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مطالعه اپیدمیولوژی کریپتوسپوریدیوز در کودکان مبتلا به اسهال در استان چهارمحال و بختیاری

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چکیده

مقدمه: عفونت کریپتوسپوریدیوم به‌عنوان عامل اصلی شیوع بیماری‌های منتقله از راه غذا و آب شناخته شده که نگرانی‌های بهداشت عمومی را در کشورهای توسعه‌یافته و درحال توسعه ایجاد کرده است. در مطالعه حاضر، شیوع و عوامل خطر ابتلا به کریپتوسپوریدیوم در کودکان مبتلا به اسهال در جوامع شهری و روستایی استان چهارمحال و بختیاری بررسی شدند.

مواد و روش‌ها: در مجموع، ۴۰۰ نمونه به‌صورت تصادفی از مدفوع کودکان مبتلا به اسهال مراجعه‌کننده به بیمارستان‌های شهرکرد و آزمایشگاه‌های تشخیص طبی گرفته شدند. بخشی از نمونه برای تست PCR، فریز و بخشی برای رنگ‌آمیزی زیل نلسون تغییریافته استفاده شد. نمونه‌هایی که از لحاظ میکروسکوپی آلودگی به کریپتوسپوریدیوم در آنها مثبت تلقی شدند، برای تعیین سویه انگل نیز با تست PCR ارزیابی شدند.

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نتایج: میزان شیوع کریپتوسپوریدیوم در نمونه‌های گرفته‌شده ۳/۵ درصد بود. همچنین نتایج نشان دادند ۸۵ درصد کودکان مبتلا به کریپتوسپوریدیوز در سن زیر ۶ سال و تنها ۱۵ درصد در سن ۶ تا ۱۲ سال بوده‌اند ($P<0.05$). براساس نتایج، میزان شیوع کریپتوسپوریدیوم در روستاییان، ۵ درصد و در شهر حدود ۲ درصد است ($P<0.05$). همچنین، ۹۵ درصد روستاییان مبتلا و ۸۵ درصد افراد شهری مبتلا، در یک ماه قبل از مراجعه، حداقل یک بار تماس با حیوان داشته‌اند ($P<0.05$). نتایج بیان می‌کنند میزان شیوع در فصل‌های بهار و تابستان بیشتر از فصول سرد بوده است ($P<0.05$)؛ اما بین دو جنس مذکر و مؤنث تفاوت آماری معنی‌داری مشاهده نشد ($P>0.05$). نتایج آزمون PCR نشان دادند همه نمونه‌های مثبت تک‌یاخته کریپتوسپوریدیوم پاروم بودند.

بحث و نتیجه‌گیری: به‌طور کلی نتایج نشان دادند میزان شیوع و بروز کریپتوسپوریدیوز در کودکان با عواملی مانند سن، سبک زندگی، تماس با حیوان و فصل ارتباط دارد. همچنین گونه غالب در این منطقه کریپتوسپوریدیوم پاروم است.

واژه‌های کلیدی: کریپتوسپوریدیوز، اسهال، کودکان، PCR آشیانه‌ای، چهارمحال و بختیاری

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An Epidemiological Study of Cryptosporidiosis in Children with Diarrhea in Chaharmahal and Bakhtiari Province

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Abstract

Introduction: Cryptosporidium infection, which has been identified as a major cause of outbreaks of food and waterborne diseases, has raised public health concerns in developed and developing countries. In the present study, the prevalence and risk factors of cryptosporidium in diarrheal children in urban and rural communities of Chaharmahal and Bakhtiari province were investigated.

Materials and Methods: A total of 400 samples were randomly collected from the feces of children with diarrhea referred to Shahrekord hospitals and medical diagnostic laboratories. Stool samples were taken from the patients and part of the sample was frozen for the PCR test and part was used for modified Ziehl Neelsen staining. Samples in which cryptosporidium contamination was considered positive in terms of microscopy were also evaluated by the PCR test to determine the parasites species.

Results: The prevalence of Cryptosporidium in the collected samples was 3.5%. The results also showed that 85% of children with cryptosporidiosis were under 6 years old and only 15% of patients were between 6 and 12 years old ($P < 0.05$). According to the results, the prevalence of Cryptosporidium was 5% in rural areas and 2% in urban areas ($P < 0.05$). Also, 95% of infected villagers and 85% of infected urban children had contact with animals ($P < 0.05$). The results showed that the prevalence was higher in spring and

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summer than in cold seasons (P <0.05). But the results revealed that there was no statistically significant difference between males and females affected (P> 0.05). The PCR test results showed that all positive samples were Cryptosporidium parvum.

