Review Article

Cryptosporidium spp. infection in Iranian children and immunosuppressive patients: A systematic review and meta-analysis

Narges Kalantari (PhD) [1]
Salman Ghaffari (PhD) [* 2]
Masomeh Bayani (MD) [3]

1. Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran.
2. Department of Parasitology and Mycology, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran.
3. Infectious Diseases and Tropical Medicine Research Center, Babol University of Medical Sciences, Babol, Iran.

* Correspondence:
Salman Ghaffari, Department of Parasitology and Mycology, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran.

E-mail: s3ghaffari@yahoo.com
Tel: 0098 1132199592
Fax: 0098 1132199936

Received: 23 April 2017
Revised: 21 June 2017
Accepted: 3 July 2017

Abstract

Background: Cryptosporidiosis is an important cause of diarrhea in children and immunosuppressive patients. The current study was intended to evaluate the prevalence rate of *Cryptosporidium* infection and clarify the epidemiological characteristics of the infection in both children and immunosuppressive patients in Iran.

Methods: Five English electronic databases including PubMed, Google Scholar, Science Direct, Scopus and Cochrane, and two Persian language databases Magiran and Scientific Information Database were searched. Additionally, reports from the Iranian congresses of parasitology and graduate student thesis dissertations were assessed manually.

Results: Out of 1856 studies from the literature search, our search resulted in a total of 27 articles published from 1991 to 2016. These include 14 reports on cryptosporidiosis in children and 13 papers regarding immunosuppressive patients. 8520 children and 2015 immunosuppressed cases were evaluated. Oocysts of *Cryptosporidium* were found in 3.8% and 8% children cases and immunosuppressed patients, respectively. There was a relatively high variation in the prevalence estimates among different studies, and the Q statistics was high among articles regarding children (p<0.0001) and also between records regarding immunosuppressed patients (p<0.0001). Findings showed that the prevalence rates of *Cryptosporidium* infection are significantly higher in children under 5 years (P=0.00).

Conclusions: In summary, the present study provides a comprehensive view of the epidemiology of *Cryptosporidium* in children and immunosuppressive patients in Iran. Furthermore, a multidisciplinary and multicenter study to evaluate the real prevalence of *Cryptosporidium* infection and to determine its risk factors using an adequate sample size and standardized methods is highly recommended.

Keywords: Children, *Cryptosporidium* spp., Cryptosporidiosis, Immunosuppressive patients, Iran, Prevalence

Cryptosporidiosis refers to the disease caused by several species of obligate, intracellular protozoan parasites known as *Cryptosporidium*. All species are able to infect the microvilli border of the gastro-intestines and respiratory epithelium of a variety of vertebrate hosts, including humans (1). A wide spectrum of clinical symptoms are observed in infected individuals because the pathogenicity of *Cryptosporidium* varies in relation with the species of parasites involved along with the age, and immune status of the host (2). The main symptoms and signs are watery diarrhea, abdominal cramps, anorexia, weight loss, nausea, vomiting, fatigue and low-grade fever (2, 3). Cryptosporidiosis affects several groups of humans including children under five years and immunocompromised individuals, particularly HIV-infected patients (4).

Generally, this infection occurs following oocyst ingestion through the fecal–oral route. However, transmission of *Cryptosporidium* is complex and may occur from host to host (animal to human, person-to-person); through the ingestion of contaminated water or food and possibly airborne. *Cryptosporidium* produces resistant oocysts, like many other parasites which are passed in the feces into the environment. These parasites have mechanical vectors such as insects or even birds which play a role in the transmission cycle (5, 6). Until now, thirty-one *Cryptosporidium* species have been recognized as valid, and of these, the most common species reported in humans throughout the world are *C. parvum* and *C. hominis (1).* This infection is highly prevalent and several factors contribute to the distribution of the parasite. These include shedding large numbers of oocysts from infected cases (7), resistance to the concentration and exposure times of disinfectants commonly used in the drinking water industry (5, 8), highly infectious oocysts (9) and the lack of treatment options (1).

However, the prevalence and distribution of *Cryptosporidium* spp. infection in humans differs in geographic regions of the world and also within a country. In industrialized countries, the prevalence rate of cryptosporidiosis ranges from 0.1- 9.1% (10) and the most prevalent species is *C. hominis* (11, 12). In some developing countries, the prevalence rate of *Cryptosporidium* infection ranges from 2.98-25.9% and the most frequent species is *C. hominis* (13). In Iran, the range of cryptosporidiosis is 0.3 in children (14) to 26.7% in immunosuppressive patients (15).

Nevertheless, there are some publications on cryptosporidiosis in Iran but no systematic review and meta-analysis is available to describe the status of *Cryptosporidium* infection in this country. The current systematic review and meta-analysis was intended to evaluate the weighted prevalence of *Cryptosporidium* infection and clarify the epidemiological characteristics of the infection in both children and immunosuppressive patients in Iran.

