

A review of giardiasis and its parasite genotypes in Iran

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Abstract

Giardia lamblia is one of the most prevailing intestinal protozoa in many vertebrates like humans, domestic and wild animals. Molecular studies show that *Giardia lamblia* is a complex parasite. Currently, giardiasis disease is one of the main problems of social and personal health in different countries around the world. *Giardia* is known as a zoonotic parasite that is divided into eight genetic assemblages (A to H). In this review study, by referring to the molecular epidemiology of *Giardia lamblia* and emphasizing its zoonotic factors, the background of *Giardia* and its genotypes in Iran have been studied. The study population consisted of indexed articles in reputable databases such as Scopus, Magiran, SID, Science Direct, PubMed, and Google Scholar in Iran from 1990 to 2020. Based on limited studies in Iran, assemblage BIII

and AII are the most common types of *Giardia* assemblages. Therefore, it is necessary to conduct comprehensive studies on various human and animal isolates in different areas of the country, especially places where no researches have been done.

Introduction

Giardia parasite is an intestinal protozoan in humans and a wide range of vertebrate hosts.¹ This organism is one of the ten major parasites of human and one of the most prevailing non-viral causes of diarrhea in humans and domestic ungulates.^{2,3} *Giardia duodenalis* is the only species found with a zoonotic significance.⁴ This parasite has two different forms in its life cycle. The first form is a flagellate trophozoite that lives in the upper part of the small intestine and causes clinical symptoms. The other form is two or four nuclei cysts that excrete with the host feces and remain in the environment for weeks. The disease transmission occurs through a person to another or through contaminated water and food. Although this protozoan has been reported from all over the world, it is more prevalent in tropics and areas with few health facilities and high populations.³

In developing countries, the rate of prevalence and incidence of giardiasis infection is high. It is estimated that about 200 million people in Asia, Africa, and Latin America are infected by symptomatic giardiasis, and about 500,000 new cases are reported annually.² Clinical symptoms of the disease are varied from asymptomatic infections to chronic diarrhea. Symptomatic cases are usually accompanied by weakness, weight loss, watery diarrhea, stinky stools, fatty or steatorrhea diarrhea, abdominal cramps, abdominal bloating, burping, nausea, vomiting, and malabsorption syndrome.^{3,5,6} Allergic skin manifestations like urticaria and angioedema are the other symptoms.⁶⁻⁸

There is little information about the risk factors of giardiasis, some of them include the status of the immune system, age, sex, and environmental, social, economic, occupational, and nutritional conditions, and recently the type of genotypes.⁹⁻¹⁷ Although relatively numerous and scattered studies have been performed by Iranian researchers on giardiasis in Iran, still the current status of this disease is not exactly clear. Therefore, this study is designed for awareness of the status of this disease in Iran. This review presented articles related to giardiasis and its various epidemiological aspects in Iran using keywords, *Giardia*, giardiasis, genotype, assemblage, prevalence, Iran (alone or in combination) have been searched in reputable and accessible information databases like Science Direct, SID, Scopus, and Magiran from 1990 to 2020.

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Species and genome

Several species of *Giardia* have been defined based on differences in morphological features and specific hosts including *Giardia duodenalis*, *Giardia muris*, *Giardia agilis*, *Giardia ardeae*, *Giardia psittaci*, and *Giardia microti* (Appendix, Table 1).

Among them, *Giardia duodenalis* (synonym with *Giardia intestinalis* or *Giardia lamblia*) is the only species detected in humans.⁸ Possibly rare parasite species similar to *Giardia duodenalis* is present in reptiles.

Although reported *Giardia* in lizards lacks a median body and two nuclei cysts, it is considered as *Giardia varani*.¹⁸ *Giardia duodenalis* genome is estimated at approximately 2.2×10^7 bp, and its C+G content 49%. This genome has four central histones commonly used in eukaryotes to form chromosomal DNA.¹⁹

Target genes

Although simple methods have been introduced to identify *Giardia* in clinical and environmental specimens, recently various molecular tools have been used to differentiate this parasite by species, assemblage (subspecies), and genotype. In this regard, different target genes are used, which GDH (Glutamate dehydrogenase), SSU rRNA Vsp (Variant surface protein), TPI (Triose-phosphate isomerase), and BG (β -giardin) have more use. By reviewing the studies of researchers, we found that the SSU rRNA gene is mostly used to differentiate species and assemblages, and the TPI gene (with the highest number of variable locus) for parasite subtypes. But in general, the GDH and BG genes are more widely used than other target genes.¹⁹⁻²³

