Tehran University of Medical Sciences Publication
http://tums.ac.ir

Iran J Parasitol

Open access Journal at
http://ijpa.tums.ac.ir

Iranian Society of Parasitology
http://isp.tums.ac.ir

Original Article

Occurrence and Molecular Identification of *Cryptosporidium* spp. and *Giardia duodenalis* in South Khorasan Province, Eastern Iran

Mahmoodreza Behravan [1,2], *Ali Haghighi [1], Rahmat Solgi [2], Masood Ziaee [2], Alireza Abadi [3], Mohammad Farjami [4]

1. *Department of Medical Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
2. *Infectious Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran*
3. *Department of Community Medicine, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
4. *Department of Biostatistics, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

Received 20 May 2026
Accepted 18 Sep 2026

Keywords:
Cryptosporidium parvum;
Giardia duodenalis;
Genotype;
Assemblage;
Iran

***Correspondence**
Email:
a_haghighi@sbmu.ac.ir

Abstract

Background: *Cryptosporidium* spp. and *Giardia duodenalis* (*G. duodenalis*) are two protozoan parasites responsible for gastrointestinal disorders in both people and animals worldwide. The genetic diversity of *G. duodenalis* is reflected through the identification of different assemblages and *Cryptosporidium* spp. is a major cause of moderate-to-severe diarrhea. This study aimed to pinpoint the molecular epidemiology of *Cryptosporidium* and *G. duodenalis* in the South Khorasan Province in the eastern Iran.

Methods: From Apr 2020 to Mar 2021, 1,800 stool samples were collected from individuals aged 1–78 yr referred to 10 medical laboratories in four cities of South Khorasan Province, eastern Iran. All samples were microscopically examined using direct wet mount smear and modified Ziehl–Neelsen staining. DNA was extracted from microscopy-positive samples, and nested PCR was performed for identification of *G. duodenalis* using the glutamate dehydrogenase (*gdh*), triosephosphate isomerase (*tpi*) and β-giardin (*βg*) genes. Nested PCR targeting the 60 kDa glycoprotein (*gp60*) gene was also performed for *Cryptosporidium* species identification. Data were analyzed using SPSS software.

Results: Twenty-two (1.22%) out of 1800 samples were identified microscopically positive for *Cryptosporidium* spp. and 72 (4%) positive for *G. duodenalis*. Nested-PCR method revealed all isolates as *Cryptosporidium parvum* (*C. parvum*) IIa and IId subtype families. All of the 72-microscopy positive samples were tested and revealed specific *G. duodenalis* band. Sequencing of 30 PCR *G. duodenalis* positive identified all as assemblage AII.

Conclusion: This study provides preliminary evidence of potentially zoonotic genotypes, warranting future One Health investigations to clarify transmission pathways.

Copyright © 2026 Behravan et al. Published by Tehran University of Medical Sciences.
This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International license.
(https://creativecommons.org/licenses/by-nc/4.0/). Non-commercial uses of the work are permitted, provided the original work is properly cited

Introduction

The protozoan parasites *Cryptosporidium* spp. and *G. duodenalis* (Syn. *G. lamblia* and *G. intestinalis*) are two important enteric public health concerns for humans, animals and they are major foodborne and waterborne pathogens along with high economic costs worldwide (1-6). *Cryptosporidium* is the second cause of moderate to severe diarrhea in children under 5 yr of age in developing countries and *G. duodenalis* is one of the most common enteric protozoan infection with an estimated annual incidence of more than 280 million cases worldwide (1, 4).

Currently, about 50 *Cryptosporidium* spp. valid species and 120 genotypes are estimated in the genus *Cryptosporidium*. Among of this parasite, 21 have been found in humans and the majority of human infections caused by *C. hominis* and *C. parvum* {*Bujila, 2024 #115*}(4, 7, 8). Microscopy, is considered by many to be suboptimal for both protozoa. Although modified Ziehl–Neelsen (mZN) staining remains a cornerstone for *Cryptosporidium* diagnosis, it is increasingly being supplemented or replaced by more sensitive techniques such as molecular-based methods in many clinical and research settings (5, 9). Therefore, for valid diagnosis, molecular methods, particularly PCR has been prompted (8-10). The 60 kDa glycoprotein gene (*gp60*), is frequently used for tracking infection sources and examining the population structure of *Cryptosporidium* spp., primarily *C. parvum* and *C. hominis* (11, 12).