Methods

Search strategy: To evaluate the epidemiological status of *Cryptosporidium* infection in humans in Iran, we designed a systematic review based on English and Persian literature released online articles published from 1991 to 2016. English electronic databases including PubMed, Google Scholar, Science Direct, Scopus and Cochrane, and two Persian language databases, Magiran and Scientific Information Database were searched. Additionally, reports from the Iranian parasitology congresses and graduate student dissertations were assessed manually. The current review was performed using medical subject headings (MeSH) terms including: "*Cryptosporidium*", "Cryptosporidiosis", "Prevalence", "Epidemiology", "Iran", "Children" and "Immunosuppressive patients" alone or combined together.

Study selection: All cross-sectional studies which estimated the prevalence of *Cryptosporidium* infection and cryptosporidiosis in children and immunosuppressive patients (HIV, severe kidney failure, cancers and multiple sclerosis patients) in the Iranian population were assessed in the current review. The criteria for the diagnosis of *Cryptosporidium* infection were based on staining, serological and molecular methods. All identified studies were imported to EndNote software to remove duplicates and were also independently assessed for eligibility and inclusion by both authors.

Figure 1 shows how the studies were categorized and the reasons for exclusion. A total of 27 studies met the present study criteria, out of 1856 that were published as journal articles and presented in national conference proceedings and graduate student dissertations.

Data extraction: All articles which met the criteria for inclusion were carefully investigated and information on population, sample size, age distribution, gender, *Cryptosporidium* diagnostic methods, and year of publication, study location, and number of subjects with positive test results were withdrawn using a data extraction form. Furthermore, data on human risk factors such as pet ownership, close contact with animals, occupational group, fruit or vegetable consumption, fruit and vegetable washing methods, place of residence, and educational level was collected.

Statistical analysis: The prevalence in total and specific groups was calculated by age group, gender, residency and geographical region. In order to assess heterogeneity among the studies, a forest plot was used. The heterogeneity and quantifying variations were performed by statistical methods, I^2 and Cochrane Q-statistics for the purpose of meta-analysis. A random effects model was employed as it assumed that the included studies were a random sample from a population of studies. The aforementioned plot

presented proportions of individual studies and overall prevalence. The trial version of StatsDirect statistical software was used to perform this meta-analysis (www.statsdirect.com).
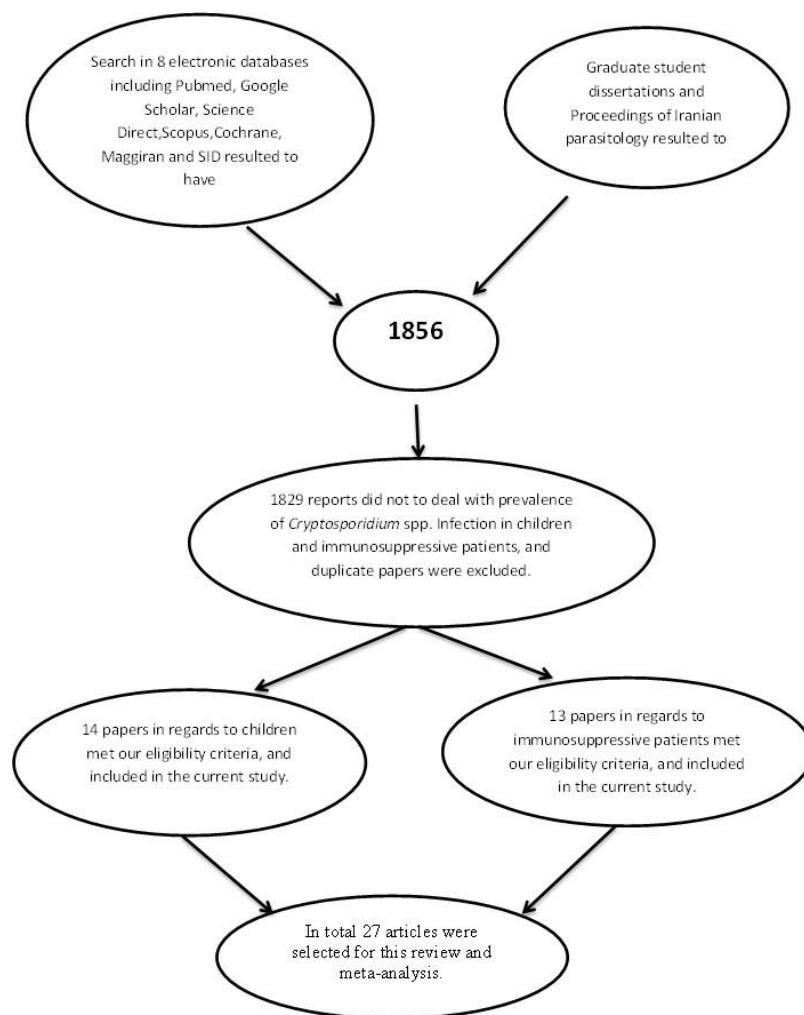

Figure 1. Flow diagram describing the study design process.

Results

Out of 1856 studies from the literature search, 14 articles regarding children and 13 records about immunosuppressed patients were considered appropriate for inclusion in this systematic review and meta-analysis. Table 1 shows the results of the literature search on demographic data associated with the prevalence of *Cryptosporidium* infection in Iranian children. In total, 8520 child cases and 2015 immunosuppressed individuals were included in the current study.