Discussion and Conclusion: *Cryptosporidium parvum* is the predominant species in Chaharmahal and Bakhtiari province, which is more prevalent in children under 6 years old, in warm seasons and rural areas, and in people who have more contact with animals.

Key words: Cryptosporidiosis, Diarrhea, Children

Introduction:

Cryptosporidiosis is caused by the species Cryptosporidium, a parasite that has been classified as a pathogen by the Centers for Disease Control and Prevention (CDC). The organism infects the gastrointestinal epithelium and causes diarrhea, which is a self-limiting infection in healthy people but is life-threatening in people with immunodeficiency such as children, and people with acquired immunodeficiency syndrome (AIDS). Infection with this parasite accounts for up to 6% of all diarrheal diseases in people with weakened immune systems. The infection is also present in 24% of people with AIDS and diarrhea worldwide (1, 2). Cryptosporidium is an intracellular parasite in the Apicomplexa group. Ten species are now known based on differences in host characteristics and oocyte morphology. *Cryptosporidium parvum* is the most common species in humans. *C. felis*, *C. muris*, and *C. meleagridis* have also been identified in immunocompromised individuals. Cryptosporidium is can complete all stages of its development (asexual and sexual) in one host. Humans become infected when they ingest cryptosporidium oocytes, oocytes rupture in the gastrointestinal tract, releasing infectious sporozoites. The sporozoite attaches to the apical membrane of the host epithelial cell. Such a transplant reorganizes the host cell actin cytoskeleton and protrudes the host cell membrane around the sporozoite to form a vacuole in which the organism remains intracellular but outside the cytoplasm (3, 4, 5). The sexual form of the parasite repeats the life cycle after fertilization in the gastrointestinal tract, producing thin-walled oocysts that release sporozoites upon re-sporulation. This condition can lead to spontaneous infection and severe and persistent infections with widespread oocyst shedding in the feces of an infected patient (6, 7, 8). Cryptosporidium became one of the most common intestinal pathogens in immunocompromised individuals worldwide in 1976, when it was first reported as human infection. The total proportion of egg-laying populations in developed countries is 1 to 3% and in developing countries 10%. Cryptosporidiosis is an important clinical problem in patients without access to antiretroviral therapy and in malnourished children, as well as in people undergoing transplantation or chemotherapy. Infection is often transmitted from person to person, by animals, and indirectly through the environment (especially water). Transmission from cattle and sheep to humans is well known and these animals are currently the most important reservoirs of human disease. Cryptosporidium oocysts can be found in all types of water, including untreated surface water, swimming pool water, and even chlorinated or disinfected drinking water, or food (9, 10, 11). The widespread prevalence of contaminated water and food has affected almost the entire world. Although its pathogenesis is weak, there is currently no fully effective treatment for this infection. Currently, there

are limited and scattered reports of the frequency of this protozoan in Iran, but the results showed that the rate of cryptosporidium infection in Iran has a wide range between 0.83 to 24% (12).

Due to the health importance of this protozoan, especially in children with diarrhea, the present study was designed and performed to investigate the prevalence of cryptosporidiosis in children with diarrhea in Chaharmahal and Bakhtiari province.

Materials and Methods

Sampling: The present cross-sectional study was performed in Shahrekord. Stool samples of children with diarrhea during autumn, winter, spring, and summer of 1399 (2020) were collected from the pediatric ward of Ayatollah Kashani Hospital and Shahrekord Central Laboratory. In addition to urban children, rural children were also referred to this hospital. Sampling was performed in the age group under 12 years which was divided into two age groups of 1-6 and 7-12 years. In each season, 100 samples and a total of 400 samples were taken from patients with diarrhea for one year. To find out the factors affecting cryptosporidia infection, as well as a comparison between the factors considered in this study, a questionnaire containing information such as sex and age of the infected person, referral season, contact with the animal, place of residence was completed by patients.