The identification of distinct assemblages or genotypes within *G. duodenalis* is crucial to better understanding its taxonomy, epidemiology, transmission, and potential zoonotic risks (8, 13, 14). Several specific genetic markers like glutamate dehydrogenase (*gdh*), triosephosphate isomerase (*tpi*) and β-giardin (*βg*) genes are commonly targeted for detection (13, 14). To date, eight assemblages (A-H) have been identified. Assemblages A and B are known to infect humans and some animals, while the other assemblages are typically found in specific animal species (14, 15).

South Khorasan Province is characterized by frequent cross-border movement of populations between Iran and Afghanistan. The rationale for this study is rooted in the significant lack of epidemiological data regarding the occurrence of *Cryptosporidium* spp. and *G. duodenalis*. Therefore, this study aimed to address this critical information gap in a region with high potential for cross-border parasite circulation. This study primarily aimed to perform a demographic analysis of patients with cryptosporidiosis and giardiasis, alongside molecular characterization and genotyping of these infections in South Khorasan.

Materials and Methods

Geographical Location of the Study Area

The province of South Khorasan is in the eastern Iran and at the border of Afghanistan with 898,000 populations. Birjand is center of the province in which about half of the population lives in this city. Birjand, Darmian, Nehbandan, and Ferdows cities were selected for the study.

Specimen Collection and Microscopic Examination

This cross-sectional survey was conducted from Apr 2020 to the end of Mar 2021. By using random integers, four cities of the province were selected for the study. Totally during 4 seasons of the year, 1,800 stool samples from referred individuals to the 10 medical laboratories from those four cities were obtained. With a sample of 1,800 and a two-sided 95% confidence interval, the study had >80% power to detect prevalence rates as low as [X%] (absolute precision of ±Y%), confirming that the sample size was adequate for the main analyses.

For each participant a demographic questionnaire including age, sex, education and their gastrointestinal symptoms was completed. Fecal samples were transferred to the Department of Medical Parasitology of Birjand University of Medical Sciences, and were tested microscopically through direct slide smear and formalin-ether concentration techniques. All the samples were also stained using modified Ziehl-Neelsen method to identify *Cryptosporidium* spp. oocysts.

DNA Extraction, Nested-PCR and Sequencing

Genomic DNA was extracted from approximately 200 µL of stool from all microscopy-positive isolates of *Cryptosporidium* spp. and *G. duodenalis*. In addition, 55 microscopy-negative samples were randomly selected from the negative specimens and included in the molecular analysis to evaluate the potential presence of false-negative microscopic results. DNA extraction was performed using the Favorgen Biotech DNA Stool Mini Kit (Taiwan) according to the manufacturer's instructions, and the extracted DNA was stored at −20 °C until further analysis.

Using the previously described Nested-PCR and genus-specific primers, the *gp60* gene of *Cryptosporidium* spp. was amplified. (16, 17). A Nested-PCR for *G. duodenalis* was also conducted by amplifying the *ßg*, *gdh* and *tpi* genes (18-21) (Table 1). Both positive controls (DNA from previously confirmed positive samples) and negative controls (sterile distilled water) were included in all PCR procedures.

All the Nested-PCR products of *Cryptosporidium* and 30 from the *gdh*, *tpi*, and *ßg* genes were chosen at random and subjected to sequencing using a Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems) on a55n ABI PRISM-3130xl Genetic Analyzer. All sequences were edited manually using the Chromas program version 1.0.0.1. The results were compared using Basic Local Alignment Search Tool (BLAST).