Oocysts of *Cryptosporidium* were found in 294 and 125 of child cases and immunosuppressed patients, respectively. There was a relatively high variation in the prevalence estimates among the different studies, and the Q statistic was high among articles regarding children (Q=51.87, df=14, p<0.0001; I2= 74.9% (95% CI=54.1% to 83.9%) and also between records regarding immunosuppressed patients (Q=151.9, df=12, p<0.0001; I2=92.1% (95% CI=88.8% to 94.1%)) (figures 2, 3).

(A)

(B)

Figure 2. Forest plot diagram (A) and bias assessment graph (B) of 14 studies showing the prevalence rates of *Cryptosporidium* infection in Iranian children (first author, year and province of study).
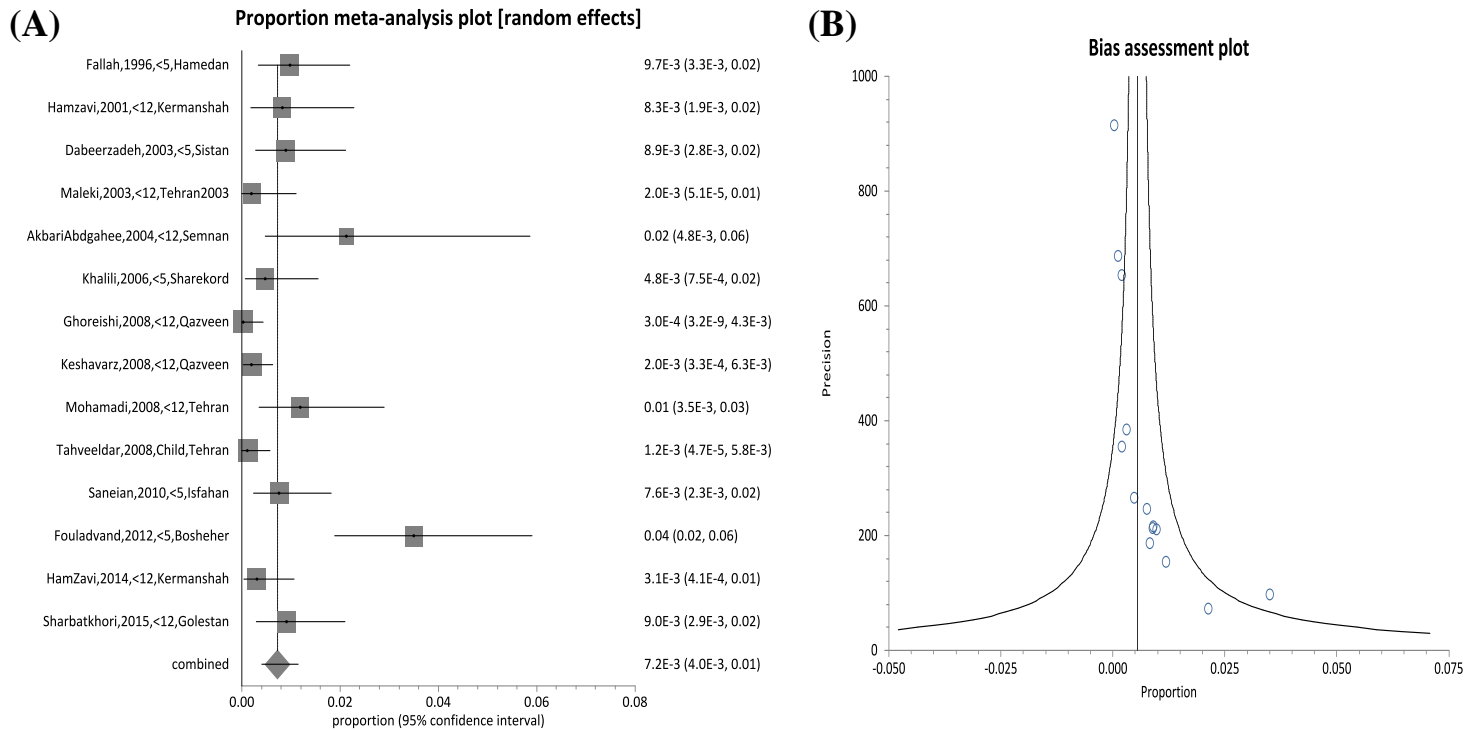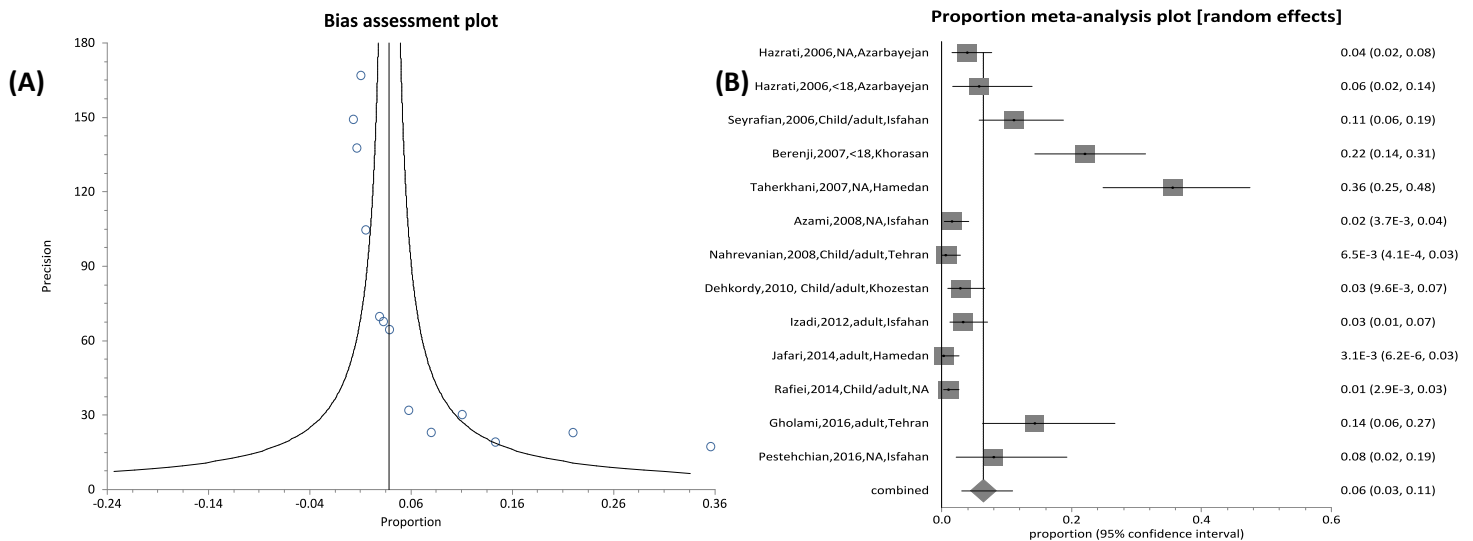
(A)