Identification of Cryptosporidia: The Modified Ziehl Neelsen staining was used to detect Cryptosporidium in feces. Briefly, one gram of feces was first mixed well with 11 ml of 1.5% saline and then filtered and transferred to centrifuge tubes. One millimeter of ether was then added to each tube and centrifuged at 35 rpm for 11 minutes. A smear of the precipitate was prepared on the slide, and after drying, a few drops of ethyl alcohol were added and placed in an oven at 50 ° C for 2 minutes to dry completely. The smears were then immersed in 96% methanol for 3 minutes to be completely fixed, heated over a flame for a few seconds, and immersed in Karbel Fuchsin (0.34% Fuchsin and 4% Phenol) for 20 minutes. In the next step, they were placed in 5% sulfuric acid, and finally, 5% malachite green was used. Stained smears were evaluated by light microscopy for the presence of cryptosporidium parasite oocysts. In the microscopic evaluation for each sample, five microscopic fields were studied, and based on the average number of oocysts counted, the severity of infection was + (1-4 oocysts), ++ (5-25 oocysts) and +++ (more than 25 oocysts) were determined in each microscopic field (13).

DNA Extraction: The QIAamp mini stool test kit was used according to the manufacturer's instructions with minor modifications. Briefly, 200 mg of each stool sample was poured into an Eppendorf tube and stored frozen at minus 20 ° C. Before thawing, completely dissolved in 1.4 ml of ASL buffer contained in the extraction kit. The samples were then boiled for 5 minutes and then subjected to three cycles of freezing in liquid nitrogen for 1 minute and boiling for 2 minutes to destroy the cyst wall and release DNA. It was then centrifuged at 13,000 rpm for 1 minute and 1.2 ml of supernatant was pipetted into a new tube, and an inhibitory tablet was added to remove possible PCR inhibitors. After incubation at room temperature for 1 minute and two centrifuges at 13000 rpm for 3 minutes, 200 mg of supernatant was mixed with proteinase K (final concentration 1 mg/ml) and 200 µl of AE buffer. After vortexing the samples, incubation was performed at 70 ° C for 10 minutes and 200 µl of 96% ethanol was added, and the samples were vortexed again. They were then centrifuged at 13,000 rpm for 1 minute. After washing

with buffers and centrifugation twice, DNA was removed from the silica column for 1 minute using 200 µl AE buffer and centrifugation at 13,000 rpm.

To control the mechanical disturbance, the negative stools were divided into several sections of 200 mg and used for DNA extraction according to the above protocol in parallel with the samples without three freezing and boiling cycles (14).

Nested PCR: PCR reaction with a volume of 50 µl including 10 mM Tris-HCl (pH 8.3), 50 mM KCl solution, 2.5 mM MgCl2 solution, 0.5 µM of each external primer (Table 1), 1.5 U of Taq polymerase enzyme, and µM 100 nucleotides were performed. For the second step, the solutions were the same as mentioned, except that 1µl of the first PCR product and 0.5 µM of each of the internal primers (Table 1) were added. Reaction mixtures with external primer set once at 94 ° C for 5 minutes, then 35 times at 94 ° C for 30 seconds, 50 ° C for 30 seconds, and 72 ° C, respectively. The steps were performed for 1 minute and then the final extension step was performed at 72 ° C for 5 minutes. For Nested PCR products, the thermal cycle was performed as follows: once at 94 ° C for 5 minutes, 30 times at 94 ° C for 30 seconds, and at 72° C for 1 minute, then Once at 72 ° C for 5 minutes (14, 15).

Table 1- Primer Sets used in the Identification of *C. parvum* 18S rDNA

Primers	Sequence (5'-3')	Size (bp)
Outer set	F: AAA CCC CTT TAC AAG TAT CAA TTG GA R: TTC CTA TGT CTG GAC CTG GTG AGT T	676
Inner set	F: TGC TTA AAG CAG GCA TAT GCC TTG AA R: AAC CTC CAA TCT CTA GTT GGC ATA GT	285

Data Analysis: The data are presented by descriptive statistics and related to factors such as age, sex, location, and contact with animals using the Chi-square test and Fisher's exact test by Sigma stat 4 statistical software. P <0.05 is considered a significant difference.

Results

The results obtained from the modified Ziehl Neelsen staining showed that out of 400 samples, 14 samples (3.5%) contained Cryptosporidium protozoa (Fig. 1).

Based on the results, the rate of infection was 3.7% and 3.3% in males and females, respectively. The results of the statistical test showed no statistically significant relationship between sex and the rate of cryptosporidium infection (P> 0.05). (Table 2).