Table 1: The oligonucleotide primer sets used for the Nested-PCR of *G. duodenalis* and *Cryptosporidium*

Protozoa	Gene	Sequences of forward and reverse primers	Product size (bp)
G. duodenalis	*gdh*	GDHeF 5'-GACGCATCAACGTCAACC-3' GDHeR 5'-GAGCTTCTCGCAAGCAAAC-3'	539
		GDHiF 5'-CAGTACAACTCTGCTCTCGG-3' GDHiR 5'-GTTGTCCTTGCACATCTCC-3'	432
	tpi	AL3543 5'-AAATIATGCCTGCTCGTCG-3' AL3546 5'-CAAACCTTITCCGCAAACC-3'	620
		AL3544 5'-CCCTTCATCGGIGGTAACTT-3' AL3545 5'-GTGGCCACCACICCCGTGCC-3'	530
	ßg	Gia7 5'-AAGCCCGACGACCTCACCCGCAGTGC-3' Gia759 5'-GAGGCCGCCCTGGATCTTCGAGACGAC-3'	753
		GiarF 5'-GAACGAACGAGATCGAGGTCCG-3' GiarR 5'-CTCGACGAGCTTCGTGGTT-3'	511
Cryptosporidium	*gp60*	Gp60-eF 5'-ATAGTCTCCGCTGTATTC-3'	854
		Gp60-eR 5'-GCAGAGGAACCAGCATC-3'	
		Gp60-iF 5'-TCCGCTGTATTCTCAGCC-3'	467
		Gp60-iR 5'-GAGATATATCTTGGTGCG- 3'	

Statistical Analysis

Using SPSS software ver. 21, (IBM Corp., Armonk, NY, USA), the data were analyzed using the chi-square (X2) test. Statistics were considered significant for *P*-values below 0.05.

Results

Microscopy results

Out of 1800 referred individuals, *Cryptosporidium* oocysts were found in 22 samples (1.22%) ranging age from 1 to 78 yr old. Eighteen specimens (81.80%) were referred to Birjand medical centers, while the other four (18.20%) were from three different cities in the province (*P*=0.00) (Table 2). Eighteen males (81.80%) and four females (18.20%) were positive (*P*=0.12). The majority of infected people were between the ages of 18 to 30 (31.8%) (*P*=0.29), lived in rural areas (54.5%) (*P*=0.00), and had at least completed their secondary school (50.5%) (*P*=0.00). Seventy-seven percent (17 instances) of the verified cases had gastrointestinal symptoms such as stomach aches, nausea, vomiting, and diarrhea (*P*=0.12). Nine cases (40.9%) were reported over the summer (*P*=0.22). Statistical analysis of the obtained data using the Chi-square test showed that there was a significant unadjusted association between *Cryptosporidium* infection and county, type of laboratory, location, literacy level, contact with livestock and job (*P*<0.05), but no significant unadjusted association was observed between *Cryptosporidium* infection and sex, reason for referral, season, and age (*P*>0.05).

Table 2: Frequency of cryptosporidiosis according to the demographic characteristics of patients (N=1800) in 10 medical laboratories in South Khorasan Province

Demographic variables		No. and (%) of the referred individuals	No. and (%) of positive *Cryptosporidium* spp.	% from 22 positive	*P* value
County	Birjand	720 (40)	18 (2.5)	81.8	0.00
	Darmian	360 (20)	2 (0.56)	9.1	
	Nehbandan	360 (20)	1 (0.38)	4.5	
	Ferdows	360 (20)	1 (0.28)	4.5	
Sex	Female	652 (36.22)	4 (0.61)	18.2	0.12
	Male	1148 (63.78)	18 (1.57)	81.8	
Age (yr)	0-6	65 (3.61)	2 (3.1)	9.1	0.29
	6-12	78 (4.33)	1 (1.28)	4.5	
	12-18	157 (8.72)	2 (1.27)	9.1	
	18-30	512 (28.44)	7 (1.37)	31.8	
	30-50	691 (38.40)	4 (0.58)	18.2	
	50>	297 (16.5)	6 (2)	27.3	
Reason for referral	Gastrointestinal discomfort	1032 (57.33)	17 (1.65)	77.3	0.12
	Non- Gastrointestinal	444 (24.67)	4 (0.9)	18.2	
	Check up	324 (18)	1 (0.3)	4.5	

