Shiraz E-Med J. 2022 October; 23(10):e116536. https://doi.org/10.5812/semj-116536.

Published online 2022 August 19. **Review Article**

An Overview on the Main Assemblages and Sub-assemblages of *Giardia intestinalis* in the Western Half of Iran

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Received 2021 September 05; **Revised** 2022 June 08; **Accepted** 2022 July 09.

Abstract

Context: Studies have shown that various Assemblages, sub-assemblage (subtypes) and genotypes of *Giardia intestinalis* have multiple hosts; therefore, their distribution reservoirs as well as pattern of epidemiological distribution are different. The present study was conducted to summarize the results of genetic studies on *G. intestinalis* in Iran.

Evidence Acquisition: To investigate the articles on the sub-assemblage of *G. intestinalis* in Iran, a systematic search was conducted in Persian and English databases. The search process led to the entry of 23 articles into this systematic review. Also, in this study, to estimate the ratio of the dominant sub-assemblage of *Giardia* parasite in Iran, meta-analysis was used and a significant level of 0.05 was considered.

Results: The results of this study showed that investigations on assemblages and sub-assemblage of *G. intestinalis* had been mostly made in the western half of Iran. The overall prevalence of A, B, and the mixed assemblages of *Giardia intestinalis* in these areas of Iran was estimated to be 0.56, 0.27, and 0.16, respectively. Also, the prevalence of AI and AII sub-assemblage of *G. intestinalis* in the same areas of Iran was 0.34 and 0.5, respectively. The prevalence of BIII and BIV sub-assemblage of this parasite in mentioned areas was further found to be 0.2 and 0.06, respectively. In addition, the total prevalence of all sub-assemblage (AI, AII, BIII, & BIV) was calculated to be 0.18 ($P < 0.001$).

Conclusions: Given that assemblage A of *G. intestinalis* has the highest prevalence in the human society of western half of Iran, in this area of the country, the main epidemiological pattern of *Giardia* transmission is zoonotic. However, to identify the source of this parasite spread, more studies are needed.

Keywords: Assemblage, Iran, Genotype, *Giardia intestinalis*, Molecular Epidemiology, Sub-assemblage

1. Context

*Giardia*sis is a global disease caused by a flagellate protozoan called *Giardia*. The only pathogenic species of this parasite in humans is *Giardia intestinalis* (also known as *G. duodenalis* and *G. lamblia*). Although this disease is often asymptomatic, the most common symptom is diarrhea. The causative agent of the disease is resistant cysts, which are excreted from the host intestine and transmitted by oral-fecal, water, and food (1, 2). Thus, identifying the parasite's reservoir is very important to prevent it.

Studies have shown different hosts and as a result different reservoir for various assemblages of *G. intestinalis*. Based on its protein and DNA polymorphism, eight major genetic groups or assemblages (A, B, C, D, E, F, G, and H) have been identified for this parasite (3-5). Numerous studies have been also performed to determine the sub-assemblages of this parasite around the world (6). Most of human isolates are in assemblages A and B (3, 4, 6). Because these two assemblages could also infect other mammals, the zoonotic transmission potential of *G. intestinalis* is epidemiologically important (3). In some parts of Iran, studies have been conducted to identify the sub-assemblages of *G. intestinalis* in humans. So, it is valuable to summarize these studies to determine the predominant sub-assemblages (subtypes) and epidemiological transmission pattern of this parasite in Iran. On the other hand, such studies could identify information gaps regarding the sub-assemblage of this parasite, thereby opening new avenues for further research. Hence, the present study aimed to provide an overview on the determination of genotypes of *G. intestinalis* and summarize the studies on this area in Iran.

