Gastroenterology and Hepatology From Bed to Bench.

SYSTEMATIC REVIEW

Subtype distribution of *Blastocystis* sp. isolated from humans in Iran: a systematic review and meta-analysis

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ABSTRACT

Aim: This systematic review and meta-analysis evaluated the subtyped *Blastocystis* sp. isolated from humans in Iran.

Background: *Blastocystis* sp. is an anaerobic intestinal protozoan that infects humans as well as domestic and wild animals, i.e. mammals, amphibians, reptiles, and arthropods.

Methods: A comprehensive search for papers published before April 2022 was undertaken utilizing English and Persian databases. The following MeSH keywords were used in the electronic search: (*Blastocystis* sp.) AND (molecular OR subtype) AND (prevalence OR epidemiology) AND Iran. The quality of the included studies was evaluated. Thereafter, a random-effects meta-analysis was conducted to estimate the pooled prevalence and odds ratios regarding the included studies.

Results: A total of 32 studies comprised of five case-control studies and 27 cross-sectional studies met the eligibility criteria. The overall pooled prevalence of subtyped *Blastocystis* sp. in Iran was estimated to be 10% (95% confidence interval: 6 to 15%). Eight subtypes of *Blastocystis* sp. (ST1- ST7 and ST9) were identified in our study, of which ST3 was the most common subtype (0.04); 0.02-0.07). The difference in subtypes between two case and control groups in reported studies was not significant, but the odds ratio of infection by ST3 (0.98; 95% CI, 0.30 to 3.20) was higher in cases.

Conclusion: The current systematic review showed that with the exception of ST8 and ST12, all human *Blastocystis* sp. subtypes reported in the world are found in different parts of Iran.

Keywords: *Blastocystis*, Subtype, Meta-analysis, Iran.

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Introduction

Blastocystis sp. is an anaerobic intestinal protozoan that infects humans as well as domestic and wild animals, such as mammals, amphibians, reptiles, and arthropods. People become infected via the fecal-oral route through direct or indirect ingestion of infectious cysts (1, 2).

Based on molecular epidemiological data, the prevalence of this parasite is still unknown in many parts of the world. Geographic variations in some parts of the world affect the prevalence of subtypes; moreover, some reports have pointed to associations between specific subtypes and disease (3). Based on the findings of a systematic review and meta-analysis study by Javanmard et al., low levels of socioeconomic status

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and unsuitable climatic conditions, such as insufficient humidity and high temperatures, increase the chance of *Blastocystis* sp. transmission in society (4). Several studies that applied DNA-based methods pointed out that genetic diversity, so-called subtypes (STs), exists based on polymorphic regions across the small subunit ribosomal RNA (SSU rRNA) or 18S rRNA gene (5, 6). On the other hand, morphologically, they are indistinguishable under the microscope, because various subtypes have similar morphological forms (7). Currently, at least 22 subtypes have been described, among which ST1-ST9 (originally human) and ST12

