Medical Journal of the
Islamic Republic of Iran

Volume 8
Number 1
Spring1373
May 1994

SOME EPIDEMIOLOGICAL ASPECTS OF CRYPTOSPORIDIOSIS IN AHWAZ, CAPITAL OF KHOOZESTAN PROVINCE, ISLAMIC REPUBLIC OF IRAN

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ABSTRACT

From December 1990 to June 1992 in Ahwaz, capital of Khoozestan province, 1333 patients referred to Khoozestan Parasitology Center, in 29 cases of whom *Cryptosporidium parvum* was detected. All infected patients were less than eight years of age. The parasite was most prevalent in the 0-1 year old age group. Though some *C. parvum* infected cases harboured *Giardia lamblia*, there was no relationship between the two infections. Similarly, there was no relationship with *Blastocystis hominis* either. Females (19/29) were significantly more commonly infected than males (10/29). The majority of cases (19/29) were detected from October to April when the weather
70%. However, no infected case was found during August and September, when the weather temperature was above 45°C and humidity was below 30%. Only 2 out of 29 were in direct contact with cattle and sheep. It is probable that the consumption of contaminated drinking tap-water was the main factor of cryptosporidium oocyst dissemination in this area.

MJIRI, Vol. 8, No. 1, 17-22, 1994.

Keywords: *Cryptosporidium parvum*, cryptosporidiosis, giardiasis, age-groups.

INTRODUCTION

Cryptosporidium parvum, a coccidian parasite, has been shown to cause diarrhea, abdominal pain, anorexia, and vomiting in immunodeficient individuals, as well as in immunocompromised and even in immunocompetent persons.[1,2,3,4,13,18,21,27,28,29] So far, although humans from a few months of age to over 90 years of age have been found infected, most reports indicate that children less than five years old are more susceptible to this infection. Many investigators reported no sex difference, but some observed sex differences among *C. parvum* infected cases.[1,2,11,27]

To clearly define the epidemiology of *C. parvum* in Ahwaz, a subtropical area in southwest Iran where few reports existed concerning animal and human infections due to this parasite, a survey was carried out on *C. parvum* infection in persons who referred to Khoozestan Parasitology Center for protozoan and metazoan parasitic diseases.

MATERIALS AND METHODS

From December 1990 to June 1992, 1333 patients were referred by their physicians to Khoozestan Parasitology Center, a medical diagnostic laboratory located in Ahwaz. Clinical symptoms as well as age, sex, financial status, type of drinking water and raw food consumption were recorded. The occupations of all patients and/or their parents were also noted for any correlation existing between cryptosporidiosis and domestic animals (e.g. sheep, goats

and cattle) in the area.

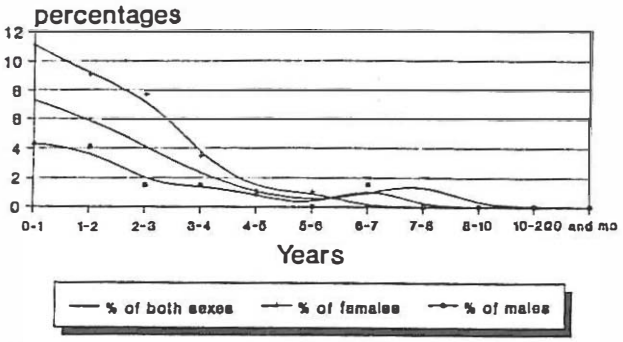
All patients were examined at least three times, with intervals of 2-3 days. Fecal samples were collected in small plastic containers with no preservative and were examined within 2 hours of collection, according to Melvin and Brook.[4] Wet mount preparations were made using physiologic saline (8.5g/1000) and D'Antoni's solution (1.5%), in order to detect the protozoan trophozoites and helminth larvae. Concentration procedures were carried out on all samples. Two grams of fecal sample were emulsified in 10 mL. of 10% formol-saline and filtered through two layers of gauze. Then, 3 mL. of diethyl-ether was added to the filtrate. After thoroughly shaking the mixture, the emulsion was centrifuged at 2000 g for 5 minutesand the supernatant decanted. A drop of the sediment was placed on a glass slide and covered by a coverslip and examined for protozoan cysts and/or helminth ova. Another drop of the sediment was used for preparing smears on a second glass slide. In other words, for each sample, at least two smears, fixed by methyl-alcohol, were heated or air dried. The fixed slides were stained by modified Ziehl-Neelsen method, according to Henriksen and Pohlenz.[7] The red or pink colour of cryptosporidium oocysts was easily seen against the green background. The oocysts of cryptosporidium were measured and their sizes recorded.