G. duodenalis cysts and trophozoites were detected in 72 cases (4.00%) out of 1800 referred individuals using direct and concentration methods. 57 of them (79.20%) were referred to Birjand medical centers (*P*=0.002) (Table 3). The remaining 15 cases (20.80%) came from three other cities. 41 (56.90%) were males and 31 (43.10%) were females

(P=0.16). The largest group of individuals infected consisted of people between the ages of 30 and 50, comprising 27.80% of the total cases (*P*=0.29). Additionally, the majority of the infected individuals lived in urban areas, accounting for 90.30% of the cases (*P*=0.21). The majority of patients (93.10%) experienced gastrointestinal symptoms, such as bellyache, nausea, vomiting, and diarrhea and a small percentage of them (6.90%) were asymptomatic (*P*=0.12). Statistical analysis of the obtained data using the Chi-square test showed that there was a significant unadjusted association between *G. duodenalis* infection and county, type of laboratory, and literacy level (*P*<0.05), but no significant unadjusted association was observed between *G. duodenalis* infection and sex, age, location, job, reason for referral, contact with livestock and season (*P*>0.05).

Table 3: Frequency of giardiasis according to the demographic characteristics among 1800 referring to the selected South Khorasan Province laboratories, Iran

Demographic variables		No. and (%) of the referred individuals	No. and (%) of positive *G. duodenalis*	% from 72 positive	*P*
County	Birjand	720 (40)	57 (7.9)	79.2	0.002
	Darmian	360 (20)	6 (1.66)	8.3	
	Nehbandan	360 (20)	4 (1.1)	5.5	
	Ferdows	360 (20)	5 (1.38)	7	
Sex	Female	652 (36.22)	31 (4.75)	43.1	0.16
	Male	1148 (63.78)	41 (3.57)	56.9	
Age (yr)	0-6	65 (3.61)	2 (3.1)	2.9	0.29
	12-Jun	78 (4.33)	8 (10.25)	11.1	
	18-Dec	157 (8.72)	9 (5.7)	12.5	
	18-30	512 (28.44)	19 (3.7)	26.4	
	30-50	691 (38.40)	20 (2.9)	27.8	
	50<	297 (16.5)	14 (4.7)	19.4	
Reason for referral	Gastrointestinal discomfort	1032 (57.33)	67 (6.49)	93.1	0.12
	Non- Gastrointestinal	444 (24.67)	3 (0.68)	4	
	Check up	324 (18)	2 (0.62)	2.9	

Molecular results and genotyping of Cryptosporidium spp.

The Nested-PCR technique with the *gp60* gene confirmed the 22 positive microscopic samples Furthermore, none of the 30 negative samples and 25 positives for other parasites except *Cryptosporidium* were positive. The *gp60* gene sequence analysis confirmed the identity of all 22 isolates as *C. parvum*. Mainly, ten IId (10/22) and twelve (12/22) *C. parvum* IIa subtype families were observed. 11 of the 12 isolates of the IIa subtype were IIaA15G2R1 (95%), and one was IIaA16G1R1 (5%). Nine isolates from the 10 IId were IIdA21G1 (95%) and one was IIdA22G1R1 (5%). The representative sequences have been submitted to GenBank and have been assigned accession numbers as OM273059 to OM273080.

PCR and sequencing of the G. duodenalis

All of the 72 microscopy-positive isolates and 2 from 55 negative samples were identified and confirmed as *G. duodenalis* using the Nested-PCR method with the *gdh* gene and/or *tpi* or *ßg* genes.. Therefore, there have been a total of 74 molecular positive cases detected, accounting for 4.11% of the total individuals.

Of the PCR-positive *G. duodenalis* samples, 30 isolates with sufficient DNA quality and clear amplification bands were randomly selected for *gdh* gene sequencing. All the sequenced isolates were identified as assemblage AII. Additionally, the remaining 30 samples, which included *tpi* and *ßg* genes also exhibited assemblage AII. The representative sequences have been submitted to GenBank and have been assigned accession numbers as listed in Table 4.