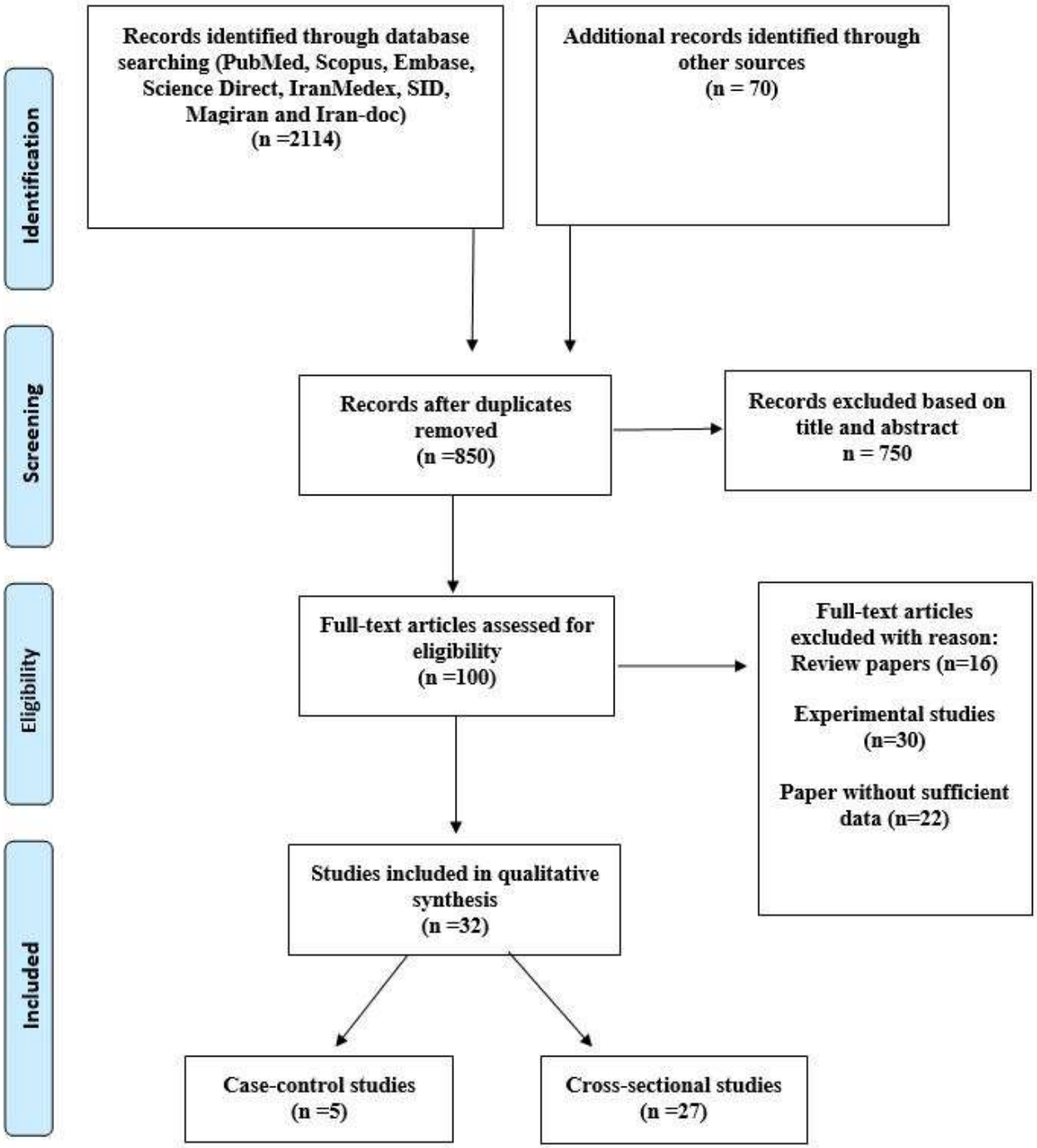

Figure 1. PRISMA chart describing the study design process.

have been isolated in humans with zoonotic potential, while the others are found exclusively in animals (8-10).

Furthermore, genetic diversity differs substantially among *Blastocystis* sp. subtypes and at the intra-subtype level. In previously conducted studies on humans across the globe, ST1-ST4 were the most frequently detected subtypes identified in >90% of investigations, whereas ST5-ST9 were rarely detected (11-14). Nonetheless, the predominant subtype is different in various parts of the world and even in the same country (1). Up to now, some aspects of the association between clinical manifestations and *Blastocystis* sp. subtypes have remained unclear. Healthy individuals are commonly without gastrointestinal symptoms (15), whereas such clinical symptoms as diarrhea, abdominal pain, flatulence, anorexia, nausea, and vomiting have been reported in infected patients with *Blastocystis* sp. (16, 17). Some studies (in vitro and in vivo) have shown the pathogenic potential of *Blastocystis* sp. to be related to secretory cysteine proteases, which induce enterocytes apoptosis and increase gut permeability (18, 19). Studies on the molecular diagnosis use sequence-gagged sites (STS) primers, barcoding region from the SSU rRNA gene, and restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) (5, 20, 21). The identification of *Blastocystis* sp. subtypes is necessary to control and prevent infection (1, 22).

The present study is a systematic review and meta-analysis conducted to assess the distribution of *Blastocystis* sp. subtypes in various parts of Iran.

Methods

Search strategy

The present study was designed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA) (23). Based on published articles on subtypes of *Blastocystis* sp., the present study aimed to identify the frequencies of specific subtypes in Iran and survey the association