(B)

Figure 3. Forest plot diagram (A) and bias assessment graph (B) of 13 studies showing the prevalence rates of *Cryptosporidium* infection in Iranian immunosuppressive patients (first author, year and province of study).

Modified Ziehl-Neelsen technique and enzyme-linked immunosorbent assay (ELISA) were used in the included studies. Molecular methods were also used in some studies to confirm the results of the Ziehl-Neelsen technique or to genotype the parasite. Results of the meta-analysis showed that the prevalence rates of *Cryptosporidium* infection in

children under 5-years old are higher than children over 5-years old (4.21% versus 1.22%, respectively; P=0.000). Considering stool form, positive rate in the diarrheal stool and both diarrheal and non-diarrheal cases was 4.03% and 1.1% (P=0.000). The results of the literature search on demographic data associated to the prevalence of *Cryptosporidium* infection in Iranian children are shown in table 1. The results of this meta-analysis showed that the average of *Cryptosporidium* infection rate was higher in immunosuppressive cases (8%), in comparison with children

(3.8%) (P= 0.038, 95% CI= -8.856041 to 0.460436). A possible association between *Cryptosporidium* infection and gender was evaluated in 10 out of 27 studies. The mean and standard deviation of prevalence in males and female cases were 5.52%±2.5 and 4.72±3.2, respectively (P=0.5). In the children group, the mean and standard deviation of prevalence was 4.83±2.2 in males and 3.52±1.4 in females (P=0.29). In the immunosuppressive patient group, the mean and standard deviation of prevalence rates were 6.22±2.8 and 5.92±4.2 (P=0.9) in male and female cases, respectively.

Table 1. Demographic data in relation to prevalence of *Cryptosporidium* infection in Iranian children.

Variables	Participants	Positive cases	Prevalence	P-value	References
Gender					
Male	2001	77	3.85	0.062	28, 44, 46, 47,
Female	1629	84	5.2		49, 51, 52
Age					
<5	3495	147	4.21	0.000	14, 28, 44,45, 46, 47,49, 51, 52
>5	982	12	1.22		
Form of stool					
Diarrheic	2456	99	4.03	0.000	14, 28, 44,45, 46, 47,49, 51, 52
Non-diarrheic	798	9	1.1		
Residency					
Urban	878	12	1.4	0.515	14, 49
Rural	493	6	1.2		
Animal contact				0.000	46, 47
Yes	86	10	11.6		
No	685	11	1.6		
Seasons					
Spring	283	24	8.5	0.038	28, 44
Summer	178	11	6.2		
Autumn	287	35	12.2		
Winter	156	8	5.1		