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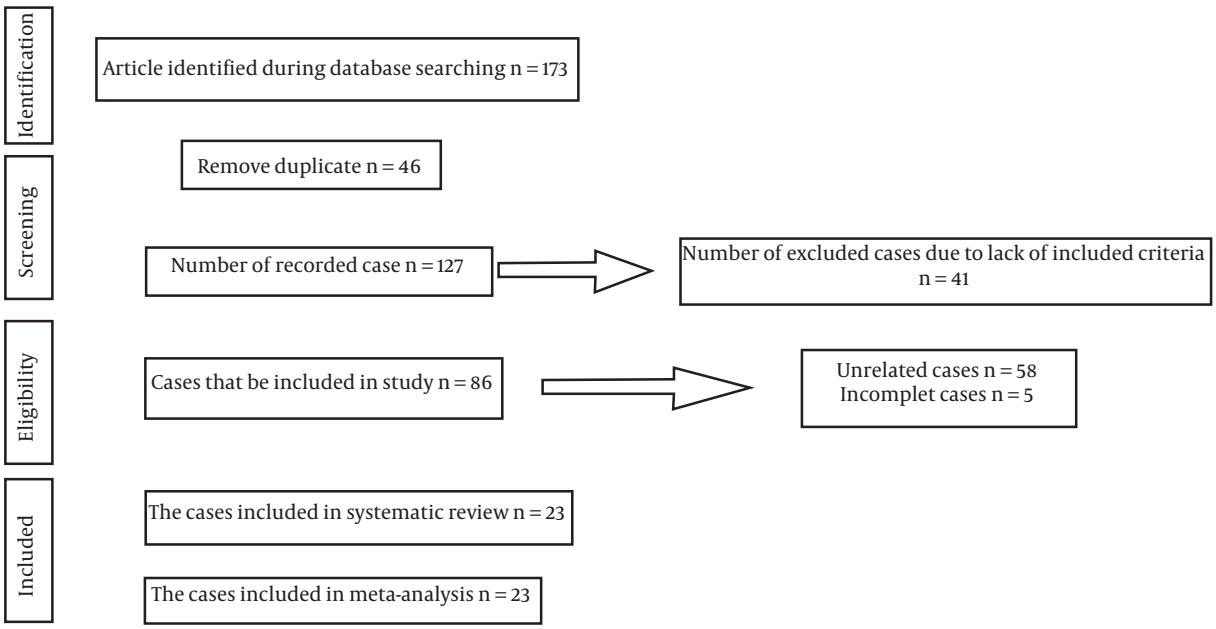

Figure 1. Steps to select and approve of articles for systematic review and meta-analysis

2. Evidence Acquisition

2.1. Search Process

All English or Persian articles and dissertations performed on *Giardia* genotype in Iran (from 1980 to 2020) indexed in PubMed, Google scholar, Science Direct, Scopus, Medline, Medlib, Scientific Information Database (SID), IranMedex, IranDoc, and Magiran were collected and reviewed. The keywords were a combination of *Giardia*, intestinal protozoa, assemblage, genotype, *G. intestinalis*, *G. duodenalis*, *G. lamblia*, *Giardia* genotype, Iran, Islamic Republic of Iran, genotype of *Giardia* in Iran. Figure 1 shows the search process.

2.2. Selection criteria

The following inclusion criteria were considered for this study. Full papers and dissertations related to determining *Giardia* genotypes based on different genes of this parasite, genetic characters' and human *Giardia* genotype in Iran published from 1980 to 2020 were included in this work. In contrast, case studies, experimental studies, and animal studies, as well as duplicate articles were excluded.

2.3. Data Extraction

Selected papers were carefully reviewed by two researchers and information, such as first author, year of publication, type of study, location of study, language, subjects, sample size, diagnosis test, assemblage or genotype detection technique, and genotype were extracted and recorded.

2.4. Statistical Analysis

In this study, meta-analysis was used to estimate the proportion of dominant genotypes of *Giardia* parasite in Iran (7). Confidence intervals were also calculated using the exact binomial distribution. Further, publication bias was investigated using the Egger test (8). Heterogeneity among the studies was considered by benefiting from the Cochran's Q test with a significant level lower than 0.1 and I^2 statistic greater than 60% (9). Due to the observed heterogeneity, we used random effects model to estimate the prevalence of predominant genotype of *Giardia* parasite in Iran.

The meta-analysis of proportions was further performed by using "Metaprop" commands on STATA software (Ver.14). The significance level was considered as 0.05 (7). Freeman-Tukey double arcsine transformation was used in the random effects model when the proportion in some studies was very large and close to 100% (7). Additionally, a Forest plot was drawn based on the random effects model for the estimated proportion of assemblages with confidence interval of 95%.

Figure 2. The geographical areas related to the reviewed articles (Provinces in which has been studied the *Giardia intestinalis* genotype)

3. Results

After searching the databases, 173 articles were found related to the *Giardia* exploration in Iran. After excluding the unrelated and duplicated studies, a total number of 23 associated articles to the sub-assemblages of human *G. intestinalis* in Iran were included. Figure 1 shows the process of designing and searching for articles, and Figure 2 illustrates the geographical areas of the included articles (Figures 1 & 2).

From 23 studies, one was cohort study and the rest were cross-sectional. These studies had been conducted in 13 provinces of Iran. Four studies in Khuzestan, three studies in Fars, three studies in Isfahan, two studies in East Azerbaijan, two studies in Tehran, two studies in Kerman, and one study in other provinces (7 provinces) (Figure 2). Totally, 1059 human specimens were examined in all the 23 included studies. The PCR-RFLP technique had been used for detecting of *Giardia* assemblages in 11 studies, while in others, the sequencing technique had been used (Table 1).

The results of statistical analysis to estimate the prevalence of A, B, as well as the mixed assemblages of *G. intestinalis* in the Iranian population are as follows.