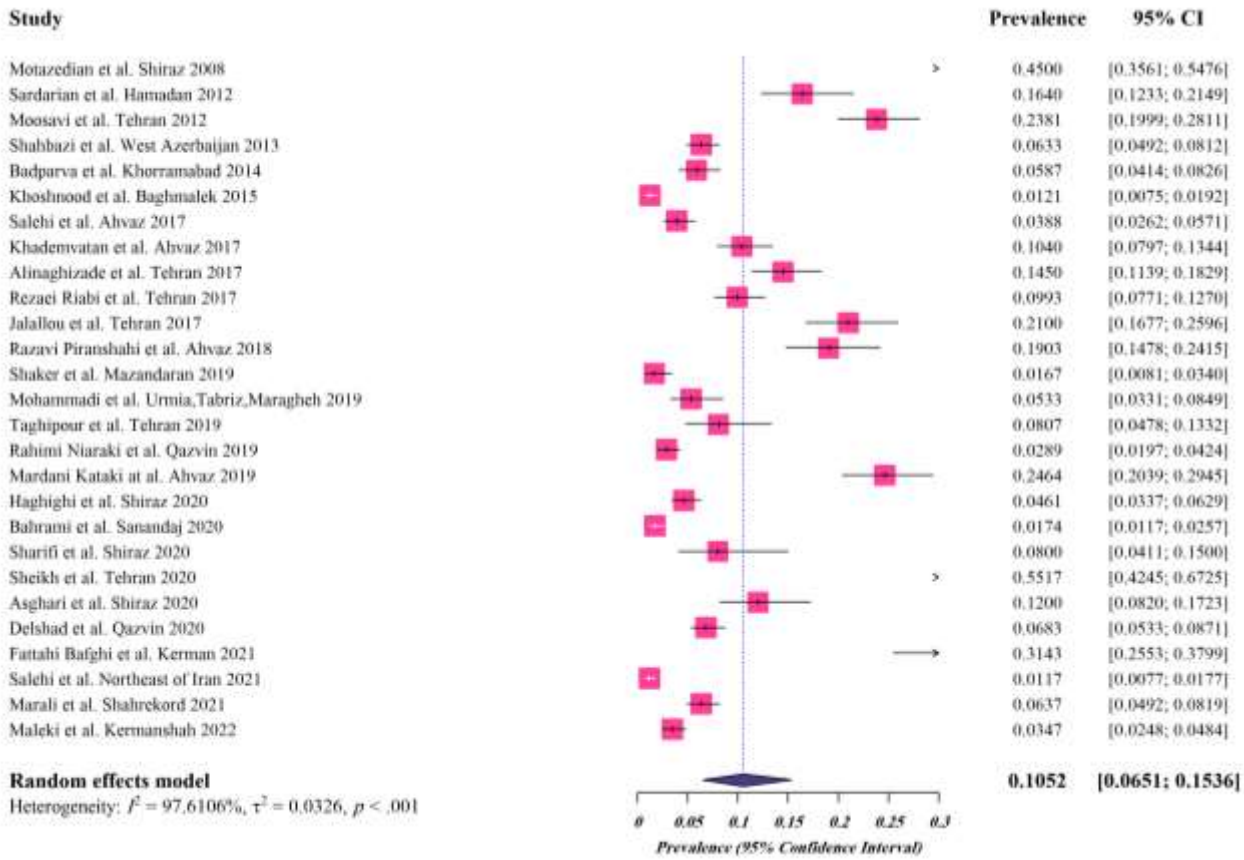

Figure 2. Forest plot for a random effect meta-analysis of subtyped *Blastocystis* frequency isolated from human in Iran

between subtypes and participants (study population) as well as the province or city of each patient's residence and its humidity conditions, and to compare the frequency of subtypes in case-control groups by odds ratio (OR). For this aim, we conducted a systematic search using English (PubMed, Science Direct, Scopus, and ProQuest) and Persian (SID, IranMedex, Magiran, and IranDoc) databases for articles published prior to April 2022. The MeSH terms used in the electronic search in the present study were: (*Blastocystis* sp.) AND (molecular OR subtype) AND (prevalence OR epidemiology) AND Iran. The search was limited to English and Persian languages.

Inclusion and exclusion criteria

The inclusion criteria comprised: (1) publications published prior to April 2022, (2) case-control and cross-sectional studies on subtypes of *Blastocystis* sp. in Iran, and (3) articles containing information on total sample size, positive samples, participants, molecular technique, and investigated city/province. Subsequently, the exclusion criteria were: (1) papers with no specific information, (2) review articles, and (3) animal studies.

Study selection and data extraction

First, all searched studies were imported into the EndNote library (version X9). Then, after deleting duplicates, titles and abstracts were checked for the first screening stage, followed by full-text screening performed by two authors (SD and BM) for the second screening stage to make the final article selection. The third author (MO) settled any disagreements between the two authors. To avoid missing valuable data, the reference lists of chosen publications and published reviews were also examined. From each eligible study, the following information was extracted: general features such as first author, type of study (case-control and cross-sectional), year of publication, province/city and its humidity rate, participants, method, sample size, number of positive samples, identified subtype, and frequency of each subtype. The extracted data was recorded into an Excel spreadsheet, and any discrepancies in the results were discussed and checked between three authors (BM, SD, and MO).

Quality assessment

The quality of the included studies was assayed based on The Newcastle-Ottawa Scale with separate criteria for case-control and cross-sectional studies to measure the selection, comparability, and outcome categories of the included studies (24, 25). In case-control studies, based on this checklist, included studies were divided into categories of good quality (score of 7–9), moderate quality (score of 4–6), and poor quality (score of less than 3). In cross-sectional studies, based on this checklist, included studies were divided into categories of good quality (score of 6–7), moderate quality (score of 3–5), and poor quality (score of 1-2).

Statistical analysis

The meta-analysis procedure was performed using R software (version 4.1.2). The pooled regional and national prevalence of *Blastocystis* sp. subtypes in Iran and associated 95% confidence intervals were calculated by random-effects meta-analysis. Heterogeneity was also checked using the Cochran Q and I2 statistics. Furthermore, publication bias was assessed based on the LFK index and Doi plot (26), in addition to the funnel diagram and Egger regression test. Finally, to evaluate the sources of heterogeneity, subgroup analysis was performed for the studied geographic region, climate and humidity conditions, as well as the type of participants.

Results

Study characteristics

After removing duplicates and non-eligible papers from 2114 publications found in eight databases, 32 papers comprising 5 case-control studies (27-31) and 27 cross-sectional studies (32-58) were included in our study, totaling 16,638 samples surveyed (Table 1 and Table 2). Figure 1 presents the flow diagram of the study selection process.

Pooled prevalence of *Blastocystis* sp. subtypes in Iran

Based on the results of the meta-analysis, the overall pooled prevalence of subtyped *Blastocystis* sp. was estimated to be 10% (95% CI: 6-15%) in Iran (Figure 2), indicating significant evidence of heterogeneity among studies (I2=97.0%; p<0.001).

Our meta-analysis findings identified eight subtypes of *Blastocystis* sp. (ST1-ST7 and ST9). Among them, subtype ST3 had the highest prevalence (0.4; 95% CI: 0.01-0.07), whereas the lowest prevalence was related to ST7 (Table 3).

Table 1. Baseline characteristics of relevant studies to prevalence of subtyped *Blastocystis* sp. in human in Iran.