RESULTS

Of 1333 persons, 29(2.27%) had *C. parvum* oocysts in their stool. Of 1333, 538 were females and 795 were males; 19 (3.53%) of the former and 10 (1.26%) of the latter group were infected. The difference between sexes in distribution of cryptosporidiosis was significant (X^2= 7.40, 0.005<p<0.01) (Tables I and II and Figure 1).

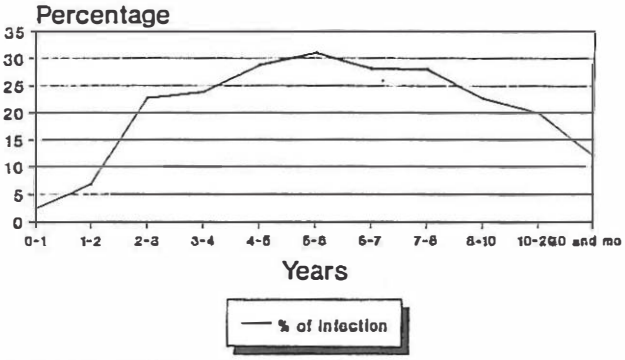
When patients were classified according to age, cryptosporidiosis was observed only in children less than 8 years of age (Table I). In addition, the 0-1 year age group had the highest infection rate (11.1% in females and 4.3% in males) (Figure 1 and 2). Of29 cryptosporidium infected cases, 6 had giardia, 4 *Blastocystis hominis*, 1 *Trichomonas intestinalis* and 1 *Enterobius vermicularis* as well. To facilitate the comparison of cryptosporidiosis and giardiasis, all 1333 persons were classified by age and sex (Table III). Overall, 23.7% of both sexes were infected with *G. lamblia*. Giardiasis in females (27%) was almost significantly different from males (21.5%) (X= 5.2: 0.01<p<0.02) (Table III). Regarding the age-group specific rates for giardiasis, 5-6 year olds were most infected, whereas 0-1 year olds were the least infected group. The prevalence of giardiasis in other age-groups of both males and females is given in Table III.

Of 1333 persons, 704 were diarrheic and 600 persons non-diarrheic. Although both groups were suffering from some clinical symptoms, 22 of the former and 7 of the latter group harboured *C. parvum* oocysts. The comparison of groups showed a significant difference in infection rates among cases of cryptosporidiosis with diarrhea as compared



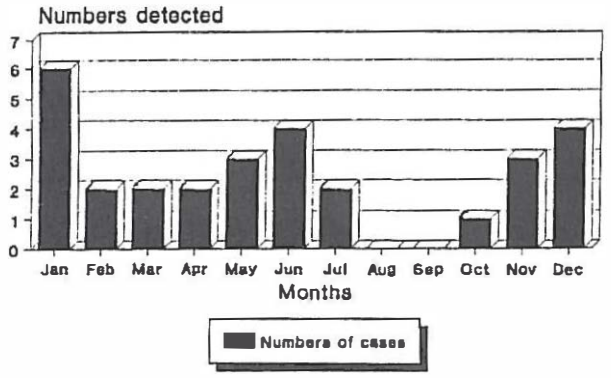
The first group was classified from 0-11 months, the 2nd group from 12-23 months, etc.

Fig. 1. Age distribution of male and female cases infected with *Cryptosporidium parvum*.



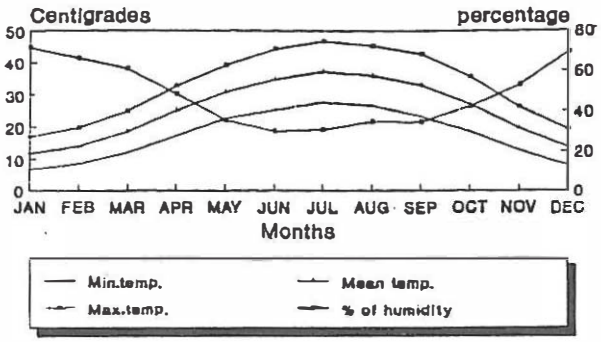
1. A total of 1333 cases were tested.
2. Age-groups were arranged as 0-11 months, 12-23 months, etc.