Among those who tested positive for cryptosporidium, 12 (5.5%) were under 6 and 2 (1.1%) were over 6 years old. There was a statistically significant difference between the infection in the ages under 6 and more than 6 years old (P <0.05) (Table 3).

Out of 400 samples, 208 samples were related to rural cases and 192 samples were related to urban cases. Among the positive cases of Cryptosporidium, 4 samples (2.1%) were related to urban individuals, and 10 samples (4.9%) were related to rural communities. The results of the statistical test showed a significant relationship between habitat and cryptosporidiosis (P <0.05) (Table 4).

The results showed that the highest prevalence of cryptosporidiosis was in summer (6%) and the lowest was in winter (1%) (Table 5).

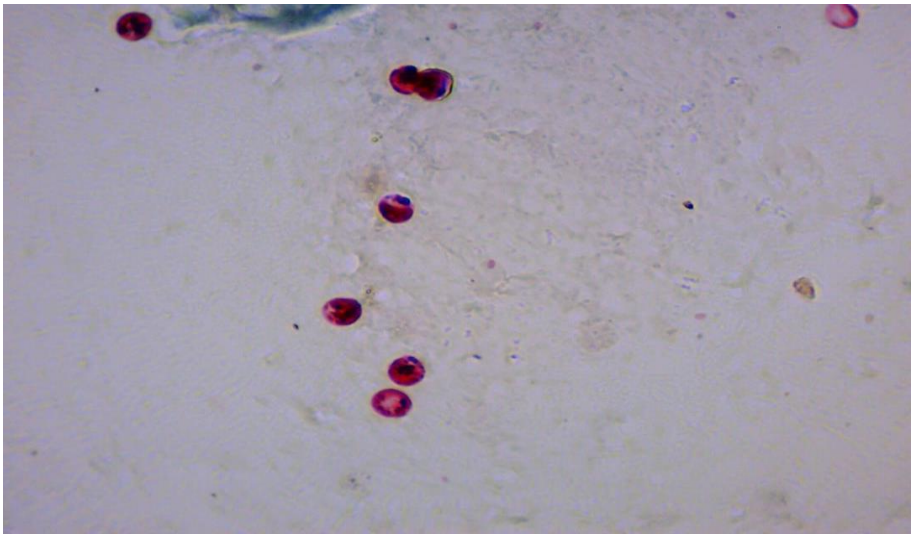

Fig. 1- Cryptosporidium oocysts Staining by the Modified Ziehl Neelsen Method

Table 2- Frequency of Cryptosporidium Infection between the Sexes in Children with Diarrhea

Gender	No of sample	Positive	Negative
Male	220	8 (3.7%) [a]	212 (96.3%)
Female	180	6 (3.3%) [a]	174 (96.7%)
Total	400	14 (3.5%)	386 (96.5%)

Similar letters in each column indicate no statistically significant relation (P> 0.05)

Table 3- Frequency of Cryptosporidium Infection at Different Ages in Children with Diarrhea

Age	No of sample	Positive	Negative
Up to 6	221	12 (5.5%) [a]	209 (94.5%)
6-12	179	2 (1.1%) [b]	177 (98.9%)
Total	400	14 (3.5%)	386 (96.5%)

Different letters in each column indicate a statistically significant relation (P< 0.05)

Table 4- Prevalence of Cryptosporidium Infection in Urban and Rural Children with Diarrhea

Location	No of sample	Positive	Negative
Rural	208	10 (4.9%) [a]	198 (95.1%)
Urban	192	4 (2.1%) [b]	188 (97.9%)
Total	400	14 (3.5%)	386 (96.5%)

Different letters in each column indicate a statistically significant relation (P< 0.05)

Table 5- Frequency of Cryptosporidium Infection in Children with Diarrhea in Different Seasons

Season	No of sample	Positive	Negative
Spring	100	4 (4%) [a]	96 (96%)
Summer	100	6 (6%) [b]	94 (94%)
Autumn	100	3 (3%) [a]	97 (97%)
Winter	100	1 (1%) [c]	99 (99%)
Total	400	14 (3.5%)	386 (96.5%)

Different letters in each column indicate a statistically significant relation (P< 0.05)

Data analysis showed that 6.7% of infected children had been in contact with the animal in the previous month, and 1.9% had no contact with the animal. The results of the statistical test showed that there was a significant relationship between the contact with the animal and infection with Cryptosporidium (P <0.05) (Table 6).

