CRYPTOSPORIDIUM PARVUM PREVALENCE IN A GROUP OF TURKISH CHILDREN*

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SUMMARY: Akyön Y, Ergüven S, Arıkan S, Yurdakök K, Günalp A. (Departments of Microbiology and Clinical Microbiology, and Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Cryptosporidium parvum prevalence in a group of Turkish children. Turk J Pediatr 1999; 41: 189-196.

Stool samples from two hundred children with diarrhea and from 50 healthy children were examined, by modified Kinyoun's acid-fast staining (MAF), Giemsa staining and direct (DFA) and indirect immunofluorescence antibody (IFA) methods, in order to determine cryptosporidiosis prevalence under the age of 12 and to detect the most efficient identifying method for use in our country. Cryptosporidium oocysts were detected in seven (3.5%) of the cases. None of the samples from the control subjects was found to be positive for Cryptosporidium. Our results indicate that Cryptosporidial oocysts should be detected in children with diarrhea. Modified Kinyoun staining method is practical and reliable for this purpose. Immunofluorescence staining methods can be applied for conformation of the results, if available. *Key words: cryptosporidiosis, children.*

A small intracellular protozoon, Cryptosporidium parvum, which causes infection in humans and animals, belongs to the phylum Apicomplexa, subclass Coccidiasina[1]. The first case of human cryptosporidiosis was reported in an immunocompetent three-year-old girl, in 1976. Now, "cryptosporidiosis" is accepted as a zoonose, causing infection in humans[2]. Cryptosporidium parvum infects epithelial cells of the gastrointestinal tract in humans and animals. Transmission of cryptosporidiosis from person-to-person is well defined. Day-care centers and nosocomial outbreaks play an important role in the spread of Cryptosporidium parvum oocysts[3, 4].

Cryptosporidiosis is mostly prevalent in developing countries where malnutrition is a predisposing factor. It causes persistent diarrhea in children and the elderly and prolonged severe or fatal diarrhea in immunocompromised individuals.

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Infection is self-limited in immunocompetent individuals. It is the causative agent of diarrhea in children, especially under the age of two, during the summer and fall[5].

The prevalence of cryptosporidiosis is one to two percent and three to 20 percent in developed and developing countries, respectively[6]. In Turkey, the prevalence varies according to the geographic region[7,8]. In this study, our aim was to detect this protozoon in children under the age of 12 with diarrhea and to determine the most efficient identifying method for use in our country.

Material and Methods

Between July 1995 and January 1997, stool samples were collected from 200 children with diarrhea, who were referred to the Children's Diarrhea Unit, Hacettepe University İhsan Doğramacı Children's Hospital. They were aged between 13 days and 12 years. Fifty healthy children in the same age group were also included in the study as the control group. A questionnaire, requesting the age, gender, weight of the child, duration of diarrhea, daily number of defecations, form of the stool, presence of diarrhea in any other member of the family, usage of antibiotics, presence of fever, vomiting, nausea, dehydration, or underlying illness, and usage of breast-feeding, was presented to the patient and control groups.

Three stool samples were collected from each patient, separately preserved in 10 percent formalin and stored at -30 °C until examined. In order to provide the same conditions the stool samples were examined in groups of 20.

The stool preparations were stained with Giemsa and modified Kinyoun's acid-fast (MAF) methods (Fig. 1) and were examined under light microscope as described previously[9].

