Gastroenterology and Hepatology From Bed to Bench.

ORIGINAL ARTICLE

Prevalence of *Cryptosporidium* spp. in children and the elderly in southwestern Iran

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ABSTRACT

Aim: The current study investigated the prevalence of *Cryptosporidium* spp. among children under 6 and adults over 60 years of age with diarrhea in the southwest of Iran.

Background: Cryptosporidiosis is an opportunistic parasitic infection caused by the species *Cryptosporidium* that causes gastrointestinal complications and diarrhea.

Methods: This cross-sectional study was conducted in Khuzestan province between January 2020 to December 2020. Out of 350 patients referring to medical centers with clinical signs of diarrhea, 57.4% were under six years of age and 42.6% were more than 60 years old. Fecal samples were examined using Modified Ziehl-Neelsen (MZN) staining and nested-PCR techniques.

Results: The overall prevalence of *Cryptosporidium* spp. infection in the study population was 0.9% as determined by microscopic and molecular methods (3/47).

Conclusion: The study results confirm the prevalence of parasitic infections as reported in previous studies in other regions of Iran. Preventive health measures are necessary.

Keywords: *Cryptosporidium* spp., Diarrhea, Molecular detection, Iran.

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Introduction

Cryptosporidiosis is a parasitic infection caused by the coccidian protozoan parasite *Cryptosporidium* spp., leading to acute or chronic diarrheal complications (1). Gastrointestinal disorders in immunocompetent individuals are often self-limiting, whereas, in immunocompromised patients they can be life-threatening (2). The main cause of clinical symptoms is the replacement of intracellular parasites in the intestinal villi (3). *Cryptosporidium* spp. can infect a wide range of hosts, and its zoonotic and anthroponotic importance has been well proven (4). *Cryptosporidium parvum* (*C. parvum*) and *C. hominis* are two medical important species reported frequently in humans (5). *Cryptosporidium* spp. transmission occurs more through water sources contaminated with oocysts and less through contaminated food sources (6); parasite oocysts, however, have been frequently isolated from soil and vegetables (7). The prevalence of cryptosporidiosis seems to be 3 cases per 100,000 people (8), although this probably represents the tip of the iceberg. The prevalence of this protozoan is estimated to be much higher, especially in developing and underdeveloped countries where personal and drinking water hygiene statuses are poorer (9). The

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global prevalence in non-human hosts has been estimated by microscopic and molecular methods (10, 11). Studies in Iran have shown that the prevalence rates of *Cryptosporidium* spp. in healthy people, people with immunodeficiency, and children are estimated to be 2.94%, 4.54%, and 3.65%, respectively (12). The Ziehl–Neelsen staining is currently a routine method used for the microscopic detection of *Cryptosporidium* spp., however, using a molecular technique with high sensitivity and specificity can be very helpful in diagnosing (13). *Cryptosporidium* is thought to be responsible for many cases of diarrhea in endemic areas. Scattered studies have been conducted in Iran, a developing country, especially in tropical regions (14, 15). In populations with adequate immune systems, even children and the elderly are at greater risk for cryptosporidiosis (16). The present study aimed to investigate the prevalence of *Cryptosporidium* spp. in children and the elderly with diarrhea in southwestern Iran.

Methods and materials

Ethical approval

Approval for the current study was obtained from the Alborz University of Medical Science Research Ethics Committee (No: IR.Absums.rec.1398.131).

Study design, location, and population

The present cross-sectional study was carried out between September 2019 and August 2020 and investigated *Cryptosporidium* spp. in Khuzestan, located in southwestern Iran. Andimeshk and Dezful are ancient cities located in the northern part of Khuzestan province. With populations of over 135 thousand and 265 thousand, respectively, these cities have hot semi-arid climates with a combined annual rainfall of 600 millimeters.

After obtaining informed consent forms for participation, demographic characteristics and diarrheal samples (~100 g) were collected in each of the four seasons (autumn, winter, spring, and summer) from patients who referred to Andimeshk-Dezful hospitals and medical health centers. Out of 350 samples, 184 (52.6%) were from males and 166 (47.4%) were from females; 201 (57.4%) were from children <6 years of age, and 149 (42.6%) were from adults over 60 years of age (Table 1).

Microscopic detection

The fresh diarrheal stool samples were transported to the Dezful Medical Science University laboratory for parasitological examination. Specimens were concentrated using formalin-ethyl acetate method, and smears prepared with fecal pellet were examined to detect *Cryptosporidium* spp. oocysts using a modified Ziehl-Neelsen (MZN) technique. Briefly, slides were exposed to Carbol Fuchsin for 20 min, then washed with sulfuric acid (10%) for 40 to 60 sec and re-stained with methylene blue for 1 min. *Cryptosporidium* spp. 4-6 µm oocysts appeared in a red color under a light microscope with magnifications of 40X and 100X. All samples for molecular examination were stored in a refrigerator at -70 °C.