Results of the Egger test revealed that in 23 studies, the proportion of assemblage A lacked publication bias (P = 0.72), the proportion of assemblage B, had publication bias (P = 0.086), while the proportion of both assemblage (mix) was deficient in publication bias (P = 0.71). On the

Table 2. Extraction of Information About Sub-assemblages of *Giardia intestinalis* from the Reviewed Articles [a]

Line	First Author	Place of Study	Sample Size	Assemblage (Genotype or Sub Assemblage)						
				A		B			Mix	Neg
				AI	AII	BIII	BIV	B Novel		
1	Babaei et al. (10)	Tehran	38	33 (87)	-	3 (7.8)	-	-	2 (5.2)	-
2	Fallah et al. (11)	Tabriz	34	-	6 (33.3)	8 (44.4)	4 (22.2)	-	-	16
3	Etemadi et al. (13)	Kerman	30	5 (16.6)	18 (60)	7 (23.4)	-	-	-	-
4	Pestehchian et al. (14)	Esfahan	67	-	40 (59.7)	23 (34.32)	2 (2.98)	-	2 (2.98)	-
5	Sarkari et al. (15)	Fars province	205	-	128 (74.41)	30 (17.44)	6 (3.49)	-	8 (4.66)	33
6	Rafiei et al. (17)	Ahwaz	100	-	18 (18)	28 (28)	-	-	54 (54)	-
7	Roointan et al. (18)	Ahwaz	50	-	5 (10)	8 (16)	-	-	37 (74)	-
8	Etemadi et al. (19)	Kerman	30	5 (16.6)	18 (60)	7 (23.4)	-	-	-	-
9	Rayani et al. (21)	Shiraz	50	-	32 (80)	4 (10)	4 (10)	-	-	10
10	Rayani et al. (22)	Shiraz	40	-	32 (80)	4 (10)	4 (10)	-	-	-
11	Bahrami et al. (23)	Kurdistan	23	4 (17.4)	8 (34.8)	5 (21.7)	4 (17.4)	2 (8.7)	-	-
12	Hooshyar et al. (25)	Kashan	47	-	24 (51.1)	7 (14.9)	2 (4.2)	-	11 (23.4)	3
13	Effati et al. (26)	Alborz	4	-	1 (25)	2 (50)	1 (25)	-	-	-
14	Shahnazi et al. (28)	Qazvin	20		11 (55)	6 (30)	-	-	-	3
15	Mahmoudi et al. (30)	Rasht	41	-	38 (92.6)	-	3 (7.4)	-	-	-
16	Rafiei et al. (31)	Shushtar	24	-	12 (50)	12 (50)	-	-		

[a] Values are expressed as No. (%) unless otherwise indicated.

other hand, results of the Cochran's Q test and I^2 statistic showed a heterogeneity among studies for assemblages A (I^2 = 91.06%, Q = 246.02, P-value<0.001), B (I^2 = 71.37%, Q = 73.35, P-value < 0.001), and mixture of both assemblages (I^2 = 96.34%, Q = 218.55 I2, P-value < 0.001). So, we applied the random effects model in this meta-analysis. Figure 3A & B) displays the forest plot for the estimated proportions of assemblage A and B with confidence interval of 95% in western half of Iran. As shown in this figure, the overall proportion of assemblage A in this region of country is 0.56 with a confidence interval of 95% (0.46, 0.67) (P < 0.001), whilst the overall proportion of assemblage B has been estimated at 0.27 in the same region with a confidence interval of 95% (0.22, 0.33) (P < 0.001). Moreover, Figure 3C presents the forest plot for the mixed proportion of both A and B assemblages. This figure shows that the overall mixed proportion of both cases is 0.16 in mentioned area with a confidence interval of 95% (0.04, 0.35) (P < 0.001).

As noticed in Table 2, out of 23 selected studies, sub-assemblages of *G. intestinalis* have been presented in 16 studies.

Results of statistical analysis are presented below to estimate the proportion of sub-assemblages A, B, and mixed of *Giardia intestinalis* in the population of the western half of Iran.

Furthermore, results of the Egger test indicated that the proportion of *Giardia intestinalis* sub-assemblages A (AI, AII) in the mentioned area lacked publication bias (P > 0.05). On the other hand, the Cochran's Q test and I^2statistic showed a heterogeneity among the studies for AI sub-assemblages (I^2 = 97.13%, Q = 209.02, P-value <0.001), and AII sub-assemblages (I^2 = 92.44%, Q = 185.11, P-value <0.001). So, we applied the random effects model in this meta-analysis. As seen in Figure 4A-D, the overall proportion of AI (Figure 4A) was estimated 0.34 with a confidence interval of 95% (0.04, 0.74) (P< 0.05). In addition, the overall proportion of AII (Figure 4B) was estimated to be 0.5 with a confidence interval of 95% (0.36, 0.64) (P< 0.001).