Discussion

Human cryptosporidiosis is an important zoonotic infection that causes diarrhea in immunocompromised individuals and children. It also causes extra-intestinal infection in severe immunodeficiency (2). This systematic review and meta-analysis study gives a general estimate for the prevalence of *Cryptosporidium* in children and immunosuppressive patients in Iran. The overall prevalence obtained in the aforementioned population was relatively high (6%). The prevalence rates of the infection with regard to children and immunosuppressive cases were 3.8%

and 8%, respectively. The total prevalence (6%) was approximately similar to some studies conducted in Malaysia (5.2%) (16), South Africa (5.59%) (17). Higher rates of infections have been reported in other studies such as in Afghanistan (14.1%) (18), Palestine (11.5%) (19), Jordan (8.3%) (20), India (12%) (21), Saudi Arabia (11%) (22) and Pakistan (10.9%) (23); and the lower rate of infection has been reported in Kenya (4%) (24) and North-West Ethiopia (4.6%) (25). Moreover, the prevalence rates of *Cryptosporidium* infection have been reported as less than 1

percent to more than 30 percent worldwide (26). In 2012, Fletcher et al. reviewed the relative prevalence of *Cryptosporidium* spp. in several developed countries and revealed that the relative prevalence rate of this infection ranges from 0.1% to 9.1% of cases. They also reported that *Cryptosporidium* infection is responsible for about 20% of diarrheal episodes in children in developing countries and up to 9% of episodes in developed regions (10). In fact, global *Cryptosporidium* infection distribution has been associated with various risk factors but its risk factors in developing nations are very different to those in industrialized countries (3). These risk factors include situation of sanitation, health status, exposure to pets and animals, nutritional **behavior** (consumption of raw vegetables and contaminated drinking water), unsafe sexual activity, geographical climate, and location of residence (3, 27).

Our study results reveal that there is a high prevalence rate of *Cryptosporidium* infection in children (13.1%) in Bushehr (28), and immunosuppressive patients (26.7%) in Hamedan (15). It also demonstrated low prevalence of this infection in children (0.3%) in Qazveen (14), and immunosuppressive patients (0.56%) in Hamedan (29). A disagreement in the prevalence of *Cryptosporidium* infection in immunosuppressive patients is seen in Hamedan. A possible explanation is the type of the studied population and time. Taherkhani et al., 2007 studied HIV patients and Jafari et al., in 2014 evaluated renal transplantation cases. **However,** the prevalence of *Cryptosporidium* infection, like other intestinal infections, is closely related to cultural, environmental, social and economic factors (30).

Our data shows that *Cryptosporidium* prevalence in children under five years old (4.21%) was significantly higher than children above five years old (1.2%). These findings are supported by various studies which demonstrate that *Cryptosporidium* infections occur frequently in children under than 5 years where the peak of infections and diarrhea appear in children younger than 2 years old (31, 32).

In regard to diarrhea in 4/14 studies evaluated, *Cryptosporidium* infections in both diarrheic and non-diarrheic children found that the prevalence of this infection are significantly higher in diarrheic cases. Overall, the statistical analysis of 9 studies showed that the prevalence rate of *Cryptosporidium* infection was significantly higher in diarrheic children compared with non-diarrheic children (table 1). These findings are in line with several studies,

indicating that this parasite is one of most important causes of diarrhea-associated pathogens in children (33, 34).

Our data showed that the *Cryptosporidium* prevalence rate is slightly higher in male cases than in female subjects and no significant difference was observed between the two sexes. A study reviewed cryptosporidiosis in developing countries and indicated that no significant differences were observed by sex in Kenya (13). The study of Painter et al., in the United States showed that the rates of cryptosporidiosis were higher among males than females in cases under15 years old. In contrast, cryptosporidiosis rates were higher among females aged ≥15 years (35).

This meta-analysis study was suffering from inadequate analysis for the prevalence of *Cryptosporidium* infections regarding contact with animals, level of education, occupation, residency, fruit or vegetable consumption, fruit and vegetable washing methods, and season. Therefore, the risk factors associated to cryptosporidiosis are not well known in Iran and indeed the roles of humans, livestock and wildlife in the *Cryptosporidium* transmission cycle remain largely unknown.

Different methods for the detection of *Cryptosporidium* infections are used globally. The most common techniques are microscopic, immunological and molecular methods (36, 37). Our data showed that the microscopy method, acid-fast Ziehl-Neelsen (ZN), was the most common assay. Three and six studies used enzyme immunoassay test and molecular methods, respectively. The molecular assays revealed that *C. parvum* (84.4%) is the most common species found in human cases in Iran, followed by *C. hominis* (13.74). These findings are supported by some studies indicating that *C. parvum* and *C. hominis* are the most prevalent species in humans and that *C. parvum* is the dominant species (1, 38, 39). In contrast, several studies demonstrated that *C. hominis* is the most prevalent species in humans (7, 13, 40). Yet, the distribution of *cryptosporidium* species in humans is different amongst geographic regions and socioeconomic conditions. For example, both *C. hominis* and *C. parvum* are the commonly detected species in humans, in European countries and New Zealand but *C. parvum* is the dominant species found in humans in the Middle Eastern countries (7, 39). Only one study reported *C. meleagridis* in a child (one out of 16 positive cases) (Rafiei et al., 2014). *C. meleagridis* infection in a child in Mazandaran province, northern Iran was first reported in our previous study, focused on the molecular epidemiology of *Cryptosporidium* spp. in some

developing countries and in the United Kingdom using restriction fragment length polymorphism (PCR-RFLP) and sequencing of the 18S rRNA gene (40), and PCR-RFLP and sequencing of *Cryptosporidium* oocyst wall protein (COWP) and 70-kDa heat shock protein (HSP70) genes (41).