Based on various molecular methods such as PCR-RELP and sequence analysis, *Giardia duodenalis* is divided into three genotypes named groups 1, 2, 3 (WB, JH, GS). In conducted studies, *Giardia* isolates divides into two assemblages (A and B assemblages). Most researchers believe that groups 1 (WB) and 2 (JH) belong to assemblage A, and group 3 (GS) belongs to assemblage B.¹⁹ On the other hand, some believe that group 2 (JH isolate) has a close and similar but distinct dependency from assemblage A.²¹

Currently, many molecular studies show that *Giardia lamblia* is a complex species that consists of at least eight main assemblages (A to H), which are morphologically similar but genetically different from each other.²² Among these eight assemblages, only assemblages A and B are obtained from humans. These two assemblages can also infect animals. Assemblage A is categorized into smaller genetic groups named AII and AI. Assemblage B also includes smaller genetic groups named BII and BIV. Assemblages C and D are specifically for dogs, and it seems that assemblages E, F, G, and H are specific for livestock, cats, rats, and marine vertebrates such as whales, dolphins, seals and shore birds, respectively.^{23,24}

The relation between assembly and clinical signs

Although the presence of a relation between clinical symptoms and assemblages of *Giardia duodenalis* has not been confirmed yet,²⁵ researchers intend to reveal which genotype has a higher effect on diarrheic patients.²⁶

Zoonotic transmission (zoonosis)

Several studies have examined the importance of zoonotic transmission in the occurrence of human giardiasis.²⁷ In late 1977, zoonotic potential of *Giardia* was identified, but this has not been confirmed yet. Possible sources for zoonotic transmission are dairy cattle, dogs, cats, and wild animals. Giardiasis is common among domestic animals (especially cattle and sheep) and infected calves can excrete 10^5 to 10^6 cysts per gram of feces. A European network called NETWORK PROTOZOA ZOONOTIC (ZOOPNET) has evaluated although the majority of human isolates are assemblage B (56%) and assemblage A (43%), about one percent of zoonotic assemblage isolates are C, D, E, F. Also a study in Europe shows that assemblage B is unique for humans, while assemblage A, in addition to humans, appears in dogs, cats, domestic and wild animals.²⁸ Studies have shown the concentration of cysts that are excreted in the feces of livestock is more than sewage, although the highest concentration of *Giardia* cyst is related to sewage. Even though the general prevalence of *Giardia* is low in wild animals; it is more common in aquatic mammals.⁴

Genotypes of Giardia in animals

Several studies on livestock-associated *Giardia duodenalis* have been performed. The results of the studies on *Giardia duodenalis* positive isolates revealed genotypes A (AI, AII), C, and E were present in sheep and goats.²⁹⁻³¹

Also, the study on infected household dogs and cats revealed the presence of genotypes A (AI, AII), C, D, and F.³²

Prevalence of Giardia in domestic animals

Various studies have been performed to compare the prevalence of *Giardia lamblia* infection by age, sex, and diarrhea in infected dogs, cats, rabbits, sheep, goats, and cattle.³³⁻³⁷ A significant difference between infection rates in various age groups or between gender was not observed in dogs.^{33,34}

While the prevalence of *Giardia lamblia* is appreciably higher in diarrheic cats, rabbits and cattle compared with non-diarrheic ones. Also the infection is more prevalent in younger animals than older animals.³⁵⁻³⁷ Although these results specified that *Giardia duodenalis* infection is more prevalent in male prenominate animals compared with female animals, but the difference is not significant.³⁵⁻³⁷ In the study of the abundance of *Giardia* in Kashan canines, Mohsen Arbabi et al. have reported *Giardia canis* in dogs and jackals at 5.7% and 5%, respectively, and *Giardia felis* in fox at 22.7%.³⁸ Dariush-Shirvani et al. reported the abundance of *Giardia* among 120 domestic dogs in Isfahan, 4 dogs in a similar study (3.33%).³⁹

Geographical distribution of giardiasis and Giardia assemblages in Iran

Giardiasis is one of the most significant personal and social health problems in different countries.⁴⁰ This disease is endemic in Iran and its average prevalence in different parts is estimated to be 17%.⁴¹ Various epidemiological studies have been conducted on the prevalence of *Giardia* in diverse climatic regions of Iran.