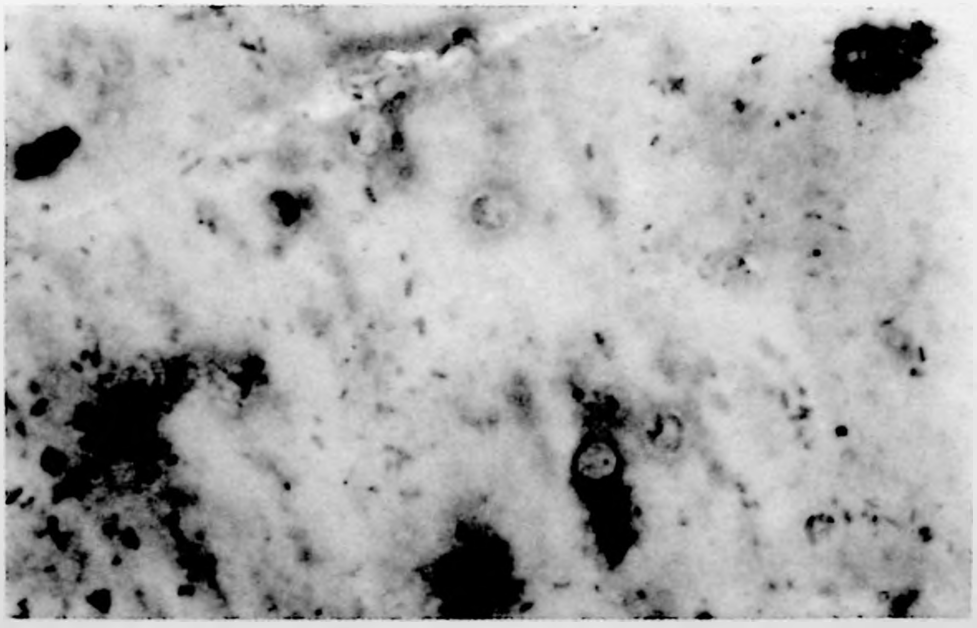

Fig. 1: Cryptosporidium oocysts by MAF (x 1000).

Direct (DFA) and indirect immunofluorescence antibody (IFA) staining methods were performed for each specimen. Merifluor Cryptosporidium/Giardia kit (Meridian Diagnostics Inc., Ohio 45244) was used for DFA and examined under the fluorescence microscope according to the manufacturer's instructions[10]. For the IFA test, mouse monoclonal antibodies (IgG and IgM culture supernatants) specific to Cryptosporidium oocysts, which were kindly provided from San Francisco General Hospital, Divison of Infectious Diseases, and the fluorescein isothiocyanate (FITC) conjugate "anti-mouse polyvalent immunoglobulins IgG, IgA, IgM (Sigma F1010) were used. The test was performed according to the instructions of Merifluor Cryptosporidium/Giardia kit and the results were interpreted under the fluorescence microscope, as described previously[11].

For routine parasitological examination stool samples of all of the children included in the study were examined by standard methods for the existence of egg and/or cyst forms of commonly encountered parasites other than Cryptosporidium[12].

Results

Stool specimens from 200 children with diarrhea were examined. In seven of the cases (3.5%) Cryptosporidium oocysts were detected. In 10 children, Giardia intestinalis cysts were detected (in 1 case together with Cryptosporidium oocysts). Taenia eggs, Hymenolepis nana eggs and Entamoeba histolytica trophozoites were detected, on each in the stool samples of three separate cases.

Cryptosporidium oocysts were not detected in any of the 50 children who were included in the study as the control group. Ascaris lumbricoides and Hymenolepis nana eggs were detected in one child and Giardia intestinalis cysts in four children.

Table I summarises the clinical features and results of the Cryptosporidial oocyst detection methods. As can be seen, there was an underlying disease in three of the patients whose stool samples yielded Cryptosporidium oocysts (hyper IgM syndrome, hypogammaglobulinemia, celiac disease). In these three cases the duration of diarrhea was 30 days, 10 years (intermittent) and 20 days, respectively. Cryptosporidium positivity could be detected using all the methods performed.

The Cryptosporidium oocysts were detected in May, June, July, September and October. Except for the seventh case, in whom there was also moderate dehydration, none were breast-fed. In the third case, Giardia intestinalis cysts, Hymenolepis nana eggs and Entamoeba histolytica trophozoites were detected in addition to the Cryptosporidium oocysts. In the second case, Cryptosporidium oocysts were detected for four months. While treated with Paromomycin, oocysts were absent in the stool samples, but upon cessation of treatment, diarrhea and oocyst excretion resumed. In the fifth and sixth cases, positivity was determined by staining and indirect IFA methods; in the 1st case it was shown only by staining.

Table I: Clinical Features and Detection Methods of Diarrheal Cases with Cryptosporidium Oocysts

Case	Age	Gender	Clinical Features	Staining Methods		Immunofluorescence	
				Giemsa	MAF	Direct	Indirect
1	2.5	F	Watery, mucus-containing diarrhea, for only one day 5-6 times/day	±*	+	–	–
2	4	M	Prolonged, yellowish, watery diarrhea, vomiting, hyper IgM syndrome	+	+	+	+
3	12	M	Watery intermittent diarrhea, for 10 years 4-5 times/day, hypogammaglobulinemia, malabsorption	+	+	+	+
4	2.5	F	Mucus-containing diarrhea, vomiting for 3 days 4-5 times/day	±*	+	+	+
5	16 mo**	M	Watery, mucus-containing diarrhea, vomiting, for 5 days 7-8 times/day	±*	+	–	+
6	3	M	Mucus-containing diarrhea, for only one day 3 times/day	±*	+	–	+
7	5	M	Watery diarrhea, for 20 days 14 times/day, celiac disease	+	+	+	+

* Structures resembling Cryptosporidial oocysts, but not exactly typical.
** months.