Table 4: Accession numbers of AII sub-assemblages of *G. duodenalis* from three studied genes

Giardia sub-assemblages	Gene	Accession Number
AII	*gdh*	OM407500-OM407529
AII	*gdh*	OQ446151-OQ446156
AII	*tpi*	OQ446157-OQ446166
A	*ßg*	OQ446141-OQ446150

Discussion

C. parvum and *C. hominis* are the two species of *Cryptosporidium* spp. reported most frequently in people (22). Direct contact with an infected person, consumption of contaminated drinking water or food and contact with infected animals are the main factors in the prevalence of cryptosporidiosis (23). The occurrence rate of cryptosporidiosis in this study area was 1.22% in which is less than some recently reported data from Lorestan province, in western and Zabol in south east of Iran (24, 25). However, there are also some published articles close to this study (26, 27). About half of the population in the South Khorasan Province are living in Birjand City and this could be an explanation that why more samples and more patients obtained from this city. The disease was significantly higher in male (1.6%) in compare to the women. In Lorestan Province (24) and Bandar-Abbas city (28), males have made up the majority of positive instances of the disease. Men are more likely to be exposed to infectious diseases at work and in the community. Another significant factor in the frequency of cryptosporidiosis is contact with animals, particularly cattle, and this factor helps to define the zoonotic nature of the illness (29). In this study, 45.50% of patients had a history of contact with livestock (P<0.05); in studies conducted in Yemen (30) and Sanandaj City (17), the majority of patients had a history of contact with livestock. Additionally, this study revealed that the summer and fall months have 63.60% of all positive cases of *Cryptosporidium*. According to comparable research conducted in Sanandaj City (17) the disease was more prevalent in the summer and fall.

C. parvum species was detected in all the sequenced samples. It has been identified as the dominant species as well (17, 28,). Subtype IIaA has been more prevalent in most molecular epidemiology investigations (17, 31). The IIdA subtype is also prevalent in several reports (26, 28, 31). We exclusively detected *C. parvum* and found no evidence of *C. hominis*. This finding is noteworthy and may reflect the specific epidemiological dynamics of the study area. The predominance of *C. parvum* suggests a significant zoonotic component in the transmission cycle within South Khorasan, potentially driven by close contact between humans and livestock or contamination of water sources by animal feces.

Regarding to the microscopic examination of *G. duodenalis*, the occurrence of giardiasis is detected 4.11%. Several prior studies, including those conducted already (32, 33, 34), have also reported the prevalence of giardiasis ranging about 2% to 7%. Out of all the reported cases of this study, 80.50% were identified from Birjand medical centers. The main reason is that 1) half of the population of the South-Khorasan Province are living in Birjand City, and 2) usually patients refer from other cities to this city in order to access better healthcare and laboratory services. The relatively lower occurrence of *G. duodenalis* observed in South Khorasan Province compared to certain other provinces in Iran may be attributed to differences in environmental conditions, demographic characteristics, or methodological approaches.

The statistical analysis of the collected data revealed a significant correlation between the occurrence of giardiasis and the underlying reasons for referral, such as heartache, heartburn, diarrhea, and abdominal bloating. This implies that individuals who experience gastrointestinal symptoms have a higher likelihood of being affected by parasitic infections. This finding aligns with the studies carried out by others (17, 26). Most of the patients with giardiasis resided in urban areas or were individuals who had previously come into contact with livestock. Studies, including those conducted already (35, 36) have reported correlation between the prevalence of giardiasis and place of residence. Our research has revealed a seasonal trend indicating that infection rates tend to peak during the summer. This finding aligns with previous studies conducted by others (37, 38). The noticeable increase in infection cases during this period is likely attributed to the higher consumption of contaminated vegetables and fruits.

Assemblage A was detected for all of the sequenced isolates. The predominance of sub-assemblage AII aligns with the findings of several studies (39-41). Notably, assemblage B of *G. duodenalis* was detected at a lower frequency.

The findings from multiple studies conducted indicate that assemblage A is predominantly present in the northern (42), southwestern (39, 40), and western of Iran (36, 43, 44). Notably, research conducted in Southwestern Iran (39) and Bangladesh (45), has found a correlation between assemblage A and clinical symptoms. In the present study, we further analyzed symptomatic versus asymptomatic cases and found that assemblage AII was more frequently observed in individuals reporting gastrointestinal symptoms, supporting previous reports and providing stronger evidence for a relationship between giardiasis genotype and clinical presentation.