Similarly, results of the Egger test demonstrated that the proportion of BIII (Figure 4C) sub-assemblages had no publication bias (P > 0.05), while the proportion of BIV sub-assemblages did (P < 0.05). The Cochran's Q test and I^2 statistic for BIII sub-assemblages (I^2 = 66.85%, Q = 42.23, P-value < 0.001), and BIV sub-assemblages (I^2 = 45.38%, Q = 14.65, P-value <0.1) showed heterogeneity among studies. Therefore, the random effects model was used in the proportion meta-analysis of *G. intestinalis* sub-assemblages in

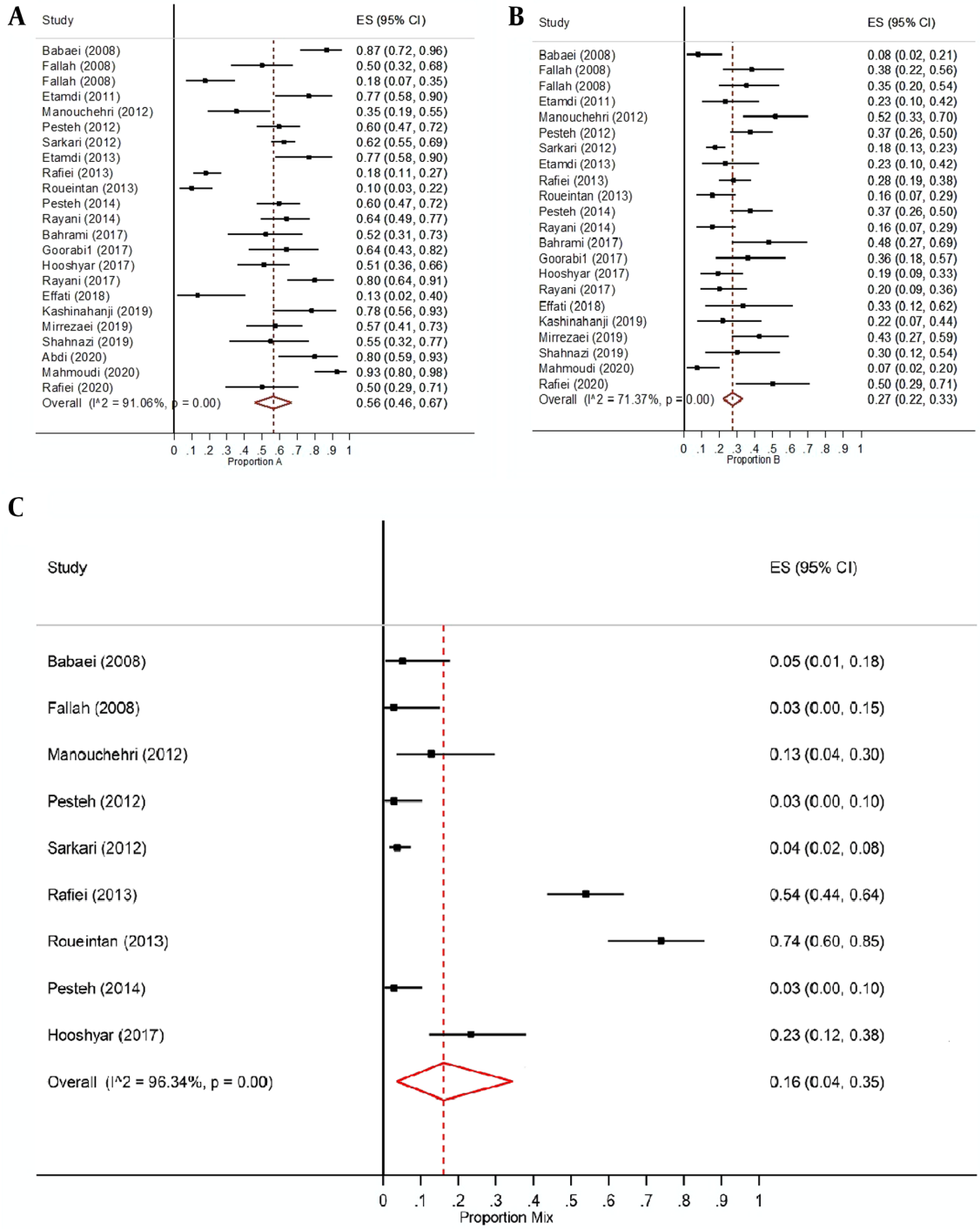

Figure 3. Forest plot based on the random effects model for assemblages of *Giardia intestinalis* in the western half of Iran. (A) assemblage A, (B) assemblage B (C) the mixed A and B assemblages

