. Inst. Med. Trop. Sao Paulo.* **40**(3) : 197-200.
- TZIPORI, S. 1988. Cryptosporidiosis in perspective. *Adv. Parasitol.* **27** : 63-129.