First author (References)	City/province	Year of Publication	Positive Number/ Sample Size	Subtypes	Participant	Method	Quality Score
Motazedian et al.(32)	Shiraz	2008	45/100	ST1(20), ST2(4), ST3(16)	Persons who referred to health centers	RFLP	5
Moosavi et al.(33)	Tehran	2012	100/420	ST1(21), ST3(25), ST6(21), Mix 1, 3(14), Mix 3,5(2), Mix 3,6(4), M 1,5(1)/ M 1,6(4)/M 1,5,3(4)/ M 1,3,6(4)	persons who referred to health centers	STS primers	5
Sardarian et al.(34)	Hamadan	2012	41/250	ST1(23), ST5(3), ST3(9), Mix 1, 3(6)	persons who referred to health centers	STS primers	4
Shahbazi et al.(35)	west Azerbaijan	2013	57/900	ST1(23), ST2(5), ST3(29)	persons who referred to health centers	STS primers	6
Badparva et al.(36)	Khorramabad	2014	30/511	ST3(17), ST5(4), ST6(6), Mix 3,5(2), Mix 3,6 (1)	persons who referred to health centers	STS primers	5
Khoshnood et al.(37)	Baghmalek	2015	17/1410	ST3(3), ST4(9), ST5(2), ST7(3),	persons who referred to health centers	PCR/Sequencing	7
Alinaghizade et al.(38)	Tehran	2017	58/400	ST1(18), ST2(21), ST3(19),	diarrheic and non-diarrheic patients	PCR/Sequencing	6
Salehi et al.(39)	Ahvaz	2017	24/618	ST1(5), ST2(5), ST3(14),	persons who referred to health centers	PCR/Sequencing	6
Khademvatan et al.(40)	Ahvaz	2017	50/481	ST1(11), ST2(3), ST3(20), ST4(1), ST5(4), Mix 1, 3(3), M 1,4(3) and M 3,4(5)	persons who referred to health centers	STS primers/ Sequencing	7
Rezaei Riabi et al.(41)	Tehran	2017	55/554	ST1(13), ST2(14), ST3(25), ST6(2), ST7(1),	Persons who referred to health centers	PCR/Sequencing	7
Jalallou et al.(42)	Tehran	2017	63/300	ST1(21), ST2(23), ST3(19)	Persons who referred to health centers	PCR/Sequencing	7
Razavi Piranshahi et al.(43)	Ahvaz	2018	51/268	ST1(11), ST2(6), ST3(29), ST6(2), Mix 1, 3(3),	HIV patients	STS primers/ Sequencing	6
Mardani Kataki at al.(44)	Ahvaz	2019	85/345	ST1(5), ST2(5), ST3(25)	Persons who referred to health centers	PCR/Sequencing	7
Taghipour et al.(45)	Tehran	2019	13/161	ST1(7), ST2(5), ST3(1)	Tuberculosis patients	PCR/Sequencing	5
Rahimi Niaraki et al.(46)	Qazvin	2019	25/864	ST1(14), ST2(7), ST3(4)	children (referred to hospital)	PCR/Sequencing	7
Shaker et al.(47)	Mazandaran	2019	7/420	ST3(7)	persons who referred to health centers	PCR/Sequencing	5
Mohammadi et al.(48)	Urmia, Tabriz, Maragheh	2019	16/300	ST1(3), ST2(3), ST3(10)	persons who referred to health centers	PCR/Sequencing	4
Bahrami et al.(49)	Sanandaj	2020	24/1383	ST1(2), ST2(6), ST3(16)	persons who referred to health centers	PCR/Sequencing	7
Sharifi et al.(50)	Shiraz	2020	8/100	ST1(2), ST2(5), ST6(1)	persons who referred to health centers	PCR/Sequencing	7
Haghighi et al.(51)	Shiraz	2020	37/802	ST1(12), ST2(9), ST3(13), ST7(3)	persons who referred to health centers	PCR/Sequencing	7
Sheikh et al.(52)	Tehran	2020	32/58	ST1(1), ST3(28), ST9(3)	Schizophrenia patients	STS primers/ Sequencing	7
Asghari et al.(53)	Shiraz	2020	24/200	ST1(5), ST2(8), ST3(9), ST7(2)	cancer children and adolescents	PCR/Sequencing	7
Delshad et al.(54)	Qazvin	2020	59/864	ST1(21), ST2(14), ST3(11)	Persons who referred to health centers	PCR/Sequencing	6
Fattahi Bafghi et al.(55)	Kerman	2021	66/210	ST1(5), ST2(1), ST3(37), ST4(7), ST5(6), ST7(3), Mix 3, 4(3), Mix 3, 5(1), Mix 1,2, 3(3), Mix 4, 5(1), Mix 1, 3(3), Mix 3, 4,7(1),	persons who referred to health centers	Real-time PCR molecular method	7
Salehi et al.(56)	Northeast of Iran	2021	22/1878	ST1(4), ST2(7), ST3(10), ST4(1)	persons who referred to health centers	PCR/Sequencing	6
Marali et al.(57)	Shahrekord	2021	55/864	ST1(16), ST2(15), ST3(20), ST7(4)	Persons who referred to health centers	PCR/Sequencing	6
Maleki et al.(58)	Kermanshah	2022	33/950	ST1(5), ST3(15), ST5(4), Mix 1, 3(2), Mix 1, 5(1), Mix 1, 6(1), Mix 3, 4(1), Mix 2, 5(1), Mix 3, 5(1), Mix 1, 5,6(1), Mix 3, 6(1),	Persons who referred to health centers	STS primers	7

Table 2. Characteristics of the included case- control studies.