The findings highlight important public health considerations, particularly due to the zoonotic potential of *Cryptosporidium* spp. and *G. duodenalis* and the cross-border movement of populations and livestock in South Khorasan Province. These results underscore the need for enhanced surveillance, preventive measures, and health education to reduce transmission risks.

This study has several limitations. The sample was restricted to laboratory-referred, microscopy-positive individuals, which limits representativeness of the general population. The small number of positive cases further affects generalizability. The lack of animal, environmental, and exposure data precludes assessment of transmission sources, and single-locus genotyping limits discriminatory power. Accordingly, our findings should be interpreted as preliminary descriptive evidence within this specific population.

Conclusion

This study documents human infections with *C. parvum* (IIa/IId) and *G. duodenalis* assemblage A in South Khorasan Province, with assemblage type correlating with clinical symptoms. However, due to the lack of animal, en-

vironmental, and exposure data, and the limitations of single-locus genotyping, our findings do not support a causal claim of zoonotic transmission. These results provide preliminary evidence of potentially zoonotic genotypes in humans and highlight the critical need for future One Health-oriented research to determine actual transmission routes.

Ethical consideration

This manuscript has been approved by the ethics committee of Shahid Beheshti University of Medical Sciences, Iran, approved the project (IR.SBMU.MSP.REC.1398.741).

Acknowledgements

The current work was a component of Mahmoodreza Behravan's Ph.D. thesis, financially supported by the Shahid Beheshti University of Medical Sciences (Grant number 25680). We want to express our sincere gratitude to all the staff members of the Department of Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, and the Deputy of Research and Technology of Birjand University of Medical Sciences, South Khorasan Province.

Funding

This study received support from the Research Department of the School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran (Grant Number 25680).

Conflicts of Interest

The author declares that they have no conflict of interests.

References

1. Squire SA, Ryan U. *Cryptosporidium* and *Giardia* in Africa: current and future challenges. Parasit Vectors. 2017;10:195.
2. Aydemir S, Barlık F, Ekici A, et. al. Molecular Characterization of *Giardia intestinalis* and *Cryptosporidium* Spp. Detected in Humans in Ağrı, Türkiye, Iran J Parasitol. 2024;19(1): 9-17.
3. Monis PT, Thompson RCA. *Cryptosporidium* and *Giardia*-Zoonoses: Fact or Fiction? Infect Genet Evol. 2003; 3(4):233-244.
4. Zahedi A, Monis P, Deere D, Ryan U. Wastewater-based epidemiology-surveillance and early detection of waterborne pathogens with a focus on SARS-CoV-2, *Cryptosporidium* and *Giardia*. Parasitol Res. 2021; 120(12):4167-4188.
5. Plutzer J, Lassen B, Jokelainen P, et al. Review of *Cryptosporidium* and *Giardia* in the eastern part of Europe, 2016. Euro Surveill. 2018; 23(4): 16-00825.
6. Sprong H, Simone M. Caccio, et al. on behalf of the ZOOPNET network and partners. Identification of Zoonotic Genotypes of *Giardia duodenalis*. PLoS Negl Trop Dis. 2009; 3(12): e558.
7. Jiang Y, Jiang A, Ren G, et al. Cross-species transmission of *Cryptosporidium* in wild rodents from the southern region of Zhejiang Province of China and its possible impact on public health. Parasite.2024; 31:34.
8. Ryan UM, Feng Y, Fayer R, Xiao L. Taxonomy and molecular epidemiology of *Cryptosporidium* and *Giardia* – a 50-year perspective (1971–2021). Int J Parasitol. 2021; 51(13-14):1099-1119.
9. Insulandr M, Ilverla CS, Lebbad M, et al. Molecular epidemiology and clinical manifestations of human cryptosporidiosis in Sweden. Epidemiol Infect. 2013; 141(5):1009-1020.
10. Jennifer K. O'Leary, Sleator RD, Lucey B. *Cryptosporidium* spp. Diagnosis and Research in the 21st Century. Food Waterborne Parasitol. 2021; 24: e00131
11. Rachel M. Chalmers RM, Robinson G, et al. Analysis of the *Cryptosporidium* spp. and gp60 subtypes linked to human outbreaks of cryptosporidiosis in England and Wales, 2009 to 2017. Parasit Vectors. 2019; 12(1):95.
12. Lebbad M, Jana Grüttner J, Beser J, et al. Complete sequencing of the *Cryptosporidium*