In the PCR test, the bands of the 676 bp and 285 bp region represent the nucleotide chain selected to confirm the presence of

Cryptosporidium parvum contamination in the samples. The results of individuals with positive results for the presence of Cryptosporidium oocysts in their feces showed that all individuals were infected with *Cryptosporidium parvum* (Fig 2).

Table 6- Frequency of Cryptosporidium Infection in Children with Diarrhea and its Relationship with Animal Contact

Contact with animal	No of sample	Positive	Negative
Contact	135	9 (6.7%) [a]	126 (93.3%)
No contact	265	5 (1.9%) [b]	260 (98.1%)
Total	400	14 (3.5%)	386 (96.5%)

Different letters in each column indicate a statistically significant relation (P< 0.05)

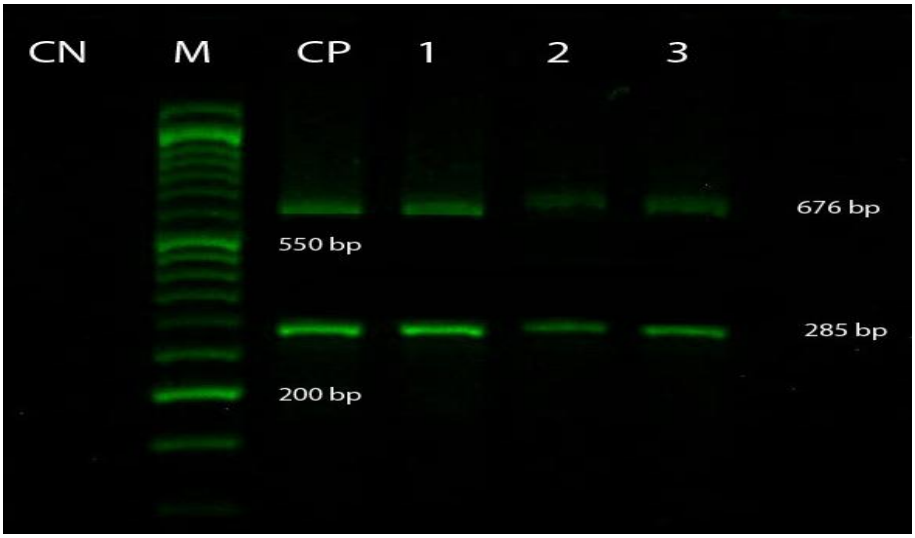

Fig. 2- The Nested PCR Test Results to Identify *Cryptosporidium Parvum*. M marker, CN negative control, CP positive control, 1, 2, and 3 positive samples. 676 bp band belongs to outer primer and 285 bp band belongs to inner primer.

Discussion

Cryptosporidiosis is caused by an obligatory intracellular parasite, which was first identified in 1907 as an opportunistic pathogen. Cryptosporidium infection raises public health concerns in developed and developing countries. Globally, it appears to be more prevalent in the United States, Canada, Australia, and Europe, especially the United Kingdom, Germany, and Ireland. Cryptosporidiosis was first reported in a rural child and an immunocompromised individual and is now reported in more than 90 countries on all continents (16). Studies show that *Cryptosporidium parvum* and *Cryptosporidium hominis* are present in children and immunocompromised ones (17). Based on the results of the present study, the prevalence of cryptosporidiosis in children with diarrhea was 3.5%. The infection rate was 3.7% in males and 3.3% in females. Other studies in Iran and other countries also show that the prevalence of this parasite varies depending on the region. In a review study in 2017, information on the prevalence of Cryptosporidium in Iran was comprehensively reviewed and summarized. The prevalence of cryptosporidiosis in children was 3.65% and in other people with diarrhea was 2.94%. Also, the infection rate in healthy individuals and immunocompromised patients was 1.29 and 54.4%, respectively (12). In another study conducted in Tehran, the rate of this infection was 2.57% in

patients with gastroenteritis (18). In Urmia, during a study, the prevalence of this parasite in people admitted to the ward of kidney patients was reported between 3.88 to 11.5% (19). In other countries, the prevalence of this parasite has been reported differently, a study in different parts of India in children with diarrhea showed that the prevalence of this parasite is from 2.55% to 18.5% (20). Also in another report from China, the cases of this infection ranged from 8% to 16.44% (21).

