

Prevalence of *Cryptosporidium* Species in Children Referred to Central and Hospital Laboratories of Zabol City, South East of Iran

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Abstract

Background

Cryptosporidiosis has a worldwide distribution, and is the commonest cause of diarrhea in children and immune compromised individuals. Since there is no data available on the prevalence of *Cryptosporidium* species (sp.) in Zabol city, thus this study was carried out to assess the disease prevalence and related factors influencing the disease.

Materials and Methods

In this cross-sectional study, 200 fecal specimens were collected from children referred to the Central or hospital labs in Zabol city, South East of Iran, during April 2014 to August 2016. Fecal examination was performed by staining with Ziel-Neelsen acid-fast to find oocysts of the parasite. The children were grouped according to the age, gender, kind of water supplies, and diarrheic and non-diarrheic condition. Data were evaluated using SPSS version 13.0 software.

Results

Among the children referred to the Central laboratory, 200 fecal samples from different age groups were collected. The prevalence of *Cryptosporidium* species was 9.7% which was higher in children under 4 years. There was a significant relationship between sources of water supply and diarrheic children infected with *Cryptosporidium* ($P<0.05$).

Conclusion

Cryptosporidiosis is the most infectious disease in Zabol city, especially in children. Healthy water supply significantly affects the disease prevalence.

Key Words: Children, *Cryptosporidium*, Diarrhea, Education, Iran.

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1- INTRODUCTION

Cryptosporidiosis causes a wide range of infections in a vertebrate host including humans (1). This parasite is mainly transmitted by the fecal-oral route (2). Water, food and direct contact are a source of infection (3). *Cryptosporidium* attacks the intestinal cell and respiratory system of vertebrate hosts (4, 5). Cryptosporidiosis can cause a self-limited diarrheal disease, but in compromised patients, it can produce serious and persistent diarrhea, and can also be chronic and potentially serious in immune-compromised patients. In some countries, community outbreaks have been reported to be associated with the consumption of polluted water (5).

In developing countries, the seroprevalence of Cryptosporidiosis is in the range of 25 to 35%, and represent up to 15% of gastrointestinal problems especially in children (6). Infection has been reported in all ages from few months old babies to a 90 year-old person. However, many reports indicate that the prevalence is more in children under 5 years old (5). Many reports from Iran show that the prevalence rate in diarrheic children (Iran) was 1.6% in Bander Abbas, 3.25% in Ramser, 5.2% in Tehran, 5.3% in Hemedan, 7.6% in Tonekabon, 9.2% in Neghadea, 10% in Oromia, 14.2% in Mashhad, and 27.2% in Rudhen and Gagerud . As there is no report on Zabol city, this research was designed to assess the disease prevalence and related factors influencing the disease.

Five species of *Cryptosporidium* including *C. hominis* (previously known as *C. parvum* human genotype), *C. parvum* (bovine genotype), *C. meleagridis*, *C. canis*, and *C. felis* have been found to be responsible for most human infections (7).

2- MATERIALS AND METHODS

2-1. Study design and population

In this descriptive study which carried out from April 2014 to August 2016, fecal samples were collected from children referred to Central or hospital labs in Zabol city, Sistan, and Blugestan provinces, South East of Iran. Also, with 5% significant level, 3% error rate and 4.7% prevalence rate (8), the sample size was estimated as 192, but for more confidence, a sample size of 200 was taken. Zabol is the capital of Zabol County, Sistan and Baluchestan provinces in South East of Iran. Zabol lies on the border with Afghanistan and was referred to as Sistan until the late 1920s. The climate of the area is: 28 °C temperature, 42 km/h NW wind, and 25% humidity. Zabol is located near Lake Hamun and Hirmand River which irrigates the region. Lake Hamun is a seasonal lake that is often dry. The Zabol area is well-known for its "120-day wind", a highly persistent dust storm in the summer which blows from North to South (8).

2-2. Methods

The information of participants including age, gender, level of education of parent, kind of water supply, diarrheic or not-diarrheic condition were obtained using a questionnaire. Fecal samples were kept in 10% formalin for further procedures and was kept frozen (at -22°C) until required. Stool smear was prepared by formalin-ether sedimentation, Sheather's sugar procedure and stained with modified acid fast and examined (7).

2-3. Measuring tools

In systemic random sampling, the samples were taken using a randomized table in clusters. In this study, a researcher-made questionnaire was used; for face and content validity, the questionnaire was administered to parasitologists and their recommendations were implemented. The questionnaire was delivered to 20 patients and using Cronbach's alpha coefficient,

the internal reliability (85%) was confirmed.

2-4. Laboratory measurements

The stool samples were collected in clean and labeled boxes and examined upon reception for consistency and concentration. The samples were then mixed with 10% formalin and kept in the refrigerator for further procedures. After smear preparation, fixing with methyl-alcohol was done. All samples were stained with Modified Ziehl-Neelsen.

2-5. Ethical consideration

Informed consent was taken from all participants and the study was approved by the Ethics Committee of the University.

2-6. Data Analysis

Data analysis was conducted with the SPSS version 13.0 software using Chi-square and or/Fisher exact test. A p-value of less than 0.05 was considered as significant.

3- RESULTS

From the 200 samples examined over a period of six months, positive oocyst specimen was detected in 18 samples and prevalence was 9.7%. For prevalence by gender, the results showed that from 200 samples, 106 (52%) children are female

while others (47%) are male. There was no significant relationship between parasite rate and gender, using the Chi-square test ($p>0.05$) (**Table.1**). The prevalence of *Cryptosporidium* oocyst showed that the highest infection was recorded in children under 4-year of age (**Table.1**). Also, Chi-square test showed that there is a significant relationship between age and parasite prevalence ($p=0.01$).