Through the investigations on intestinal and gastrointestinal

parasites in different regions of Iran, *Giardia lamblia* has been detected.⁴²⁻⁴⁶ *Giardia duodenalis* has been isolated from human stool samples of rural and urban population;⁴⁷⁻⁴⁹ Especially in northern and southern areas.^{50,51} In the study in the Boyer-Ahmad district *Giardia duodenalis* was considered the most common pathogenic protozoan infection. This study revealed associations between protozoan infection and contact with animals and educational status. Findings of this study demonstrated that protozoan infection rate in rural areas of southwestern Iran is still high and remained as a challenging health problem in these areas.⁵² Also in the study on intestinal parasitic infections *Giardia duodenalis* was the common infection in Chelgerd city. This study revealed that intestinal parasitic infections are more frequent in the tribe populations compared to the inhabitant population. Poor personal hygiene, not washing hands before and after eating, consuming raw food without washing them, play on soil and defecating on it among children and adults and flowing of sewage into rivers are some factors of high prevalence of intestinal protozoa in tribes.⁵³ *Giardia lamblia* has been detected the second most prevalent parasite in Eghbaliyeh city in Qazvin province and Jiroft city in Kerman province. Socio-economic, geographic, sanitary/hygienic, cultural, and nutritional factors may contribute to the high prevalence of intestinal parasitic infection in these cities.^{54,55} In Takestan district *Giardia intestinalis*, *Blastocystis*, and *Entamoeba coli* were the most prevalent intestinal parasitic infections. Over the past years, causes of intestinal parasitic infections including the level of literacy and health knowledge of people, easy access to anti-intestinal parasitic drugs, safe water supply and expansion of drinking water pipeline have improved. At present, it seems that the most important risk factors for intestinal parasitic infections in Iran are eating raw vegetables and close contact to animals.⁵⁶ In southern Iran during 2007-2017 *Giardia lamblia* has been found the most common parasite.⁵⁷ *Giardia lamblia* has been determined as the most prevalent parasite in Abadan and Khorramshahr cities during four successive years.⁵⁸ The most infection among diarrheic patients of Ahvaz was *Giardia intestinalis*.⁵⁹

Giardia duodenalis has been detected in students of Khorramabad. Among the infected students, the most infection belonged to the Laboratory Sciences major (32.43%) and the fewest infection belonged to the Family Health major (2.70%). Most of the infected students were dormitory residents, and this detection is probably due to the sanitary and socioeconomic conditions of this location.⁶⁰ Also *Giardia* has been found the commonest parasite among the school children of Khorasan province and Golestan province. These studies revealed that the prevalence of parasitic infection increases by family size. Giardiasis infection was the least frequent among those students whose fathers were employer (36.7%) and the highest prevalent among those students whose fathers were farmer (61.4%).^{61,62} The most frequent parasitic infection among the children in Haji-Abad was *Giardia lamblia* during 18 years (1991-2008). This conducted study showed that the prevalence of the infection was significantly higher in out-patients.⁶³ Among the school children in Sari *Giardia lamblia* was the second most prevalent parasite. Parasitic infections were negatively associated with contact with soil and eating raw vegetables that were not washed with detergent.⁶⁴ Moreover, among the primary school children of southern Iran *Giardia duodenalis* was the most prevalent protozoan. The parasitic infection was associated with parents' educational level; The infection was higher among children whose parents' education level was below a high school diploma than those whose parents had at least a high school diploma.⁶⁵ Intestinal parasites have been evaluated in bakery employees in Khorramabad including *Giardia intestinalis*. The highest para-