The results of the present study showed that the infection rate of Cryptosporidium parasite in children under 6 years is 5.5% and in children over 6 years is 1.1%. Other studies in Iran have also confirmed the relationship between children's age and infection with this parasite. In 2017, Reza Brahmat et al., in a review study reported the prevalence of cryptosporidiosis in children and healthy individuals as 3.65 and 1.29%, respectively (12). In other countries such as India and China, children are more infected with the cryptosporidium parasite than adults (20, 21). According to the results of the present study, the infection rate of Cryptosporidium parasite in children living in rural areas was 4.9% and in children living in urban areas was 2.1%. The reasons for the higher level of infection in rural areas can be due to two reasons. First, the level of hygiene and compliance with health standards in rural areas is lower than in urban areas, this is evident even in different countries that have different levels of health. Studies show that African, South, and South American countries, Asia, and other parts of the Pacific and Caribbean have the highest prevalence of the infection, while North America and Europe have the lowest prevalence of cryptosporidiosis. Also, in rural areas due to low levels of health, lifestyle, lack of facilities, etc., the prevalence of cryptosporidium is higher (22).

Also, in the present study, it was found that there is a relationship between contact with animals and the rate of infection so among infected children, 6.7% had contact with animals and 1.9% had no history of contact with animals. Other studies have shown an association between contact with animals and infection with Cryptosporidium (23). Cacciò et al.(2006) stated that the most important factor for the transmission of *Cryptosporidium parvum* is contact with animals (24). The existence of this relationship can also justify the higher level of infection in rural areas than in urban areas because due to the lifestyle and employment of villagers in agriculture and animal husbandry, the possibility of contact with animals is much higher than in urban areas. Studies also indicate the presence of this infection in livestock, especially in calves, sheep, and goats in Iran (18). However, keeping pets such as dogs and cats may also contribute to the spread of cryptosporidiosis in urban areas.

In the present study, it was shown that there is a relationship between the rate of Cryptosporidium infection and the season, so that in hot seasons, especially summer the prevalence was higher (6%) than in cold seasons, such as winter (1%). The other studies summarized the effect of season on the prevalence of cryptosporidiosis (21, 22, 25).

Based on the results of the PCR test, all positive samples were *Cryptosporidium parvum*. Other studies have shown that parvum species are more prevalent than other species of this parasite. Studies have shown that the most well-known risk factor for *Cryptosporidium parvum* infection is contact with animals (21, 23).

In conclusion, the results of the present study showed that *Cryptosporidium parvum* is the predominant species in Chaharmahal and Bakhtiari province, which is more prevalent in children under 6 years old, in warm seasons and rural areas, and in people who have more contact with animals.

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Conflict of interests

There is no conflict of interest between the authors of this article.

References

(1) Clark DP. New insights into human cryptosporidiosis. *Journal of Clinical Microbiology Reviews* 1999; 12 (4): 554-63.

(2) Griffiths JK. Treatment for AIDS-associated cryptosporidiosis. *Journal of Infectious Diseases* 1998; 178: 915-16.

(3) Roberts F., Roberts CW., Johnson JJ., Kyle DE., Krell T., Coggins JR., Coombs GH., Milhous WK., Tzipori S., Ferguson DJ., Chakrabarti D. Evidence for the shikimate pathway in apicomplexan parasites. *Nature* 1998; 393 (6687): 801-5.

(4) Keithly JS., Zhu G., Upton SJ., Woods KM., Martinez MP., Yarlett N. Polyamine biosynthesis in *Cryptosporidium parvum* and its implications for chemotherapy. *Journal of Molecular and Biochemical Parasitoloy* 1997; 88 (1-2): 35-42.

(5) Zhu G., Marchewka MJ., Woods KM., Upton SJ., Keithly JS. Molecular analysis of a type I fatty acid synthase in *Cryptosporidium parvum*. *Journal of Molecular and Biochemical Parasitology* 2000; 105 (2): 253-60.

(6) Chen XM., Levine SA., Tietz P., Krueger E., McNiven MA., Jefferson DM., Mahle M., LaRusso NF. Cryptosporidium parvum is cytopathic for cultured human biliary epithelia via an apoptotic mechanism. *Hepatology* 1998; 28 (4): 906-13.

(7) Tzipori S., Widmer G. The biology of Cryptosporidium. *Journal of Contributions to Microbiology* 2000; 6: 1-32.