In conclusion, to the best of our knowledge, the present study provides a comprehensive view of the epidemiology of *Cryptosporidium* in children and immunosuppressive patients in Iran.

Immunosuppressive individuals show a two-fold higher prevalence rate than children and also *Cryptosporidium* infection is more prevalent in diarrheic patients compared with non-diarrheic cases. The current study suggests that these patients should be monitored regularly. It is also suggested that a multidisciplinary and multicenter study to evaluate the real prevalence of *Cryptosporidium* infection and to determine its risk factors using adequate sample size and standardized methods should be performed in Iran. Furthermore, the continuous monitoring of *Cryptosporidium* in surface water, livestock, wildlife, and humans using appropriate methods particularly molecular techniques would be helpful to increase our understanding of infection and transmission patterns of this parasite in Iran.

Acknowledgments

Conflict of Interest: The authors announce that there is no conflict of interest.

References

1. Ryan U, Zahedi A, Paparini A. Cryptosporidium in humans and animals-a one health approach to prophylaxis. Parasite Immunol 2016; 38: 535-47.
2. Chalmers RM, Davies AP. Minireview: clinical cryptosporidiosis. Exp Parasitol 2010; 124: 138-46.
3. Bouzid M, Hunter PR, Chalmers RM, Tyler KM. Cryptosporidium pathogenicity and virulence. Clin Microbiol Rev 2013; 26: 115-34.
4. Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet 2013; 382: 209-22.
5. Baldursson S, Karanis P. Waterborne transmission of protozoan parasites: review of worldwide outbreaks–an update 2004–2010. Water Res 2011; 45: 6603-14.
6. Thompson RA, Olson M, Zhu G, et al. Cryptosporidium and cryptosporidiosis. Adv Parasitol 2005; 59: 77-158.
7. Ryan U, Fayer R, Xiao L. Cryptosporidium species in humans and animals: current understanding and research needs. Parasitology 2014; 141: 1667-85.
8. Burnet JB, Penny C, Ogorzaly L, Cauchie HM. Spatial and temporal distribution of Cryptosporidium and Giardia in a drinking water resource: Implications for monitoring and risk assessment. Sci Total Environ 2014; 472: 1023-35.
9. Chappell CL, Okhuysen PC, Langer-Curry R, et al. Cryptosporidium hominis experimental challenge of healthy adults. Am J Trop Med Hyg 2006; 75: 851-7.
10. Fletcher SM, Stark D, Harkness J, Ellis J. Enteric protozoa in the developed world: a public health perspective. Clin Mcrobiol Rev 2012; 25: 420-49.
11. Wielinga PR, de Vries A, van der Goot TH, et al. Molecular epidemiology of Cryptosporidium in humans and cattle in the Netherlands. Inter J Parasitol 2008; 38: 809-17.
12. Chalmers R, Smith R, Elwin K, Clifton-Hadley F, Giles M. Epidemiology of anthroponotic and zoonotic human cryptosporidiosis in England and Wales, 2004-2006. Epidemiol Infect 2011; 139: 700-12.
13. Snelling WJ, Xiao L, Ortega-Pierres G, et al. Cryptosporidiosis in developing countries. J Infect Dev Ctries 2007; 1: 242-56.
14. Ghoreyshi MS, Dalirani R, Daneshi KM, et al. Cryptosporidial infection in the children referred to Qazvin Qods Hospital (2003). J Qazvin Univ Med Sci 2008; 12: 95-9.
15. Taherkhani H, Fallah M, Jadidian K, Vaziri S. A study on the prevalence of Cryptosporidium in HIV positive patients. J Res Health Sci 2007; 7: 20-4.

16. Al-Delaimy AK, Al-Mekhlafi HM, Nasr NA, et al. Epidemiology of intestinal polyparasitism among Orang Asli school children in rural Malaysia. PLoS Negl Trop Dis 2014; 8: e3074.

17. Abu Samra N, Jori F, Cacciò SM, et al. Cryptosporidium genotypes in children and calves living at the wildlife or livestock interface of the Kruger National Park, South Africa. Onderstepoort J Vet Res 2016; 83: e1-e7.

18. Elyan D, Wasfy M, El Mohammady H, et al. Non-bacterial etiologies of diarrheal diseases in Afghanistan. Trans R Soc Trop Med Hyg 2014; 108: 461-5.

19. Abu-Alrub SM, Abusada GM, Farraj MA, Essawi TA. Prevalence of Cryptosporidium spp. in children with diarrhoea in the West Bank, Palestine. J Infect Dev Ctries 2008; 2: 59-62.

20. Hijjawi N, Mukbel R, Yang R, Ryan U. Genetic characterization of Cryptosporidium in animal and human isolates from Jordan. Vet Parasitol 2016; 228: 116-20.

21. Daniels ME, Shrivastava A, Smith WA, et al. Cryptosporidium and Giardia in humans, domestic animals, and village water sources in rural India. Am J Trop Med Hyg 2015; 93: 596-600.

22. Shalaby I, Gherbawy Y, Jamjoom M, Banaja A. Prevalence and genotyping of Cryptosporidium in stool samples collected from children in Taif City (Saudi Arabia). Trop Biomed 2014; 31: 215-24.