Discussion

In recent years Cryptosporidium species have become important protozoal infectious agents, especially in HIV (human immunodeficiency virus) infected patients. Person-to-person transmission and/or spread from water is well defined[9].

This protozoon is especially prevalent in developing countries where malnutrition is common. The agent is mostly detected in children; in adults it is mostly seen in immuncompromised patients as a causative agent of diarrhea[13].

Another water-spread agent is Giardia intestinalis. In most of the cases, these two protozoa can be detected together[14]. Giardia intestinalis prevalence is high in Turkey, therefore Cryptosporidium prevalence is also expected to be high[15]. As the routine parasitological methods are not sufficient for determining the oocysts of Cryptosporidium, this study was planned to determine the most effective method in view of our laboratory conditions[16].

In stool samples of seven of the 200 children (3.5%) with diarrhea who were admitted to Hacettepe University İhsan Doğramacı Children's Hospital, Cryptosporidium oocysts were detected. Based on the detection methods used, C. parvum is now accepted as one of the most common enteropathogens causing diarrhea in the world, mostly in developing countries. Prevalence has been reported in Europe as one to two percent and in North America as 0.6-4.3 percent. In Asia, Africa, Australia, and Central and South America it has been reported as between three to 20 percent. The studies show that the prevalence is higher in children two years old or younger when compared with adults[1]. In the United Kingdom, in acute or chronic diarrheal children, the prevalence has been reported as 3.2 percent. In Venezuela, in children under the age of two with diarrhea it was 10.8 percent. In Ghana in infants two to 12 months of age it was 21.6 percent, and in Haitian acute diarrheal children the rate was 16.7 percent[14, 17-20].

In Turkey, in a study performed in İzmir (West Anatolia) involving 600 children aged between zero to six years, Cryptosporidium oocysts were only detected in a 15-month-old child, together with Giardia intestinalis cysts[7]. In the Adana region (southeast), the percentage was 8.2 in diarrheal (n = 110) and 4.08 in non-diarrheal (n = 98) children[8]. In İstanbul (northwest) in 100 acute diarrheal cases it was two percent (17 months and 2 years of age)[21]. In Bursa region (northwest) the rate was 2.9 percent and in İstanbul (northwest) oocysts were detected in 1.36 percent of diarrheal children under five years of age. Investigators reported that this low percentage may be due to breast-feeding, good sanitary conditions and little animal contact[22, 23].

Modified Kinyoun's acid-fast staining method and the immunofluorescence technique are the most common diagnostic tools for Cryptosporidium oocyst detection in Turkey and other countries. Recently, enzyme-linked immunosorbent assay has also been used for detection of oocysts[24]. In addition, detection of oocysts from water samples by polymerase chain reaction can be used[25].

In our study, Cryptosporidium oocysts could only be detected in 3.5 percent of diarrheal children, which is similar to rates from other reports from Turkey. This result shows that Cryptosporidium can also be a causative agent of diarrhea in children.

Cryptosporidiosis can be seen in immunocompromised patients throughout the year, whereas in immunocompetent persons it is common during the summer and fall[24, 26].

Although the study was carried out for seven months, the seasonal distribution was found to be similar to the previous reports.

We defined Cryptosporidium oocysts in three immunocompromised patients (hyper IgM syndrome, hypogammaglobulinemia and malabsorption, and celiac disease). Some investigators have detected Cryptosporidium oocysts in diarrheal children with malnutrition, depressed cellular immunity, congenital hypogammaglobulinemia and primary immunoglobulin deficiency[27-30]. These findings emphasize that in chronic malabsorption cases and immunocompromised diarrheal children, Cryptosporidium oocysts should be investigated.

Laboratory diagnosis of this protozoon can be established by various techniques. Modified acid-fast (MAF) staining technique is superior to the Giemsa staining method in differentiating the oocysts from yeast cells[31]. In recent years with the discovery of the monoclonal antibodies specific to the oocyst wall, DFA and IFA methods are being used and are more sensitive.

In many studies the methods of detecting Cryptosporidium oocysts in stool samples have been compared. Some studies revealed that immunofluorescence methods are superior to the staining methods, as they can detect the oocysts even if the number is very low. Due to the prolonged application of the stain there may be false positive results; therefore, the sensitivity of the MAF staining method is low[11, 32-33].

Although immunofluorescence methods are rapid, some reports claim that there can be false positive results, because of the non-specific fluorescence[16, 34]. In our study, staining and immunofluorescence methods were positive in four cases. In one case, oocysts were detected only with the staining method, and this was considered a false positive result.

In conclusion, for the detection of Cryptosporidium oocysts, the MAF staining method is an easily performed and reliable method in immunocompromised children with chronic diarrhea. If possible, for conformation of the staining results, the immunofluorescence method should be performed.

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