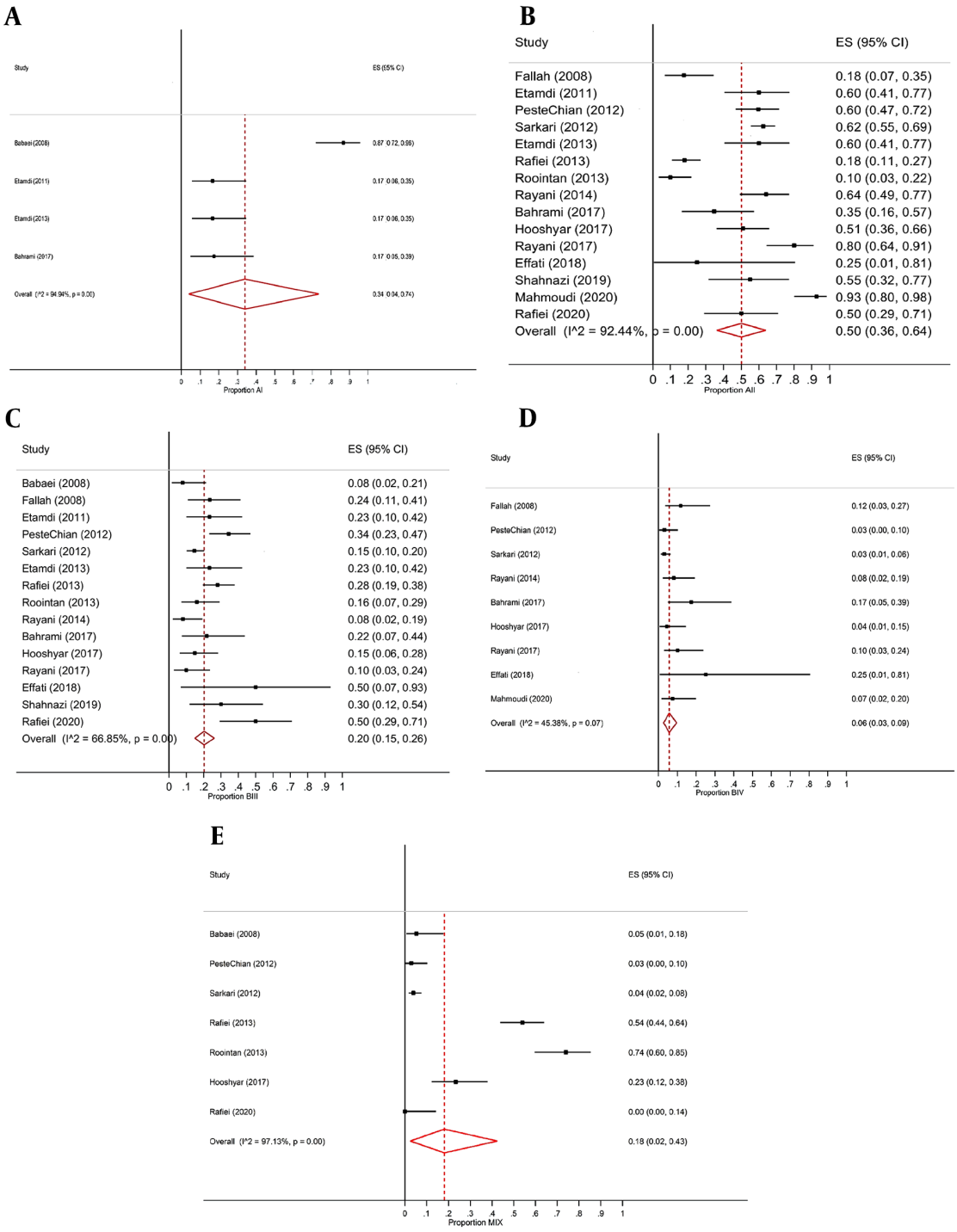

Figure 4. Forest plot based on the random effects model for genotypes (sub assemblages) of *Giardia intestinalis* in the western half of Iran. (A) AI; (B) AII (C) BIII (D) B (E) mixed A and B sub assemblages

the western half of Iran. Additionally, based on Figure 4D), the overall proportions of BIII and BIV were estimated to be 0.2 [with a confidence interval of 95% (0.15, 0.26) (P < 0.001)] and 0.06 [with a confidence interval of 95% (0.03, 0.09) (P < 0.001)], respectively.

On the other hand, results of the Egger test indicated that the mixed proportion of different sub-assemblages of *G. intestinalis* in mentioned region lacked publication bias (P = 0.675). In addition, outcomes of the Cochran's Q test and I^2 statistic verified the heterogeneity among the studies (I^2 = 97.13%, Q = 209.02, P-value < 0.001). Therefore, the random effects model was used in the proportion meta-analysis for various sub-assemblages of *G. intestinalis*. Eventually, based on Figure 4E, we can notice that the overall proportion for various *G. intestinalis* sub-assemblages is 0.18 with a confidence interval of 95% (0.02, 0.43) (P < 0.001).

4. Discussion

In this systematic study, the prevalence of assemblages and sub-assemblages of human *G. intestinalis* in different geographical regions of Iran was analyzed. The result of this study showed that most of studies on assemblages and sub-assemblages of *G. intestinalis* have been performed in the western half of Iran. There was little of the same information in the eastern half of this country. The main results of this study are as follows.