Author (References)	Province/city	Year of publication	Groups	Sample size	Subtypes									Quality Score
					ST1	ST2	ST3	ST4	ST5	ST6	ST7	Mixed 1,3	Unsubtyped	
Azizian et al.(27)	Ilam	2016	Case	40	1	1	7						5	6
			Control	40	3	4	1						6	
Beiromvand et al.(28)	Ahvaz	2017	Case	152	2	7	6						1	8
			Control	130	7	13	19							
Khademvatan et al.(29)	Ahvaz	2017	Case	122	9	0	12			1	2			6
			Control	122	8	0	9			3	1			
Mirjalali et al.(30)	Tehran	2017	Case	71	1	0	7							9
			Control	166	14	12	9							
Shirvani et al.(31)	Kerman	2019	Case	133	4	7	5						11	8
			Control	51	4	3	5						2	

Table 3. Frequency of each identified Blastocystis sp. subtype based on studied humidity, climate zone, and region.

Genotype	No. of studies	Sample size	Positive cases	Overall prevalence(95%CI)	Heterogeneity		
					I^2	χ^2	P value
ST1 (Total)	24	13270	262	0.0255(0.0151-0.0385)	91.4806%	0.0071	P <0.001
ST1 (humidity)							
20-40	15	5017	160	0.0346(0.0196-0.0535)	82.9281	0.0062	P <.001
41-61	9	8253	102	0.0142(0.0032-0.0327)	93.2755	0.0062	P <.001
ST1 (Climate zone)							
Hot, Desert and Semi-desert	15	5017	160	0.0346(0.0196-0.0535)	82.9281	0.0062	P <.001
Mountainous	9	8253	102	0.0142(0.0032-0.0327)	93.2755	0.0062	P <0.001
ST1 (region)							
South of Iran	4	1202	39	0.0471(0.0000-0.2081)	92.7683	0.0236	P<.001
Southwest of Iran	5	2576	51	0.0204(0.0088-0.0366)	63.7225	0.0011	P =.03
West of Iran	3	2583	30	0.0188(0.0000-2303)	96.7695	0.0206	P <0.01
North-central of Iran	6	1893	81	0.0421(0.0249-0.0635)	60.411	0.0014	P =.03
Northwest of Iran	4	2928	52	0.0155(0.0033-0.0367)	82.1965	0.0014	P<.001
Southeast of Iran	1	210	5	-	-	-	-
Northeast of Iran	1	1878	4	-	-	-	-
ST2 (Total)	20	11592	169	0.0178(0.0108-0.0264)	85.1719	0.0033	<0.001
ST2 (humidity)							
20-40	13	4539	112	0.02 (0.01-0.03)	82%	0.0034	<0.01
41-61	7	7053	57	0.00(0.00-0.01)	71%	0.0006	.002
ST2 (Climate zone)							
Hot, Desert and Semi-desert	13	4539	89	0.0253 (0.0144-0.0391)	82.1134%	0.0034	P <0.001
Mountainous	7	7503	57	0.0083 (0.0041-0.0139)	71.4439%	0.0006	P =0.002
ST2 (region)							
South of Iran	4	1202	26	0.0290(0.0063-0.0676)	73.0268%	0.0020	P =.01
Southwest of Iran	5	2576	37	0.0139(0.0059-0.0254)	51.8756%	0.0007	P =.08
West of Iran	1	1383	6	-	-	-	-
North-central of Iran	4	1415	63	0.0444(0.0154-0.0874)	76.8688%	0.0022	P =.005
Northwest of Iran	4	2928	29	0.0095(0.0036-0.0183)	42.5586%	0.0003	P =.16
Southeast of Iran	1	210	1	-	-	-	-
Northeast of Iran	1	1878	7	-	-	-	-

Continued							
ST3 (Total)	26	15511	431	0.04(0.02-0.07)	94%	0.0198	<0.01
ST3 (humidity)							
20-40	15	6327	283	0.07(0.03-0.12)	96%	0.0282	P <0.01
41-61	10	8764	141	0.02(0.01-0.03)	85%	0.0018	P <0.01
62-82	1	420	7	0.02 (0.01-0.03)	_	_	_
ST3 (Climate zone)							
Hot, Desert and Semi-desert	15	6327	283	0.07(0.03-0.12)	96%	0.0282	P <0.01
Mountainous	10	8764	141	0.02(0.01-0.03)	85%	0.0018	P <0.01
Mediterranean	1	420	7	0.02(0.01-0.03)	_	_	_
ST3 (region)							
South of Iran	3	1002	22	0.06 (0.00-0.32)	93%	0.0190	P <0.01
Southwest of Iran	6	2777	66	0.04(0.01-0.09)	96%	0.0097	P <0.01
West of Iran	4	2144	42	0.02 (0.01-0.04)	76%	0.0013	P <0.01
North-central of Iran	6	1039	73	0.08 (0.00-0.26)	94%	0.0533	P <0.01
Northwest of Iran	4	2064	43	0.02(0.00-0.05)	89%	0.0027	P <0.01
North of Iran	1	420	7	0.02 (0.01-0.03)	-	-	-
Southeast of Iran	1			0.18 (0.13-0.23)	-	-	-
Northeast of Iran	1			0.01 (0.00-0.01)	-	-	-
ST4 (Total)	4	3979	18	0.01 (0.00-0.03)	88%	0.0041	<0.01
ST4 (humidity)							
20-40	3	2101	17	0.01 (0.00-0.07)	82%	0.0041	<0.01
41-61	1	1878	1	0.00 (0.00-0.00)	-	-	-
ST4 (Climate zone)							
Hot, Desert and Semi-desert	3	2101	17	0.01 (0.00-0.07)	82%	0.0041	<0.01
Mountainous	1	1878	1	0.00 (0.00-0.00)	-	-	-
ST4 (region)							
Southwest of Iran	2	1891	10	0.01 (0.00-0.07)	41%	0.0003	0.19
Southeast of Iran	1	210	7	0.03 (0.01-0.06)	-	-	-
Northeast of Iran	1	1878	1	0.00 (0.00-0.00)	-	-	-
ST5 (Total)	5	3562	20	0.01 (0.00-0.02)	75%	0.0017	<0.01
ST5 (humidity)							
20-40	3	2101	12	0.01 (0.00-0.06)	86%	0.0035	<0.01
41-61	2	1461	8	0.00 (0.00-0.05)	0%	0.0001	0.39
ST5 (Climate zone)							
Hot, Desert and Semi-desert	3	2101	12	0.01 (0.00-0.06)	86%	0.0035	<0.01
Mountainous	2	1461	8	0.01 (0.00-0.05)	0%	0.0001	<0.01
ST5 (region)							
West of Iran	2	1461	8	0.01 (0.00-0.05)	0%	0.0001	0.39
Southwest of Iran	2	1891	6	0.00 (0.00-0.15)	76%	0.0010	0.04
Southeast of Iran	1	210	6	0.03 (0.01-0.06)	-	-	-
ST6 (Total)	5	1853	32	0.01 (0.00-0.04)	86%	0.0034	<0.01
ST6 (humidity)							
20-40	4	1342	26	0.01 (0.00-0.06)	89%	0.0047	<0.01
41-61	1	511	6	0.01 (0.00-0.02)	-	-	-