(8) Xiao L., Morgan UM., Fayer R., Thompson RC., Lal AA. Cryptosporidium systematics and implications for public health. *Journal of Parasitology Today* 2000; 16 (7): 287-92.

(9) Miller JR. Decreasing cryptosporidiosis among HIV-infected persons in New York City, 1995-1997. *Journal of Urban Health : Bulletin of the New York Academy of Medicine* 1998; 75 (3): 601-2.

(10) Smith HV., Patterson WJ., Hardie R., Greene LA., Benton C., Tulloch W., Gilmour RA., Girdwood RW., Sharp JC., Forbes GI. An outbreak of waterborne cryptosporidiosis caused by post-treatment contamination. *Journal of Epidemiology & Infection* 1989; 103 (3): 703-15.

(11) Lake IR., Bentham G., Kovats RS., Nichols GL. Effects of weather and river flow on cryptosporidiosis. *Journal of Water and Health* 2005; 3 (4): 469–74.

(12) Berahmat R., Spotin A., Ahmadpour E., Mahami-Oskouei M., Rezamand A., Aminisani N., Ghojazadeh M., Ghoyounchi R., Mikaeili-Galeh T. Human cryptosporidiosis in Iran: A systematic review and meta-analysis. *Journal of Parasitology Research* 2017; 116 (4): 1111-28.

(13) Ortolani EL. Standardization of the modified Ziehl-Neelsen technique to stain oocysts of Cryptosporidium sp. *Revista Brasileira de Parasitologia Veterinária* 2000; 9 (1): 29-31.

(14) Coupe S., Sarfati C., Hamane S., Derouin F. Detection of cryptosporidium and identification to the species level by nested PCR and restriction fragment length polymorphism. *Journal of Clinical Microbiology* 2005; 43 (3): 1017–23.

(15) Mahmoudi MR., Kazemi B., Mohammadiha A., Mirzaei A., Karanis P. Detection of Cryptosporidium and Giardia (oo) cysts by IFA, PCR and LAMP in surface water from Rasht, Iran. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2013; 107 (8): 511–17.

(16) Gallas-Lindemann C., Sotiriadou I., Plutzer J., Karanis P. Prevalence and distribution of Cryptosporidium and Giardia in wastewater and the surface, drinking and ground waters in the Lower Rhine, Germany. *Journal of Epidemiology &*

Infection 2013; 141 (1): 9–21.

(17) Chalmers RM., Davies AP. Minireview: Clinical cryptosporidiosis. *Journal of Experimental Parasitology* 2010; 124 (1): 138–146.

(18) Arjmandi KH., Gharavi M., Dalimi Asl A. Parasitological study of Cryptosporidium in human and animal. *2th Cong Zoono.* 1993; Tabriz-Iran.

(19) Rahbari M., Hzrati tape KH., Makhdumi KH. Prevalence of cryptosporidium in hemodialysis patients of Taleghani Hemodialysis center in Urmia. *3th Cong Iranian Parasitol.* 1995, Sari-Iran.

(20) Dupont HL. Guidelines on acute infectious diarrhea in adults. *American Journal of Gastroenterology* 1997; 92 (11): 1962-75.

(21) Yao QX., Zhang XX., Chen K., Ma JG., Zheng WB., Xu XQ., Zhu XQ. Prevalence and genetic characterization of cryptosporidium infection in Java Sparrows (Lonchura oryzivora) in Northern China. *BioMed Research International* 2017; 1: 1-5.

(22) Lal A., Baker MG., Hales S., French NP. Potential effects of global environmental changes on cryptosporidiosis and giardiasis transmission. *Journal of Trends in Parasitology* 2013; 29 (2): 83–90.

(23) Rezaeian M., Shahmoradi A., Dalimi A. Cryptosporidium in sheep as a source for human infection. *Medical Journal of the Islamic Republic of Iran (MJIRI)* 1993; 6 (4): 273-4.

(24) Cacciò SM., Pozio E. Advances in the epidemiology, diagnosis, and treatment of cryptosporidiosis. *Expert Review of Anti-Infective Therapy* 2006; 4 (3): 429–43.

(25) Jagai JS., Castronovo DA., Monchak J., Naumova EN. Seasonality of cryptosporidiosis: A meta-analysis approach. *Journal of Environmental Research* 2009; 109 (4): 465–78.