suis gp60 gene reveals a novel type of tandem repeats—Implications for surveillance. Infect Genet Evol. 2024; 122: 105614.
13. Thompson RCA and Ash A. Molecular epidemiology of Giardia and *Cryptosporidium* infections. Infect Genet Evol. 2016; 40:315–323.
14. Bahramdoost Z, Mirjalali H, Yavari P, Haghighi A. Development of HRM real-time PCR for assemblage characterization of *Giardia lamblia*. Acta Trop. 2021; 224: 106109.
15. Lee MF, Auer H, Lindo JF, Walochnik J. Multilocus sequence analysis of *Giardia* spp. isolated from patients with diarrhea in Austria. Parasitol Res. 2017; 116:477-81.
16. Nazemalhosseini-Mojarada E, Haghighi A, Taghipour N, et al. Subtype analysis of *Cryptosporidium parvum* and *Cryptosporidium hominis* isolates from humans and cattle in Iran. Vet Parasitol. 2011; 179: 250–252.
17. Bahrami B, Haghighi A, Zamini Gh, et al. Zoonotic transmission of *Cryptosporidium* and *Microsporidia* in individuals of the Kurdistan province, west, Iran. J Parasitol. 2020; 106(4):464–470.
18. Sulaiman IM, Fayer R, Bern C, Gilman RH, et al Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. Emerg Infect Dis. 2003; 9(11):1444-52.
19. Kashinahanji M, Haghighi A, Bahrami F, et al. *Giardia lamblia* assemblages A and B isolated from symptomatic and asymptomatic persons in Hamadan, west of Iran. J Parasit Dis. 2019; 43(4):616–623.
20. Hong SH, Anu D, Jeong Y-I1, et al. Molecular Characterization of *Giardia duodenalis* and *Cryptosporidium parvum* in Fecal Samples of Individuals in Mongolia. Am J Trop Med Hyg. 2014; 90(1): 43-47.
21. Caccio SM, De Giacomo M, Pozio E. Sequence analysis of the b-giardin gene and development of a polymerase chain reaction–restriction fragment length polymorphism assay to genotype *Giardia duodenalis* cysts from human fecal samples. Int J Parasitol. 2002; 32:1023–1030.
22. Bujila O, Troell K, Ögren J, et al. *Cryptosporidium* species and subtypes identified in human domestic cases through the national microbiological surveillance programme in Sweden from 2018 to 2022. BMC Infect Dis. 2024; 24:146.
23. Helmy YA and M. Hafez HM. Cryptosporidiosis: From Prevention to Treatment, a Narrative Review. Microorganisms. 2022; 10(12): 2456.
24. Ghanadi K, Khalaf AK, Jafrasteh A, et al. High prevalence of *Cryptosporidium* infection in Iranian patients suffering from colorectal cancer. Parasite Epidemiol Control. 2022; 19: e00271.
25. Dabirzadeh M, Koshsima Shahraki et al. Prevalence of *Cryptosporidium* Species in Children Referred to Central and Hospital Laboratories of Zabol City, South East of Iran. Int J Pediatr. 2017; 5(12):6359-6364.
26. Kiani H, Haghighi A, Seyyedtabaei SJ, et al. Prevalence, Clinical Manifestations and Genotyping of *Cryptosporidium* Spp. in Patients with Gastrointestinal Illnesses in Western Iran. Iran J Parasitol. 2017; 12(2):169-176.
27. Nouraftab B, Mahmoudi E, Bahadory S, et al. Prevalence of *Cryptosporidium* spp. in children and the elderly in southwestern Iran. Gastroenterol Hepatol Bed Bench. 2022; 15(4):415-420.
28. Najafi-Asl M, Faraji H, Hosseini T, et al. Prevalence and Molecular Genotyping of *Cryptosporidium* Spp. in Diarrheic Patients from Bandar Abbas City, Southern Iran. Jundishapur J Microbiol. 2020; 13(6): e102706.
29. Ryan U, Zahedi A, Feng Y and Xiao L. An Update on Zoonotic *Cryptosporidium* Species and Genotypes in Humans. Animals (Basel). 2021; 11(11):3307.
30. Al-Shamiri A, Al-Zubairy A, Al-Mamari R. The prevalence of *Cryptosporidium* spp. in children, Taiz District, Yemen. Iran J Parasitol. 2010; 5(2):26-32.
31. Osman M, El Safadi D, Benamrouz S, et al. Initial data on the molecular epidemiology of cryptosporidiosis in Lebanon. PLoS One. 2015; 10(5): e0125129.
32. Mehran S, Haghighi A, Khazan H, et al. Epidemiological Study of Intestinal Parasites in Referred Individuals to the Medical Centers' Laboratories of Haji-Abad