Continued							
ST6 (Climate zone)							
Hot, Desert and Semi-desert	4	1342	26	0.01 (0.00-0.06)	89%	0.0047	<0.01
Mountainous	1	511	6	0.01 (0.00-0.02)	-	-	-
ST6 (region)							
South of Iran	1	100	1	0.01 (0.00-0.04)	-	-	-
Southwest of Iran	1	268	2	0.01 (0.00-0.02)	-	-	-
West of Iran	1	511	6	0.01 (0.00-0.02)	-	-	-
North-central of Iran	2	974	23	0.02 (0.00-0.86)	96%	0.0127	<0.01
ST7 (Total)	6	4040	16	0.00 (0.00-0.01)	20%	0.0005	0.28
ST7 (humidity)							
20-40	5			0.00 (0.00-0.01)	33%	0.0006	0.20
41-61	1			0.00 (0.00-0.01)	-	-	-
ST7 (Climate zone)							
Hot, Desert and Semi-desert	5	3176	12	0.00 (0.00-0.01)	33%	0.0006	0.20
Mountainous	1	864	4	0.00 (0.00-0.01)	-	-	-
ST7 (region)							
South of Iran	2	1002	5	0.01 (0.00-0.08)	0%	0.0002	0.32
Southwest of Iran	2	2274	7	0.00 (0.00-0.04)	3%	<0.0001	0.31
Southeast of Iran	1	210	3	0.01 (0.00-0.03)	-	-	-
North-central of Iran	1	554	1	0.00 (0.00-0.01)	-	-	-
Mixed 1 and 3	5	2369	27	0.01(0.00-0.03)	88%	0.0031	<0.01
Mixed 1 and 3 (humidity)							
20-40	3	1169	20	0.02(0.00-0.07)	80%	0.0023	<0.01
41-61	2	1200	7	0.01(0.00-0.59)	92%	0.0065	<0.01
M1 ,3 (Climate zone)							
Hot, Desert and Semi-desert	3	1169	20	0.02(0.00-0.07)	80%	0.0023	<0.01
Mountainous	2	1200	7	0.01(0.00-0.59)	92%	0.0065	<0.01
M1 and 3 (region)							
Southwest of Iran	2	749	6	0.01(0.00-0.06)	0%	<0.0001	P =.0.48
West of Iran	2	1200	7	0.01(0.00-0.59)	92%	0.0065	<0.01
North-central of Iran	1	420	14	0.03(0.02-0.05)	-	-	-
Mixed 3 and 5	4	2091	7	0.00(0.00-0.01)	44%	0.0005	0.15
Mixed 3 and 5 (humidity)							
20-40	2	630	5	0.00(0.00-0.07)	0%	<0.0001	0.50
41-61	2	1461	2	0.00(0.00-0.01)	0%	<0.0001	0.67
M3 ,5 (Climate zone)							
Hot, Desert and Semi-desert	2	630	5	0.00(0.00-0.01)	0%	<0.0001	0.67
Mountainous	2	1461	2	0.00(0.00-0.07)	0%	<0.0001	0.50
Mixed 3 and 4	3	1641	9	0.01(0.00-0.04)	78%	0.0016	0.01
Mixed 3 and 6	3	1881	6	0.00(0.00-0.02)	61%	0.0008	0.08

Sub-group analysis based on geographic region, climate zone, and humidity

The highest and lowest pooled prevalence of subtyped *Blastocystis* sp. was found in the southeast (31%; 95% CI 25 to 37) and northeast of Iran (1%; 95% CI 0 to 1), respectively (Figure 3).