sitic infections prevalence among the bakery employees belonged to the bakery workers with no health card (12.3%) and the least prevalence rate belonged to bakery workers who had health card but were contaminated with intestinal parasites (11.73%).⁶⁶ The commonest intestinal parasite among the food handlers of Sari is *Giardia lamblia*. Parasitic infections were negatively associated with household income level, contact with dogs and cats, place of living, and eating raw vegetables that were not washed with detergent. Restaurant workers, fast-food workers and fruits/vegetables sellers had the most parasite infection among the food handlers.⁶⁷ In an investigation among the food handlers of western Iran various parasites comprising *Giardia duodenalis* have been observed. There was a significant difference between validity date of health card, awareness of intestinal parasites transmission, participation in training courses in environmental health with intestinal parasites.⁶⁸ According to a study among the food handlers of Tabriz, *Giardia* was the prevailing intestinal parasitic infection. The highest percentage of infection is related to restaurants and supermarkets, but in general, there was no statistically significant difference between different professions.⁶⁹ The results of the study among food handlers of Bandar Abbas evinced *Giardia lamblia* was the third most prevalent intestinal parasite. The least infection was found in the workers of coffee shops while the highest infection was seen in the bakery workers including delivery of bread, bread cooking and cashier. Among the office servants including butlers, chef, and cleaners, the butlers had low contamination but the cleaners had the most.⁷⁰ In the meanwhile, a few food handlers in Saqqez county were infected with *Giardia intestinalis*. The results indicated that there is no significant correlation between the status of intestinal parasitic infection and the education level or between various occupation.⁷¹ *Giardia lamblia* has been observed among the food handlers of Andimeshk.⁷²

Multiple intestinal parasitic infections inclusive of *Giardia lamblia* were prevailing among transplant recipients. According to the results there is no statistically difference in parasitic infections prevalence between renal transplant recipients, comparing to healthy individuals.⁷³ Conducted study on the prevalence of intestinal infections among AIDS patients has detected *Giardia lamblia*.⁷⁴ Besides, the conducted study in diverse groups of immunocompromised patients including cancer and HIV/AIDS patients, Renal Transplant Recipients (PTR), and Hemodialysis Patients (HD) in Qom and Kashan indicated *Giardia duodenalis* was the most common intestinal parasitic infection. The results of this study showed that intestinal protozoan infections were more frequent among immunocompromised patients, especially in HIV/AIDS and HD.⁷⁵ Although in another research among immunocompromised patients *Giardia lamblia* existed only in the control group.⁷⁶ The second prevailing intestinal parasitic infection was *Giardia duodenalis* among cancer patients.⁷⁷ Moreover, *Giardia lamblia* has been observed among immunocompromised tuberculosis patients. The highest prevalence of IPIs was seen among those TB patients who were positive for HIV, while the less prevalence of IPIs was observed among TB patients who received immunosuppressive drugs. However, statistical significance difference was not observed between presence of intestinal parasites and type of immunodeficiency.⁷⁸ Besides, in patients with irritable bowel syndrome *Giardia duodenalis* has been detected. The rate of intestinal protozoan infections was higher in patients with IBS compared with control group regarding underlying diseases, and the difference was statistically conspicuous.⁷⁹ The study performed on patients with intestinal protozoan infections diagnosed with salt and pepper retinal lesions has determined the presence of *Giardia lamblia*.⁸⁰ Examined fecal specimens among mentally retarded

individuals in Urmia contained five intestinal protozoan species including *Giardia lamblia*.⁸¹ According to the research among intellectual disability children *Giardia lamblia* was the most prevalent species of intestinal parasites.⁸² Among mentally retarded individuals of Bandar-Abbas *Giardia lamblia* has been observed.⁸³ The prevailing protozoan in the mentally disabled population of Rasht was *Giardia lamblia* as well. The causes of the infection among these patients included illiteracy, consuming raw vegetables and low self-hygiene.⁸⁴ The results of the research on evaluating intestinal parasitic infections among mentally retarded individuals revealed the presence of *Giardia lamblia*.⁸⁵

An investigation of raw vegetable contamination in the villages of Qazvin has been performed. They found *Giardia lamblia* cysts in unwashed vegetable samples.⁸⁶ *Giardia* cysts have been observed in fresh vegetable samples of Tehran as well.⁸⁷

Kia *et al.* reported the prevalence of this parasite in 21 villages of Mazandaran as 10.2%.⁸⁸ Sajjadi Study in the rural areas of northern Iran has shown that giardiasis is the highest protozoan infection in preschool children.⁸⁹

In 1993, Sharifi and Keshavarz introduced *Giardia* as the most common protozoan parasitic infection in 1 to 12 years old children in Kerman.⁹⁰