DNA extraction and nested-PCR

DNA was harvested from all fecal samples using a commercial kit (Sinaclon-Iran) according to the manufacturer's instructions. A NanoDrop (2000c-Thermo Fisher Scientific) instrument was applied to ensure purification of the DNA concentration.

Two pairs of primers (Metabion-Germany) were applied to two round nested-PCR targeting the small subunit of 18s-rRNAgene. In the initial round Crp1 forward 5′-CTATTGGAGCTGGAATTACC-3′ and Crp1 reverse 5′-GGTGACTCATAATAACTTTACGG-3′ primers, and in the second round Crp2 forward 5′-GACTTGCCCTCCAATTGATA-3′ and Crp2 reverse 5′-CGGTAGGGTATTGGCCTA-3′ were applied to amplify the *Cryptosporidium* spp. genomes, respectively. Polymerase chain reaction was carried out on 47 suspected and positive samples in the microscopic method using mastermix (Sinaclon, Iran); in the first round of PCR, of the 26 µl, the final volume in strips included 13 µl master mixes, 2 µl from each forward and/ or reverse primer (30 µM), and 5 µl DNA, which was finalized with 5.5 µl PCR-grade water. In the second round, the volume of the mixture was 25 µl for each tube, of which 21 µl of components (including mastermix: 13 µl, primers: 2 µl, and 5 µl: DW) and 5 µl of the first PCR product was added as the template DNA. The amplification process was carried out according to the following thermal and time schedule: initial denaturation at 94 °C for 5 min, 45 cycles denaturation at 95 °C (40 sec), annealing at 57 °C for 40 sec, and extension at 72 °C for 20 s.

Table 1. Summary of results according to the studied variables.

Variables	No. examined (%)	Prevalence		(*P* value) *
		Microscopic	Nested-PCR	
Age group (years)				0.06
<6	201(42.6)	2 (1%)	2 (1%)	
>60	149 (57.4)	1 (0.7%)	1 (0.7%)	
Gender				0.09
Female	166 (47.4)	1 (0.6%)	1 (0.6%)	
Male	184 (52.6)	2 (1.1%)	2 (1.1%)	
Clinical signs				0.054
Yes	141 (40.3)	1 (0.7%)	1 (0.7%)	
No	209 (59.7)	2 (0.96%)	2 (0.96%)	
Residence area				0.09
Dezful	217 (62)	3 (1.4%)	3 (1.4%)	
Andimeshk	133 (38)	0 (0.0%)	0 (0.0%)	
Season				0.62
Spring	76 (21.7)	2 (2.63%)	2 (2.63%)	
Summer	110 (31.4)	1 (0.9%)	1 (0.9%)	
Autumn	107 (30.6)	0 (0.0%)	0 (0.0%)	
Winter	57 (16.3)	0 (0.0%)	0 (0.0%)	

Results

Out of a total of 350 samples, three cases were found to be positive using the microscopic method (Figure 1-A). Forty-seven suspected and positive samples were examined by the PCR molecular technique, and the results were similar to those of the microscopic examination: three were positive (Figure 1-B). Prevalence rates based on age were found to be the same with both methods; two cases (≈1%) were reported in participants under 6 years of age (children) and one case (0.7%) was reported in participants over the age of 60 years (elderly). The results of both methods also showed the number of cases in males was double that of females (1.1% vs. 0.6%). Interestingly, all positive cases came from Dezful city, with none found in the Andimeshk samples (1.4% vs. 0.0%). Seasonal analysis revealed two positive cases (2.63%) occurred in spring and one in summer (0.9%).

No significant statistical relationship was observed between the prevalence and the studied variables. Only one case (0.7%) had clinical symptoms of diarrhea with abdominal cramps and two cases (0.96%) had no clinical symptoms other than diarrhea. The results are summarized in Table 1.