The overall prevalence of assemblages A, B, and the mixed assemblage (A and B) in the mentioned area were 0.56, 0.27, and 0.16, respectively. Also, prevalence of AI, AII, BIII and BIV sub-assemblages of were 0.34, 0.5, 0.2 and 0.06, respectively. In addition, the total prevalence of all sub-assemblages (AI, AII and BIII, BIV) was estimated to be 0.18.

*Giardia*sis is one of the most important health problems worldwide because the parasite that causes this disease, *G. intestinalis*, is a gastrointestinal protozoan common among human, domestic, and wild animals (3, 5). Hence, the epidemiological study of *Giardia*sis is important to identify the host spectrum of different species of this parasite as well as its assemblages, sub-assemblages, strains and genotypes. On the other hand, such studies, if performed using molecular methods, will help us improve our understanding about the zoonotic transmission potential of animal's *Giardia* species and to determine how many cases of human *Giardia*sis have animal source (3). Although being sporadic, molecular taxonomic studies can also clarify the relationship between the parasitic and its host genotypes, pathogenesis, and clinical symptoms (33).

Also, different species of *Giardia* parasite have different hosts. Currently, six species of this parasite have been recognized by researchers. Of these six species, only *G. intestinalis* (also known as *G. lamblia* and *G. duodenalis*) could infect humans and many other mammals. Studies have shown that these species of *Giardia* have the widest range of hosting and the greatest health importance (34). The results of epidemiological and genotypic studies have also confirmed the possibility of zoonotic transmission of *G. intestinalis* (33).

Study on allozymes has further shown that all human isolates of *G. intestinalis* belong to two genetic assemblages (A and B) each of which contains at least four genetic clusters or sub-assemblages (I to IV). On the other hand, both of these assemblages are zoonotic whose examination can help us track the source of infection (35). Further, in Brazil, the findings of two separate studies showed that in communities, where people care for pets, humans and animals (especially cats) could become infected with *Giardia* assemblage A. In such communities, there seems to be the possibility of cross-transmission of this parasite between animals and humans (36, 37). Another study in Poland verified assemblage A in *Giardia* infected cats (38). Research works in Mexico, Spain, and Jamaica approved the same assemblage of *G. intestinalis* (A) in dogs that had close connection with human (39-41). Furthermore, a study conducted to determine the sub-assemblage of *G. duodenalis* in livestock of Urmia, Iran showed that the predominant assemblage among the livestock of this city is type E, which is not zoonotic (42). Similar results were obtained in other studies conducted on Arabian horses, and ruminants in Ahvaz and Yazd province, Iran, respectively (43, 44). As well as studies performed in Turkey on horse and cattle (45, 46).

As the results of this systematic study showed, prevalence of assemblage A of *G. intestinalis* with estimated of 0.56 is the dominant assemblage in the human society of western half of Iran. By comparing *Giardia* sub-assemblages in animals and humans, it can be concluded that in mentioned region of Iran zoonotic transmission of this parasite from livestock and pets is very scarce. In contrast, a general comparison between the prevalence of *Giardia* in this region of Iran, Brazil, Mexico, Spain, and Jamaica suggested that the source of contamination in these countries may be stray domestic animals, including cats and dogs (36, 39-41). Currently, the large number of stray cats and dogs are the most important urban problems in Iran. These animals can be involved in the transmission and spread of a number of zoonotic parasitic infections including *Giardia*. Works on the prevalence and genotype determination of *Giardia* in Iranian domestic animals is so little that we had to report results of the only study in this field in Iran, which showed that the prevalence of *G. duodenalis* in cats in Ahvaz was 2% and 3.33% by microscopic method and immunochromatography assay, respectively

(47).

Moreover, studies in other countries showed that assemblage A of *G. intestinalis* had the most possibility of zoonotic transmission since it is the most common species of this parasite in animals and it also lacks a specific host. It should be also noted that humans are usually infected with genotype AII, while animals are often infected with type AI (34). Results of three studies conducted in Iran to determine the genotypes of the *G. duodenalis* in animals also showed that although the predominant assemblage of the parasite in these animals is of category E, a number of these animals were infected with assemblage A and genotype AI (42-44). Thus, it would be likely that the source of zoonotic transmission of *Giardia* is not livestock (cattle) and horses and there might be other animals responsible for this transmission in Iran.

The results of determining assemblage of *Giardia* in two neighboring countries of Iran showed that the dominant assemblage in Saudi Arabia and Turkey were A and B, respectively (4).