HIV infection may affect the prevalence of giardiasis. Although *Giardia* is not considered an opportunistic parasite, its prevalence is higher in AIDS patients.⁹¹⁻⁹³ Stool tests of 206 HIV-positive patients at a medical center in Tehran showed *Giardia lamblia* in 7.3% of cases.⁹⁴

Taherkhani has reported the frequency of *Giardia* as 21.41% in the study of the abundance of intestinal parasites among mentally retarded students of Hamadan in 191 stool samples.⁹⁵ In the study by Molavi *et al.* in 2009 in rural areas of Khuzestan province, the prevalence of giardiasis was reported at 9.1%.⁹⁶ The use of molecular methods based on PCR to identify and determine different genotypes of *Giardia* in stool samples has been increased. So far, few studies have been performed in Iran to determine the genotypes of *Giardia lamblia* (Appendix Table 2). For the first time in Iran, in 2002, Zare *et al.* showed that by using the PCR-RELP method, some *Giardia* isolates from humans can be identified.⁹⁷

In the study of Babaei *et al.*, out of 38 studied samples, 33 samples of AII assemblage, and 3 samples of BIII assemblages, and 2 samples of mixed AII and BIII infections were reported. These findings show that in Tehran, *Giardia* infection was of human origin and is mostly AII assemblage and human is a reservoir of infection.⁹⁸ A similar study was conducted in 2008 by Fallah *et al.* in East Azerbaijan to determine the genotype of *Giardia* in humans, cats, dogs, and cattle, and the GDH gene was used to determine the genotype. Among human samples, 8 samples of assemblage BIII, six samples of assemblage AII, and 4 samples of assemblage BIV and among animal samples (1 cat) AI assemblage were obtained.⁹⁹ In another investigation by Etemadi *et al.* in Kerman on the GDH gene, out of 30 studied samples, 18 AII assemblage samples, five AI assemblage samples, and 7 BIII assemblage samples were reported.¹⁰⁰ Nahavandi *et al.* conducted a study in Tabriz in 2011. In this study, 34 positive stool samples were collected for *Giardia lamblia* and the PCR-RELP method was used to identify the diversity of TPI genes. In using the TPI gene in this study, 13 samples (41.9%) were from genotype B, 17 samples (54.8%) were from genotype A and 1 sample (3.2%) was from both genotypes and 3 samples (8.8%) were negative. The results show that PCR is a convenient method of diagnosing fecal contamination.¹⁰¹ Akbarian *et al.* studied the genetic differences of *Giardia lamblia* in Khorramabad city and surrounding villages using PCR and determining the sequence. The GDH gene amplification was successful-

ly performed by PCR in only 24 out of 30 parasitic fecal samples. Alignment of the obtained GDH sequence with the gene bank sequences was performed and a total of 5 samples (3 urban samples and 2 rural samples) were a sequence that all of them were genotype B and no difference was observed between them.¹⁰² In the Sarkari *et al.* study, among 172 positive samples of *Giardia*, 128 samples (74.1%) assemblage AII, 30 samples (17.44%) assemblage BIII, six samples (3.49%) assemblage BIV and 8 samples (4.66%) Mixed (BIII and AII) were reported.¹⁰³ In Manouchehri *et al.*, investigation, PCR showed that in patients, the frequency of *Giardia* B genotype was higher than A genotype (51.6% vs. 35.5%). However, the frequency of genotype A was significantly higher among patients with diarrhea compared to asymptomatic patients.¹⁰⁴

In the end, it can be concluded that according to the studies conducted in different parts of Iran, assemblages BIII and AII are the most common type of *Giardia*, respectively (Appendix Table 2).^{25,49,52,55,99-125}

There is also no clear correlation between *Giardia lamblia* assemblages and clinical signs, and the results of the study are contradictory. Limited studies on different genotypes of *Giardia* in Iran show that the most suitable target gene for genotypic studies is glutamate dehydrogenase (GDH).

Giardia infection probably transmits in both human-animal and human-human ways in Iran, and the possibility of *Giardia lamblia* being zoonotic in Iran is also raised. Therefore, comprehensive studies on humans and different animal isolates in different parts of the country, especially in areas where no research has been done, seem necessary.

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