Discussion

Cryptosporidium is an opportunistic coccidian protozoan that can cause gastrointestinal complications in both immunocompetent and immunocompromised individuals (17). Various species of *Cryptosporidium* have been isolated from a wide range of hosts (18). According to the current results, three positive cases were observed among 350 people with diarrhea (0.9%). Tahvildar-Biderouni and Salehi (19) reported in their study that the prevalence of *Cryptosporidium* spp. in people with diarrhea was about 1.9%, which is similar to the current results (19). Cryptosporidiosis distribution varies in different geographical areas; the prevalence of parasitic infection in tropical regions has been reported to be relatively high (20). *Cryptosporidium* infection is more important in immunocompromised individuals than in persons with adequate immunity. In the general population, elderly people over 60 years of age and children are more susceptible to infection. In a two-year study in Egypt by Abdel-Messih et al., the prevalence of *Cryptosporidium* was reported as about 17%, indicating a high prevalence of infection in younger children (21). Because of the poor sanitation of drinking water sources in most tropical regions, the risk of parasitic infections, especially *Cryptosporidium*, is high (22).

As we know, cryptosporidiosis can be responsible for diarrhea. Mirzaei et al. found the prevalence of *Cryptosporidium* spp. from diarrheal and non-diarrheal patients to be 10.8%, of which 25.6% and 3.7% were related to diarrhea and non-diarrheal samples, respectively (23); there was also a significant relationship between diarrhea and cryptosporidiosis infection in this study. In the acquisition of cryptosporidiosis as well as the progress of the

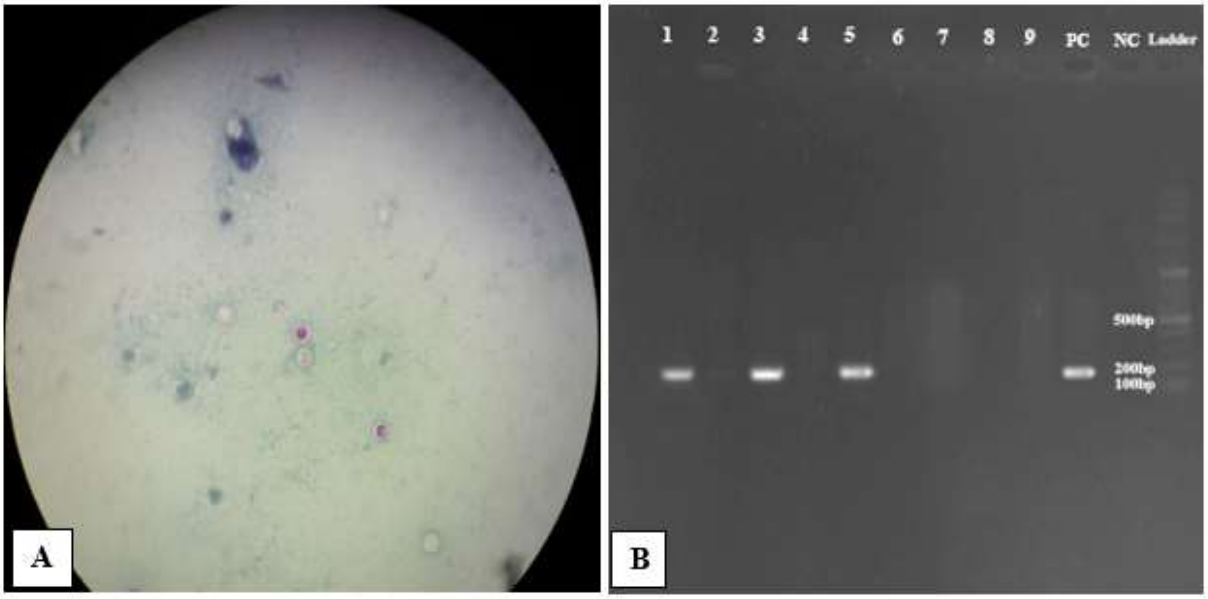

Figure 1. A) *Cryptosporidium* spp. oocysts in light microscope field with 400 x magnification, with modified Ziehl-Neelsen staining; B) Agarose gel electrophoresis showing the nested PCR products of the of 18S rRNA gene, diagnostic of *Cryptosporidium* species, three samples were positive; NC: Negative control, PC: Positive control.

infection, diverse variables such as geographical region, season, infectious species, and the individual's immune system, can be risk factors (24). Several species of *Cryptosporidium* have been isolated from humans; meta-analysis results showed that *C. parvum* and *C. hominis* are circulated among children and immunocompromised people (12). Additionally, C. parvum was reported to be the most common zoonotic species. Regarding the prevalence of *Cryptosporidium* spp. in Iranian animals, Motavalli Haghi et al. reported the highest value to be related to rodents (20.8% [95%, CI = 9.1–40.7%]) and the lowest value to dogs (4.9% [95%, CI = 2.6%, 8.8%]); it is noteworthy that the highest prevalence rate (50%) was reported from the southwestern of Iran (Khuzestan province) (25). It is also worth noting that control measures such as no contact with stray animals and fewer kept pets due to the cultural background can be possible reasons for the low prevalence rate in humans despite the high prevalence among animals in this region (25-27). Regarding the difference in prevalence in genders, although no statistically significant difference was observed, considering the relatively higher level of outdoor activity among boys, leading to the higher exposure rate of males, the higher prevalence of infections seems normal (28, 29). It is expected that increases in temperature and humidity and the favorable conditions for oocysts will further facilitate the acquisition of infection from the environment; in this regard, the present study showed that spring and summer showed higher prevalence rates, as with several other parasites (30). The importance of opportunistic parasite infections (e.g., *Cryptosporidium* spp. and Microsporidia) is very important in people with insufficient immunity, such as HIV patients, cancer patients undergoing corticosteroid treatment, children, and the elderly, because it can be dangerous and even lead to the death of the infected person (31-33).