Fig. 2. Age distribution of cases infected with *Giardia lamblia*.



A total of 1333 cases were tested by stool examinations

Fig. 3. The number of *C. parvum* infected cases detected throughout the year.



10 year meteorological data from Ahwaz Meteorological Station.

Fig. 4. Averages of temperature variations and humidity percentage of Ahwaz, capital of Khoozestan province (1980-1989).

to non-diarrheic patients (X2= 6.44, 0.01<p<0.02), (Table IV).

During August and September, no case of *C. parvum* infection was found. Figure 3 shows the number of infected cases diagnosed monthly. The ten-year weather temperature and humidity percentage of Ahwaz is illustrated in Figure 4 in order to facilitate the interpretation.

The prevalence of *B. hominis* infection in the studied population was 9.5%. This figure isnot significantly different from the 4 cases of *B. hominis* among the 29 cryptosporidium infected cases. Thus, no comparison was made. Similarly, the infection with other parasites, reported above, showed no significant differences with the cryptosporidium infected cases.

DISCUSSION

The presence of cryptosporidiosis in children was not unexpected, for this infection is prevalent throughout Iran.[15,16,17,20] Although many workers have reported that children under five years of age are most susceptible,[1,2,3,4,6,21,27,29] the age-group specific susceptibility was not fully determined. Casemore[2] and Ungar, et al.[29] emphasized a low infection rate to cryptosporidiosis in the 0-1 year age-group, whereas Pal, et al.[18] reported a high infection rate in infants less than 6 months old. The present findings are similar to those of Pal, et al.[18]

The *C. parvum* infected individuals were also infected with *Giardia lamblia* (6 cases), *Blastocystis hominis*, (4 cases) *Trichomonas intestinalis*, (1 case) and *Enterobius vermicularis* (1 case). Some workers[9,10,23,30] pointed out an association between giardiasis and cryptosporidiosis. However, others[1,2,13,25] stated that such an association may not always be significant. In the present study, 23.7% of the

Table I. Age distribution of both sexes infected with *Cryptosporidium parvum* in Abwaz.

Age-group (years)*	No. examined			No. infected %		
	Females	Males	Total	Females %	Males %	Total %
0-1	18	23	41	2(11.1)	1(4.3)	3(7.3)
1-2	44	72	116	4(9.9)	3(4.1)	7(6.0)
2-3	90	125	215	7(7.7)	2(1.6)	9(4.1)
3-4	85	125	210	3(3.5)	2(1.5)	5(2.3)
4-5	88	140	228	1(1.1)	1(0.7)	2(0.8)
5-6	96	144	240	1(1.0)	1(0.6)	2(0.8)
6-7	45	65	110	1(2.2)	0	1(0.9)
7-8	25	32	57	0	1(3.1)	1(1.7)
8-10	16	28	44	0	0	0
10-20	15	16	31	0	0	0
20 <	16	25	41	0	0	0
Total	538	795	1333	19(3.53)	10(1.26)	29(2.17)

* The age-groups are arranged as 0-11 months, 12-23 months, and so forth.

Table II. Comparison of male and female cryptosporidium infected cases.

Sex	No. examined	No. infected	%
Females	538	19	3.5
Male	795	10	1.2
Total	1333	29	2.17

$X^2 = 7.40$ $0.005<p<0.01$

population examined harboured *G. lamblia*, thus the six cases of dual infection with giardia and cryptosporidium are not significantly different from the expected rate for the Ahwaz area. The specific age-groups, with dual giardia and cryptosporidium infection, are shown in Figures 1 and 2. It was found that the most infected age-group in giardiasis were 5-6 year olds and those with cryptosporidiosis were 0-1 year olds. Thus, it seems that there is no association between these two groups in the area. While 9.5% of the population under study were infected with *B. hominis*, the presence of 4 cases of blastocystosis among 29 cryptosporidium infected cases does not seem far from expectation. A similar situation exists for *T. intestinalis*, where its general distribution among the population under study was about 3%.