City, Hormozgan Province, Iran, 2015. Novelty in Biomedicine. 2017; 5(3):119-26.
33. Hatam-Nahavandi K, Ahmadpour E, Rezaeian M, et al. Occurrence and assemblage distribution of *Giardia duodenalis* in symptomatic and asymptomatic patients in southeastern Iran (2019–2022). Gut Pathog. 2024; 16:68.
34. Asghari A, Mahdavi F, Karimi K, et al. Molecular epidemiology and multilocus genotyping of *Giardia duodenalis* in individuals attending major public hospitals in Shiraz, southwestern Iran: A public health concern. Parasite Epidemiol Control. 2024; 25: e00354.
35. Afshar MJA, Mehni MB, Rezaeian M, et al. Prevalence and associated risk factors of human intestinal parasitic infections: a population-based study in the southeast of Kerman province, southeastern Iran. BMC Infect Dis. 2020; 20(1):12.
36. Badparva E, Kheirandish F, Ebrahimzade F. Prevalence of intestinal parasites in Lorestan Province, West of Iran. Asian Pacific Journal of Tropical Disease. 2014; 4:S728-S32.
37. Yoder JS, Harral C, Beach MJ, et al. Prevention Giardiasis surveillance—United States, 2006–2008. MMWR Surveill Summ. 2010; 59(6):15-25.
38. Siyadatpanah A, Sharif M, Daryani A, et al. Spatial distribution of *Giardia lamblia* infection among general population in Mazandaran Province, north of Iran. J Parasit Dis. 2018; 42(2):171-176.
39. Rafiei A, Baghlaninezhad R, Köster PCet al. Multilocus genotyping of *Giardia duodenalis* in Southwestern Iran. A community survey. PLoS One. 2020; 15(2):e0228317.
40. Mirrezaie E, Beiromvand M, Tavalla M, et al. Molecular genotyping of *Giardia duodenalis* in humans in the Andimeshk County, southwestern Iran. Acta Parasitol. 2019; 64(2):376-383.
41. Pacheco FTF, Silva RKNR, de Carvalho SS, et al. Predominance of *Giardia duodenalis* AII sub-assemblage in young children from Salvador, Bahia, Brazil. Biomedica. 2020; 40(3):557- 568.
42. Mahmoudi MR, F, Ashrafi K, et al. Reports of *Giardia* assemblages and Giardiasis in residents of Guilanprovince-Iran. Parasitol Res. 2020; 119(3):1083-1091.
43. Bahrami F, Zamini G, Haghighi A, Khademerfan M. Detection and molecular identification of human *Giardia* isolates in the West of Iran. Biomedical Research (India). 2017; 28(13):5687-92.
44. Kiani H, Haghighi A, Rostami A, et al. Prevalence, risk factors and symptoms associated to intestinal parasite infections among patients with gastrointestinal disorders in Nahavand, Western Iran. Rev Inst Med Trop Sao Paulo. 2016:58:42.
45. Haque R, Roy S, Kabir M, et al. *Giardia* assemblage A infection and diarrhea in Bangladesh. J Infect Dis. 2005;192(12):2171-3.