Conclusion

The prevalence rates of *Cryptosporidium* spp. in southwestern regions of Iran were similar to reports from other regions of the country. Preventive health measures such as health education and drinking water sanitation seem necessary.

The present study was limited by the intense air heat in the study areas as well as the amount and volume, storage, and rapid transfer of samples to the laboratory. Moreover, because of the limited financial resources and problems caused by the COVID-19 pandemic, it was not possible to sequence the positive

samples. Therefore, it is suggested that future studies should focus on sequencing positive samples to identify subtypes.

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Conflict of interests

The authors declare that they have no conflict of interest.

References

1. The ANOFEL *Cryptosporidium* National Network, Laboratory-based surveillance for *Cryptosporidium* in France, 2006–2009. Eurosurveillance 2010;15:19642.

2. Sunnotel O, Lowery CJ, Moore JE, Dooley JSG, Xiao L, Millar BC, et al. *Cryptosporidium*. Lett Appl Microbiol 2006;43:7-16.

3. Ryan U, Hijjawi N. New developments in Cryptosporidium research. Int J Parasitol 2015;45:367-373.

4. Xiao L, Yaoyu Feng. Zoonotic cryptosporidiosis. FEMS Immunol Med Microbiol 2008;52:309-323.

5. Ryan, U., A. Zahedi, and A. Paparini, Cryptosporidium in humans and animals—a one health approach to prophylaxis. Parasite Immunology, 2016. 38(9): p. 535-547.

6. Korpe PS, Gilchrist C, Burkey C, Taniuchi M, Ahmed E, Madan V, et al. Case-control study of Cryptosporidium transmission in Bangladeshi households. Clin Infect Dis 2019;68:1073-1079.

7. Javanmard E, Mirsamadi ES, Olfatifar M, Ghasemi E, Saki F, Mirjalali H, et al. Prevalence of Cryptosporidium and Giardia in vegetables in Iran: a nineteen-years meta-analysis review. J Environ Health Sci Eng 2020;18:1629-1641.

8. Gerace E, Lo Presti VDM, Biondo C. Cryptosporidium infection: epidemiology, pathogenesis, and differential diagnosis. Eur J Microbiol Immunol 2019;9:119-123.

9. Dong S, Yang Y, Wang Y, Yang D, Yang Y, Shi Y, et al. Prevalence of Cryptosporidium infection in the global population: a systematic review and meta-analysis. Acta Parasitol 2020;65:882-889.

10. Taghipour A, Olfatifar M, Bahadory S, Godfrey SS, Abdoli A, Khatami A, et al., The global prevalence of Cryptosporidium infection in dogs: a systematic review and meta-analysis. Vet Parasitol 2020;281:109093.

11. Taghipour A, Olfatifar M, Foroutan M, Bahadory S, Malih N, Norouzi M. Global prevalence of Cryptosporidium infection in rodents: A systematic review and meta-analysis. Prev Vet Med 2020;182:105119.

12. Berahmat R, Spotin A, Ahmadpour E, Mahami-Oskouei M, Rezamand A, Aminisani N, et al. Human cryptosporidiosis in Iran: a systematic review and meta-analysis. Parasitol Res 2017;116:1111-1128.

13. Carey CM, Lee H, Trevors JT. Biology, persistence and detection of Cryptosporidium parvum and Cryptosporidium hominis oocyst. Water Res 2004;38:818-862.

14. Taghipour N, Nazemalhosseini-Mojarad E, Haghighi A, Rostami-Nejad M, Romani S, Keshavarz A, et al. Molecular epidemiology of cryptosporidiosis in Iranian children, Tehran, Iran. Iran J Parasitol 2011;6:41-45.

15. Hamedi Y, Safa O, Haidari M. Cryptosporidium infection in diarrheic children in southeastern Iran. Pediatr Infect Dis J 2005;24:86-88.