ST1 (4%; 95% CI 3 to 6) was reported as the predominant subtype in the south and north-central, ST2 (4%; 95% CI 1 to 8) was found as the predominant subtype in the north-central, and ST3 (18%; 95% CI 13 to 23) was the predominant subtype in the southeast regions of Iran (Table 3).

The results regarding climate zones showed that geographic regions with hot, desert, and semi-desert weather had a high prevalence (15%; 95% CI 9 to 24) of subtyped *Blastocystis* sp. in Iran. In contrast,

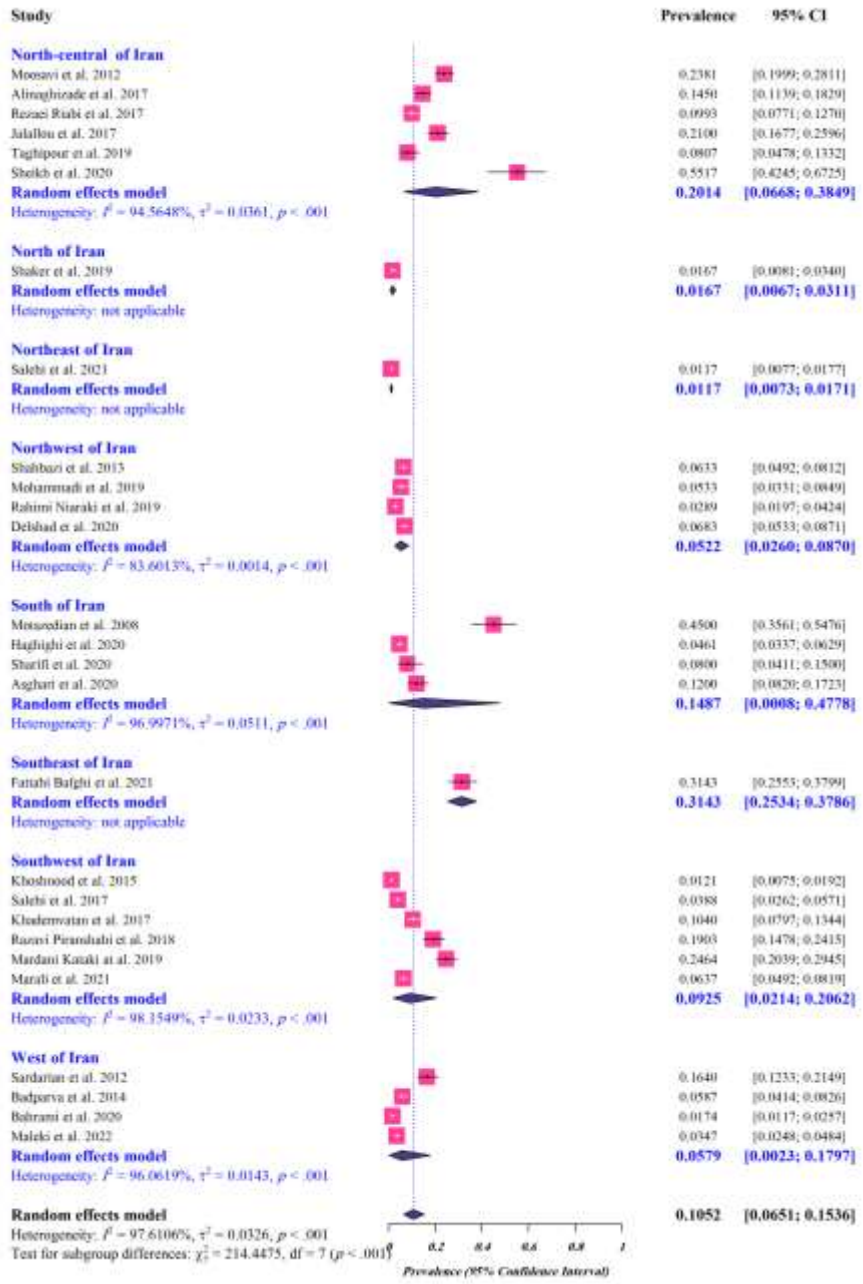

Figure 3. Forest plot for a random effect meta-analysis of subtyped *Blastocystis* frequency isolated from human in Iran according to geographic region

geographic regions with Mediterranean weather had a low prevalence (1%; 95% CI 0 to 3) (Figure 4 and Table 3).

The highest pooled prevalence of subtyped *Blastocystis* sp. was found in geographic regions with humidity of 20-40% (15%; 95% CI 9 to 24), followed by geographic regions with humidity of 41-61% (4%; 95% CI 2 to 7) and 62-82% (1%; 95% CI 0 to 3) (Figure 5). Among isolated subtypes, ST1, ST2, and ST3 had high prevalence rates in areas with humidity of 20-40%. As shown in Table 3, there seems to be a possible association between the distribution of isolated *Blastocystis* sp. subtypes and low humidity.

Sub-group analysis based on participants

Among studies surveyed on *Blastocystis* sp. subtyping in Iran, almost all (23 from 27) were done on persons who referred to health centers. Among the studies analyzed in the meta-analysis, schizophrenia patients (55%; 95% CI 42to 67) had the highest pooled prevalence, and infectious disease/tuberculosis patients had a low pooled prevalence (8%; 95% CI 4 to 12) (Figure 6). Subtypes ST1 (4%; 95% CI 1 to 8), ST2 (4%; 95% CI 1 to 7), and ST3 (48%; 95% CI 36 to 61) were predominant in infectious disease/HIV patients, cancer patients, and schizophrenia patients, respectively (Supplementary Figures 1, 2, 3). As other participant groups were surveyed in only a few studies, it was not feasible to carry out a meta-analysis; instead, we have presented the total sample and positive samples of the participant groups in Table 1.