23. Haider SS, Baqai R, Qureshi FM, Boorom K. Blastocystis spp., Cryptosporidium spp., and Entamoeba histolytica exhibit similar symptomatic and epidemiological patterns in healthcare-seeking patients in Karachi. Parasitol Res 2012; 111: 1357-68.

24. Gatei W, Greensill J, Ashford RW, et al. Molecular analysis of the 18S rRNA gene of Cryptosporidium parasites from patients with or without human immunodeficiency virus infections living in Kenya, Malawi, Brazil, the United Kingdom, and Vietnam. J Clin Microbiol 2003; 41: 1458-62.

25. de Lucio A, Amor-Aramendía A, Bailo B, et al. Prevalence and genetic diversity of Giardia duodenalis and Cryptosporidium spp. among school children in a rural area of the Amhara region, North-West Ethiopia. PloS One 2016; 11: e0159992.

26. Meinhardt PL, Casemore DP, Miller KB. Epidemiologic aspects of human cryptosporidiosis and the role of waterborne transmission. Epidemiol Rev 1996; 18: 118-36.

27. Cacciò SM, Pozio E. Advances in the epidemiology, diagnosis and treatment of cryptosporidiosis. Expert Rev Anti Infect Ther 2006; 4: 429-43.

28. Fouladvnd M, Barazesh A, Naeimi B, Najafi A. Frequency of Cryptosporidium infection and related factors under five years old children hospitalized with gastroenteritis. Afr J Microbiol Res 2012; 6: 4102-6.

29. Jafari R, Gharibi Z, Fallah M. The prevalence of cryptosporidium infection among renal transplanted patients in Hamadan city, West of Iran. Avicenna J Clin Microb Infec 2014; 1: e19570.

30. Singh A, Bairy I, Shivananda P. Spectrum of opportunistic infections in AIDS cases. Indian J Med Sci 2003; 57: 16-21.

31. Tumwine JK, Kekitiinwa A, Nabukeera N, et al. Cryptosporidium parvum in children with diarrhea in Mulago Hospital, Kampala, Uganda. Am J Trop Med Hyg 2003, 68: 710-15.

32. Steinberg EB, Mendoza CE, Glass R, et al. Prevalence of infection with waterborne pathogens: a seroepidemiologic study in children 6–36 months old in San Juan Sacatepequez, Guatemala. Am J Trop Med Hyg 2004; 70: 83-8.

33. Platts-Mills JA, Babji S, Bodhidatta L, et al. Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). The Lancet Global Health 2015; 3: e564-75.

34. Sow SO, Muhsen K, Nasrin D, et al. The Burden of Cryptosporidium diarrheal disease among children< 24 months of age in moderate/high mortality regions of Sub-Saharan Africa and South Asia, utilizing data from the global enteric multicenter study (GEMS). PLoS Negl Trop Dis 2016; 10: e0004729.

35. Painter JE, Hlavsa MC, Collier SA, et al. Cryptosporidiosis surveillance United States, 2011–2012. MMWR Surveill Summ 2015; 64: 1-14.

36. Ghaffari S, Kalantari N. Recognition of Cryptosporidium oocysts in fresh and old stool samples: comparison of four techniques. Asian Pac J Trop Biomed. 2014; 4: S570-4.

37. Chalmers RM, Katzer F. Looking for Cryptosporidium: the application of advances in detection and diagnosis. Trends Parasitol 2013; 29: 237-51.

38. Meamar AR, Guyot K, Certad G, et al. Molecular characterization of Cryptosporidium isolates from humans and animals in Iran. Appl Environ Microbiol 2007; 73: 1033-5.

39. Xiao L. Molecular epidemiology of cryptosporidiosis: an update. Exp Parasitol 2010; 124: 80-9.

40. Ghaffari S, Kalantari N. Molecular analysis of 18S rRNA gene of Cryptosporidium parasites from patients living in Iran, Malawi, Nigeria and Vietnam. Int J Mol Cell Med 2012; 1: 153-61.

41. Ghaffari S, Kalantari N. A multi-locus study of Cryptosporidium parasites isolated from patients living in Iran, Malawi, Nigeria, the United Kingdom, and Vietnam. Iran J Parasitol 2014; 9: 79-89.

42. Fallah M, Haghighi A. Cryptosporidiosis in children with diarrhea submitted to health centers in the west of iran (Hamedan). Med J Islam Repub Iran 1996; 9: 315-17.