16. Jumani RS, Blais J, Tillmann HC, Segal F, Wetty D, Ostermeier C, et al. Opportunities and challenges in developing a Cryptosporidium controlled human infection model for testing antiparasitic agents. ACS Infect Dis 2021;7:959-968.

17. Manjunatha UH, Chao AT, Leong FJ, Diagana TT. Cryptosporidiosis drug discovery: opportunities and challenges. ACS Infect Dis 2016;2:530-537.

18. Widmer G, Köster PC, Carmena D. Cryptosporidium hominis infections in non-human animal species: revisiting the concept of host specificity. Int J Parasitol 2020;50:253-262.

19. Tahvildar-Biderouni F, Salehi N. Detection of Cryptosporidium infection by modified ziehl-neelsen and PCR methods in children with diarrheal samples in pediatric hospitals in Tehran. Gastroenterol Hepatol Bed Bench 2014;7:125-130.

20. Mohammad Rahimi H, Soleimani Jevinani S, Nemati S, Sharifdini M, Mirjalali H, Zali MR. Molecular characterization of Cryptosporidium skunk genotype in raccoons (Procyon lotor) in Iran: concern for zoonotic transmission. Parasitol Res 2022;121:483-489.

21. Abdel-Messih IA, Wierzba TF, Abu-Elyazeed R, Ibrahim AF, Ahmed SF, Kamal K, et al. Diarrhea associated with Cryptosporidium parvum among young children of the Nile River Delta in Egypt. J Trop Pediatr 2005;51:154-159.

22. Lal A, Cornish LM, Fearnley E, Glass K, Kirk M. Cryptosporidiosis: a disease of tropical and remote areas in Australia. PLoS Negl Trop Dis 2015;9:0004078.

23. Mirzaei M. Prevalence of Cryptosporidium sp. infection in diarrheic and non-diarrheic humans in Iran. Korean J Parasitol 2007;45:133-137.

24. Nazemalhosseini-Mojarad E, Feng Y, Xiao L. The importance of subtype analysis of Cryptosporidium spp. in epidemiological investigations of human cryptosporidiosis in Iran and other Mideast countries. Gastroenterol Hepatol Bed Bench 2012;5:67-70.

25. Haghi MM, Khorshidvand Z, Khazaei S, Foroughi-Parvar F, Sarmadian H, Barati N, et al. Cryptosporidium animal species in Iran: a systematic review and meta-analysis. Trop Med Health 2020;48:1-15.

26. Ghasemi E, Shamsinia S, Taghipour A, Anvari D, Bahadory S, Shariatzadeh SA, et al. Filarial worms: a systematic review and meta-analysis of diversity in animals from Iran with emphasis on human cases. Parasitology 2020;147:909-921.

27. Zibaei M, Alemi M, Cardillo NM, Derafshi H, Miahipour A, Bahadory S, et al. Human toxocariasis seroprevalence among patients with uveitis in Alborz Province, Iran. Ann Agric Environ Med 2019;26;154-158.

28. Khatami A, Bahadory S, Ghorbani S, Saadati H, Zarei M, Soleimani A, et al. Two rivals or colleagues in the liver? Hepatit B virus and Schistosoma mansoni co-infections: a systematic review and meta-analysis. Microb Pathog 2021;154:104828.

29. Razizadeh MH, Khatami A, Zarei M. Global status of Bufavirus, Cosavirus, and Saffold Virus in gastroenteritis: a systematic review and meta-analysis. Front Med 2022;8:775698.

30. Raissi V, Saber V, Zibaei M, Bahadory S, Akhlaghi E, Raiesi O, et al., Comparison of the prevalence of Toxocara spp. eggs in public parks soils in different seasons, from 2017 to 2018, Tehran Province, Iran. Clin Epidemiology Glob Health 2020;8:450-454.

31. Hosseini Parsa M, Bahadory S, Heidari A, Khatami A, Bairami A. Molecular and microscopic prevalence of intestinal microsporidia among HIV+/AIDS patients in the Alborz province, Iran. Trans R Soc Trop Med Hyg 2021;115:1445-1449.

32. Khatami A, Pormohammad A, Farzi R, Saadati H, Mehrabi M, Kiani SJ, et al. Bovine Leukemia virus (BLV) and risk of breast cancer: a systematic review and meta-analysis of case-control studies. Infect Agent Cancer 2020;15:1-8.

33. Mohammad Rahimi H, Mirjalali H, Zali MR. Molecular epidemiology and genotype/subtype distribution of Blastocystis sp., Enterocytozoon bieneusi, and Encephalitozoon spp. in livestock: concern for emerging zoonotic infections. Sci Rep 2021;11:1-16.