In correlation with kinds of water supplies, there was a significant relationship between children who consumed untreated pipe-borne drinking or recreational water and those who used purified water from bottle or used treated pipe drinking water in the villages around the city with purified water system ($p <0.05$) (**Table.2**). In children who have diarrhea and non-diarrhea, there was a significant relationship between infected and diarrheic children ($p<0.05$) (**Table.3**).

Children who were not breast-fed were significantly more commonly infected (83.3%). Although some of them have both *Cryptosporidium* spp. and *Giardia lamblia*, but there was no relationship between the two infections ($p>0.05$). In relation to the severity of cryptosporidiosis, children who were breast fed, only two children (1.4 %) had a slight infection (**Table.4**).

Table-1: The prevalence of *Cryptosporidium* spp. infection in participants (by gender and age group)

Gender	Positive number	Negative number
Female	11(10.4)	95(89.6)
Male	7(7.4)	87(92.6)
Age group, (year)	Positive number	Negative number
0-4	16(8%)	105(52.5)
>4	2(1%)	77 (38.5)

Table-2: The prevalence of *Cryptosporidium sp.* infection in the participants (related to kinds of water supplies)

Water supplies	<i>Cryptosporidium</i>		P-value
	Positive, n (%)	Negative, n (%)	
Water supplies other than pipe	16(14.5%)	94(85.5%)	0.009
Pipe water supplies	2(3.3%)	58(96.7%)	
Mineral water or/filtered water	0	30 (100%)	

Table-3: The prevalence of *Cryptosporidium spp.* infection in the participants (related to diarrheic and non-diarrheic condition)

Variables	<i>Cryptosporidium</i>		P-value
	Positive, n (%)	Negative, n (%)	
With diarrhea	16	50	0.000
Without diarrhea	2	132	

Table-4: The severity of Cryptosporidiosis in the participants (in relation to breastfeeding)

Mother milk condition/Cryptosporidiosis	Slight infection, Number (%)	Moderate infection, Number (%)	Sever infection, n (%)	Summation, Number (%)
Mother- milk used	2	1	0	3
No mother- milk used	7	5	2	14

4- DISCUSSION

It is very difficult to determine the prevalence rate of cryptosporidiosis, since there are no data available from many countries. In one estimate, the prevalence of *Cryptosporidium* in patients with gastroenteritis was 1-4% in Europe and North America, and 3-20% in Africa, Asia, Australia, and South and Central America (9). Laberge et al. estimated that the prevalence rates of *Cryptosporidium spp.* were 1- 3% in industrialized countries, and up to 10% in developing countries (based on oocyst excretion) (10). Cryptosporidiosis exists in more than 106 countries in the world (10). At current study, the prevalence rate of *Cryptosporidium spp.*, in children was 9.75%. The majority of infection appeared to be correlated with water consumption. Many studies have emphasized that

Cryptosporidium spp. is occasionally found in untreated water (2). Results of present study showed that children who used treated or purified water, the infection rate of *Cryptosporidium* was zero as the majority of infection was seen in children who used unsafe (untreated) water. In this study, children were randomly selected and two presented with no diarrheic and gastrointestinal signs. Thus there was a significant association between diarrhea occurrence and *Cryptosporidium spp.* infection ($P < 0.05$). Results also showed that mineral water and pipe water are moderately good, but water supplies other than pipe is not suitable. The high prevalence of one subtype of *Cryptosporidium* in calves and the frequent detection of this subtype in humans suggests that parasite fitness probably plays an important role in the

transmission of cryptosporidiosis among cattle and in zoonotic infections (10). The vast majority of human cryptosporidiosis is mainly caused by two species, *C. parvum*, and *C. hominis*, but some other species of this parasite can infect humans too; *C. parvum* is more prevalent in rural or agricultural regions, probably as a result of zoonotic transmission (11, 12). Since Zabol is a cow breeding region, the study predicts a high probability of *C. parvum* species infection. This study reports a prevalence rate higher than in Zahedan and Gonbad-e Kavos and Isfahan cities with prevalence rates of 4.7%, 4.9%, and 4.6%, respectively (5, 13, and 14). In Bandar, Abbas Pediatric Hospital, Southeastern Iran, the prevalence rate of *Cryptosporidium* infection was 7% (13).

One study (15) emphasized that the infection rate of *Cryptosporidium spp.* in children less than 1 year is the least whereas Pal et al. (16) emphasized that infection rate in children between 0-6 months old is more than in other groups (5). With regards to the prevalence of Toxoplasma antibodies in Ahwaz, the seropositive rate was lowest in new-born and children under 6 months of age and highest in persons 14-19 years old (15). In the present study, the most diarrheic children were under 4 year-old which is similar to previous study (15) that reported in Ahwaz city, only 3 cases were less than six months of age. Conversely, in the study of Tahira (India), the highest prevalence of *Cryptosporidium spp.* was found in the age group of 2– 4 years (18.36%) (17).

In our study, it was detected that breast-fed infants were less susceptible to infection. Interestingly, this study identified a smaller percentage of asymptomatic infections. Some researchers confirmed that the effect of asymptomatic cryptosporidiosis was less severe in these children, but they also gained less weight than the controls (18). The epidemiology of this parasite is uncertain in many parts

of the world, including Iran and more studies are needed to establish a relationship with the prevalence and control, especially in microhabitat without any systemic water treatment.

4-1. Limitations of the study

Some children and infants were not accessible due to migration or expatriation and some information had to be dismissed.

5- CONCLUSION

The present study is the first record of Cryptosporidiosis among children in Zabol city, in South East of Iran. It clearly demonstrated a high prevalence rate of Cryptosporidiosis among children of less than 4-year old in this region. More caution should be shown, especially in water consumption which is one of the most important sources of disease.

6- CONFLICT OF INTEREST: None.

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