Cryptosporidiosis is also regarded as a zoonotic infection because domestic and wild animals are able to transmit *C. parvum* to man.[2,3,16,22,24] However, with the exception of two children who were in contact with sheep, goats and calves, the remainder of the cryptosporidium infected cases had no contact with domestic animals, as far as their parents remembered, indicating that animals were not the source of infection of the human population in the area under study.

The majority of *C. parvum* infected cases (28/29), similar to the population under study, used tap-water for drinking. According to the local health authorities, in most parts of Ahwaz, the pipe lines are adjacent to the sewage system. The pollution of tap-water due to the leakage of sewage canals and penetration of their contents into the broken water pipe lines, is not unusual. This finding seems similar to the reports of some workers.[1,2,3,11,12,19,23,26] Thus, filtration of tap-water and/or its boiling may diminish the spread of infection.

During the present survey, we were unable to find cryptosporidium infected cases through August and September (Figure 3), during which the weather temperature is above 45°C and the humidity is below 35% (Figure 4). The seasonal variation of cryptosporidiosis has been reported by some workers,[2,3,21,26] who stated that during the hot months, the cryptosporidium oocysts cannot tolerate temperatures of 45°C and more. During the hot months, the population of flies, especially those of the *Musca* sp.,

Table III. Age distribution of both sexes of *Giardia lamblia* infected patients in Ahwaz.

Age-group (years)*	No. examined			No. infected			
	Females	Males	Total	Females	Males	Total %	%
0-1	18	23	41	0	1	1	2.4
1-2	44	72	116	3	5	8	6.8
2-3	90	125	215	29	24	50	22.8
3-4	85	125	210	34	17	51	24.7
4-5	88	140	228	30	30	60	26.3
5-6	96	144	242	31	46	77	31.8
6-7	45	65	110	9	22	31	28.2
7-8	25	32	57	5	11	16	28
8-10	16	28	44	2	8	10	22.7
10-20	19	16	33	4	3	7	20
20 <	16	25	41	1	4	5	12.2
Total	538	795	1333	145	171	316	23.7

Table IV. Cryptosporidiosis in diarrheic and non-diarrheic cases.

Symptoms	Uninfected	Infected	Total
Diarrheic	704	22	726
Non-diarrheic	600	7	607
Total	1304	29	1333
	X^2= 6.44	0.01 < p < 0.02	

decreases considerably. In contrast to the present finding, Rahman et al.[21] reported that more cases of cryptosporidiosis were detected in the hot and humid months (from April to July). Similarly, seasonality of cryptosporidiosis was noted in diarrheic calves in Bangladesh.[21]

Out of 29 cryptosporidium infected children, females (19/538) were significantly more infected than males (10/795) (X^2=7.40, 0.005<p<0.01), (Table II). The same finding was reported from north-west Iran.[15] These findings are in agreement with the reports of Kwage et al.[11] from Nigeria and Casemore[2] from England. However, Pal et al.[18] indicated no sex differences in Calcutta. Meanwhile, it may be worthwhile to mention that there was a significant difference among sexes infected with *G. lamblia* in our study, (Table III) (X^2= 5.2; 0.01<p<0.02). The reasons for this sex difference are unexplained at present.

Diarrhea was one of the most common clinical features of cryptosporidiosis in the present study. 22 out of 29 cases had diarrhea, while the remainder gave no history of diarrhea (Table IV). Similar findings have been reported by many workers.[1,2,3,4,6,13,15,21] The difference non-diarrheic cases was significant (X^2=6.44; 0.01<p<0.02) (Table IV). All non-diarrheic cases had other clinical symptoms with varying intensity, such as abdominal pain, anorexia, vomiting, headache, and weakness.

With the exception of 2 cases, the remainder of cryptosporidium infected cases had had no contact with animals. This finding suggested that cryptosporidiosis was not necessarily a zoonosis in the area as some workers have previously pointed out.[1,2,3]

ACKNOWLEDGEMENTS

The author wishes to express deep gratitude towards Dr. Ronald Fayer for the confirmation of *Cryptosporidium parvum* identification, as well as his valuable comments on the manuscript, Dr. Ruhi Taghi-Kilani, Dr. K. Kowalewska-Grochowska and Dr. Ravindra Nath Sharma for their constructive and useful suggestions and comments in preparing the manuscript and all of the Khoozestan Parasitology Center staff for their technical assistance.

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