In addition, the association between the genotypes of the *G. intestinalis* and clinical symptoms was investigated in a human experimental study. The results showed that the clinical signs of *Giardia*sis appear only in people infected with the GS / M isolate of the parasite. This insolate belongs to Assemblage B of *Giardia* (3, 33). Further, results of this study led to a theory stating that pathogenesis of parasite changes with variation of parasite strain. Results of animal experimental studies also reinforced this theory (3, 33). Moreover, a study on the infected Egyptian school children confirmed the symptoms of *Giardia*sis appeared in patients with Assemblage B of *Giardia* (48). A similar study in Cuba also confirmed the same assemble (B) (48, 49).

In Iran, several studies have been performed to investigate the relationship between *Giardia* genotype and the presence or absence of clinical signs. For example, Rafiei et al. showed that the predominant genotype of *Giardia* in Ahvaz is AII and BIII, but no significant difference was observed between the presence/absence of symptoms and the incidence of these genotypes (17). In another study by Etemadi et al., patients with assemblage A suffered from milder symptoms (mild diarrhea) than those with assemblage B (13).

On the other hand, according to the findings of the current study, it seems that in most studies that have been performed to determine the genotype of *Giardia* parasite in Iran, in fact, sub-assemblages the parasite were identified and reported.

To achieve more accurate results in Iran and have the possibility for better comparisons with other countries' studies, it is necessary to conduct more comprehensive studies in different parts of Iran regarding the prevalence and genetic characteristics (assemblage, sub-assemblage and genotype) of *G. intestinalis* in humans, livestock, and pets.

4.1. Conclusions

Taking into account all the above-discussed studies, it is obvious that the epidemiological transmission pattern of *G. intestinalis* in Iran is zoonotic transmission. However, it seems important to determine the genetic characteristics of the parasite in order to discover the source of contamination in human societies. Therefore, to reduce the risk of this disease transmission from animal to human (zoonotic), revision of treatment, prevention and control guidelines of this disease in cats and other pets is a priority. On the other hand, the results of the current study shed new light on the prospects and opportunities for future research in the field of epidemiology and genetics of this parasite. Hence, we recommend researchers to use the results of this systematic study to identify areas of Iran that need research in the field of genetic and epidemiological studies of *Giardia*.

Acknowledgments

This study was conducted as a systematic review and meta-analysis at Arak University of Medical Sciences and did not require funding, so it lacked financial support. The authors would like to appreciate those who provided the opportunity to implement the project.

Footnotes

Authors' Contribution: Study concept and design: Z. E.; Acquisition of data: Z. E., and R. H.; Analysis and interpretation of data: A. M., and Z. E.; Drafting of the manuscript: A. M., and Z. E.; Critical revision of the manuscript for important intellectual content: Z. E., and R. H.; Statistical analysis: A. M..

Conflict of Interests: The authors declare that there is no conflict of interest regarding the publication of this study.

Funding/Support: This study did not require funding and lack financial support.

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Table 1. Extraction of Information About the Assemblage of *Giardia intestinalis* from the Selected Articles [a]