43. Hamzavi Y. Cryptosporidial infection in the children under 12 years old, referred to Shahid Fahmideh Hospital, Kermanshah, Iran (1995-96). J Kermanshah Univ Med Sci 2001; 4: 8 -13. [in Persian] Available at: http://www.sid.ir/En/Journal/ViewPaper.aspx?ID=55600

44. Dabirzadeh M, Baghaei M, Bokaeyan M, Goodarzei M. Study of Cryptosporidium in children below five years of age with diarrhea in referring Ali-Asghar Pediatric Hospital of Zahedan. J Gorgan Univ Med Sci 2003; 5: 54-9. [in Persian]

45. Maleki F, Sadegh Hasani S. Prevalence of cryptosporidiosis in students of elementary schools in the west Tehran-Iran from 1999-2001. Razi J Med Sci 2003; 10: 105-9. [in Persian]

46. Akbari-Eidigahi MR, Abooei-Mehrizi MM, Amin-Beidokhti ME, Shaebani AA. Evaluation of cryptosporidiosis in diarrheic children referred to Amir al Moemenin hospital, Semnan. Koomesh 2004; 5:99-104. [in Persian] Available at:http://koomeshjournal.semums.ac.ir/article-1-173-en.html

47. Khalili B, Shahabi G, Besharat M, Mardani M, Cuevas L, Hart C. Determining the prevalence of Cryptosporidium and measuring of micronutrients in cryptosporidiosis among children under 5 years in Shahrekord. J Res Med Sci. 2006; 30: 187-91. [in Persian]

48. Keshavarz A, Athari A, Haghighi A, et al. Genetic characterization of Cryptosporidium spp. among children with diarrhea in Tehran and Qazvin provinces, Iran. Iran J Parasitol 2008; 3: 30-6. Available at: http://ijpa.tums.ac.ir/index.php/ijpa/article/view/68

49. Mohammadi Ghalehbin BB, Falah E, Asgharzade M, et al. Prevalence of Cryptosporidium in children suffering from gastroenteritis in Ardabil Hospitals. J Ardabil Univ Med Sci 2006; 6: 176-82. [in Persian]

50. Tahvildar Bidrooni F, Dalimi Asl A, Kazemi D B. Using a 1055 bp fragment of 18s rRNA for differentiation of human and cattle Cryptosporidiosis. Res Med 2008; 32: 5-10.

51. Saneian H, Yaghini O, Yaghini A, Modarresi MR, Soroshnia M. Infection rate of Cryptosporidium parvum among diarrheic children in Isfahan. Iran J Pediatr 2010; 20: 343-7.

52. Hamzavi Y, Amiri M, Jalalvandi S. Cryptosporidiosis in children with and without diarrhea in Kermanshah from 2011-12. J Clin Res Paramed Sci 2014; 3: 40-6. [in Persian]

53. Sharbatkhori M, Nazemalhosseini Mojarad E, Taghipour N, Pagheh AS, Mesgarian F. Prevalence and genetic characterization of Cryptosporidium spp. In diarrheic children from Gonbad Kavoos City, Iran. Iran J Parasitol 2015; 10: 441-7.

54. Hazrati Tappeh K, Gharavi M, Makhdoumi K, Rahbar M, Taghizadeh A. Prevalence of Cryptosporidium spp. infection in renal transplant and hemodialysis patients. Iran J Public Health 2006; 35: 54-7.

55. Hazrati Tappeh K, Rahbar M, Hejazi S, Mostaghim M. Cryptosporidium in children referred to oncology center of Urmia, Imam Khomeini Hospital. J Ardabil Univ Med Sci 2001; 5: 327-32. [in Persian]

56. eyrafian S, Pestehchian N, Kerdegari M, Yousefi HA, Bastani B. Prevalence rate of Cryptosporidium infection in hemodialysis patients in Iran. Hemodial Int 2006, 10: 375-9.

57. Berenji F, Zabolinejad N, Kianifar H, et al. Cryptosporidium infection in pediatric patients with lymphohematopoietic malignancies. Iran J Pediatr 2007; 17: 247-51.

58. Azami M, Dorostkar Moghadam D. Prevalence of Cryptosporidium in children under 5 years of age, immunocompromised patients and high risk persons in Isfahan province. Iran South Med J 2008; 11: 47-54.

59. Nahrevanian H, Assmar M. Cryptosporidiosis in immunocompromised patients in the Islamic Republic of Iran. J Microbiol Immunol Infect 2008; 41: 74-7.

60. Dehkordy AB, Rafiei A, Alavi S, Latifi S. Prevalence of Cryptosporidium infection in immunocompromised patients, in South-West of Iran, 2009-10. Iran J Parasitol 2010; 5: 42-7.

61. Izadi M, Jonaidi-Jafari N, Saburi A, et al. Prevalence, molecular characteristics and risk factors for cryptosporidiosis among Iranian immunocompromised patients. Microbiol Immunol 2012; 56: 836-42.

62. Rafiei A, Rashno Z, Samarbafzadeh A, Khademvatan S. Molecular characterization of Cryptosporidium spp. isolated from immunocompromised patients and children. Jundishapur J Microbiol 2014; 7: e9183.

63. Gholami R, Gholami S, Emadi-Kouchak H, Abdollahi A, Shahriari M. Clinical characteristic of the HIV/AIDS patients with cryptosporidiosis referring to behavioral diseases consultation center, Imam Khomeini Hospital, Tehran in 2013. Iran J Pathol 2016; 11: 27-34.

64. Pestehchian N, Etemadifar M, Yousefi H, Aslani N, Chiani M. Prevalence of Cryptosporidium infection in patients with multiple sclerosis compared to control group in Isfahan city, Iran. J Isfahan Med Sch 2016, 34: 791-6. [in Persian]