Line	First Author	Type of Study	Place of Study (City)	Characteristic of the Sample Under Study	Number of Samples Molecularly Tested	Diagnosis Method	Determination Method Assemblage	Assemblage				Description and Conclusion
								A	B	Mix	Neg	
1	Babaei et al. (10)	Cross sectional	Tehran	Stool samples were reported to be positive microscopically	38	PCR (Based on the gdh gene)	RFLP	33 (87)	3 (7.8)	2 (5.2)	-	Epidemiologic transmission pattern was anthroponotic
2	Fallah et al. (11)	Cross sectional	Tabriz	Laboratory samples of Tabriz Children's Hospital laboratory and reference laboratory samples that were positive microscopic method	34	PCR (Based on the tim gene)	Seq	17 (50)	13 (38)	1 (3)	3	
3	Fallah et al. (12)	Cross sectional	Tabriz	Tabriz Children's Hospital and reference laboratory samples	34	PCR (Based on the gdh gene)	RFLP	6 (17.6)	12 (35.3)	-	16	Epidemiologic transmission pattern was anthroponotic
4	Etemadi et al. (13)	Cross sectional	Kerman	Kerman Medical Centers	30	PCR (Based on the gdh gene)	RFLP	23 (76.6)	7 (23.4)	-	-	
5	Pestehchian et al. (14)	Cross sectional	Isfahan	Isfahan Health Centers	67	PCR (Based on the gdh gene)	RFLP	40 (59.7)	25 (37.3)	2 (3)	-	Epidemiologic transmission pattern was anthroponotic and zoonotic.
6	Sarkari et al. (15)	Cross sectional	Fars province	Fars Province Laboratories	205	PCR (Based on the gdh gene)	RFLP	128 (62.4)	36 (17.6)	8 (3.9)	33	Epidemiologic transmission pattern was anthroponotic
7	Manouchehri Naeini et al. (16)	Cross sectional	ShahreKord	Shahrekord Medical Diagnosis Laboratories	31	PCR (Based on the tpi gene)	Seq	11 (35.5)	16 (51.6)	4 (12.9)	-	Association was seen between diarrhea and genotype A. And association was seen between asymptomatic infection and genotype B
8	Rafiei et al. (17)	Cross sectional	Ahwaz	Ahwaz Health Centers	100	PCR (Based on the gdh gene)	RFLP	18 (18)	28 (28)	54 (54)	-	There is a association between clinical symptoms and genotype A
9	Roointan et al. (18)	Sectional (September 2011 to July 2012)	Ahwaz	Children referred to health center clinics	50	PCR (Based on the gdh gene)	RFLP	5 (10)	8 (16)	37 (74)	-	
10	Etemadi et al. (19)	Cross sectional	Kerman	Three medical diagnostic laboratories	30	PCR (Based on the gdh gene)	RFLP	23 (76.6)	7 (23.4)	-	-	
11	Pestechian et al. (20)	Cross sectional	Isfahan province	Medical diagnostic laboratories	67	PCR (Based on the tpi gene)	RFLP	40 (59.7)	25 (37.3)	2 (3)	-	
12	Rayani et al. (21)	Cross sectional	Shiraz	Health centers and hospitals in Shiraz	50	PCR (Based on the SSU-rDNA)	Seq	32 (80)	8 (20)	-	10	Epidemiologic transmission pattern was anthroponotic
13	Rayani et al. (22)	Cross sectional	Shiraz	Health centers and hospitals in Shiraz	40	PCR (Based on the gdh gene)	Seq	32 (80)	8 (20)	-	-	Epidemiologic transmission pattern was anthroponotic
14	Bahrami et al. (23)	Cross sectional	Kurdistan province	14 Medical Diagnosis Laboratories	23	Nested-PCR (Based on the tpi gene)	Seq	12 (52)	11 (48)	-	-	Epidemiologic transmission pattern was zoonotic
15	Nasiri Goorabi et al. (24)	Cross sectional	Baharestan city	Health centers of Baharestan city in Tehran province	25	PCR (Based on the β-giardin gene)	RFLP	16 (64)	9 (36)	-	-	Epidemiologic transmission pattern was anthroponotic and anthropozoonotic
16	Hooshyar et al. (25)	Cross-sectional	Kashan	Medical Diagnosis Laboratories	47	PCR (Based on the gdh gene)	RFLP	24 (51.1)	9 (19.1)	11 (23.4)	3	Epidemiologic transmission pattern anthropozoonotic
17	Effati et al. (26)	Cross-sectional	Alborz Province	Shahid Bahonar Children's Hospital of Karaj And Medical diagnostic laboratories Fardis, Nazar abad and Eshtehard	15	Nested-PCR (Based on the tpi gene)	Seq	2 (13.3)	5 (33.3)	-	8 (53.4)	Epidemiologic transmission pattern anthropozoonotic and zoonotic
18	Kashinahanji et al. (27)	Cross sectional	Hamedan	Clients of health centers	23	Nested-PCR (Based on the tpi gene)	Seq	18 (78.2)	5 (21.7)	-	-	Epidemiologic transmission pattern zoonotic
19	Shahnazi et al. (28)	Cross sectional)	Qazvin	People who come in contact with food	20	PCR (Based on the gdh gene)	Seq	11 (55)	6 (30)	-	3	
20	Mirrezaie et al. (29)	Cross sectional	Andimeshk	Imam Ali Hospital and Health Center in Andimeshk	40	PCR (Based on the gene gdh and β-giardin)	Seq	23 (57.5)	17 (42.5)	-	-	Only the number of samples that were sequencing was listed, but 84 samples were tested for PCR.

21	Mahmoudi et al. (30)	Cross-sectional a	Rasht	Visitors to 3 educational hospitals of Gilan University of Medical Sciences	41	PCR (Based on the gdh gene)	Seq	38 (92.6)	3 (7.4)	-	-	60 samples were PCR, but 41 samples were sent for sequencing.
22	Rafiei et al. (31)	Cohort study	Shushtar	Referrals to health centers	24	PCR (Based on genes SSU-rRNA)	Seq	12 (50)	12 (50)	-	-	Based on SSU-rRNA gene 90 samples was PCR test, which confirmed 82 samples, but PCR based on gdh, Tpi, and bg genes was performed on 24 samples
23	Abdi et al. (32)	Cross sectional	Arak	*Giardia*-infected samples from Arak University of Medical Sciences Medical Diagnosis Laboratory it was gathered	25	PCR (Based on the betagiardin gene)	Seq	20 (80)	-	-	-	Assemblage of 5 samples was unknown

Abbreviations: Seq, sequencing; RFLP, restriction fragment length polymorphism; gdh, glutamate dehydrogenase; tim or tpi, triose phosphate isomerase; bg or β-giardin, Beta-giardin; SSU-rRNA, small subunit ribosomal ribonucleic acid.

[a] Values are expressed as No. (%) unless otherwise indicated.