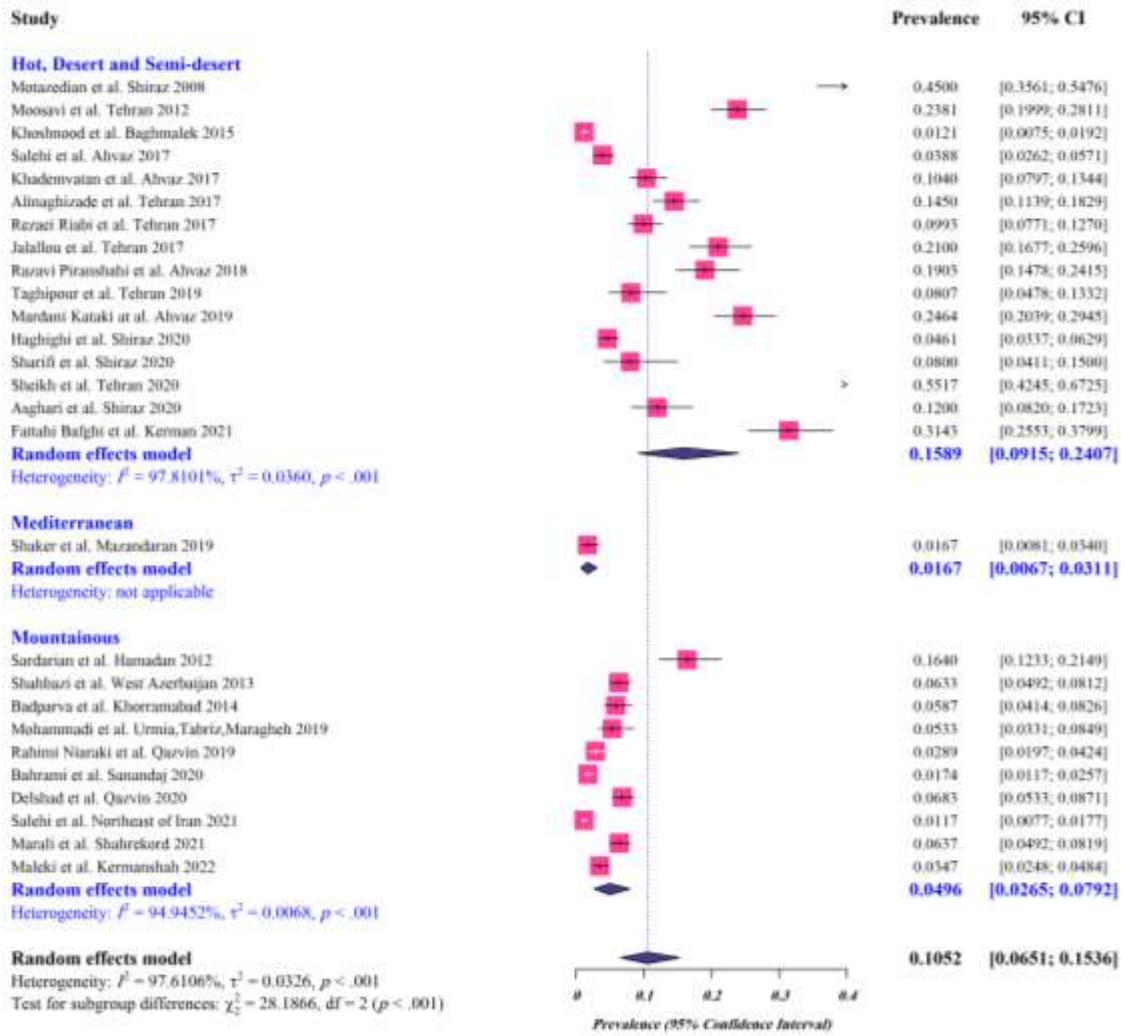

Figure 4. Forest plot for a random effect meta-analysis of subtyped *Blastocystis* frequency isolated from human in Iran according to different climate zones

Case-control studies

Based on the meta-analysis findings, the odds ratio (OR) between cases and control groups for subtypes ST1, ST2, and ST3 were 0.39 (95% CI, 0.16 -0.98), 0.37 (95% CI, 0.09-1.52), and 0.98 (95% CI, 0.30-3.20), respectively (Supplementary Figures 4, 5, and 6). Although no significant difference was seen, the findings indicated that the odds of infection by ST1 were lower in case groups than in control groups. Conversely, the odds of infection by ST3 were higher in case groups. On the other hand, the rate of infection with subtype ST2 was similar in both groups (cases and control groups).

Publication bias

The results indicated the existence of publication bias in this study, either with the Egger regression method (p<0.001, Figure 7) or the new and optimal method (LFX=3.37; major asymmetry; Figure 7).

Quality assessment

A quality evaluation of the cross-sectional studies revealed that 19 papers (70.37%) had excellent quality and eight (29.63%) had moderate quality. Among the case-control studies, three articles (60%) had high quality and two papers (40%) had intermediate quality (Tables 1 and 2).

Discussion

Infection with numerous subtypes has been widely reported in humans in both developing and developed countries around the world (59). Our results revealed a pooled prevalence of 10% (95% CI: 6-15%) for

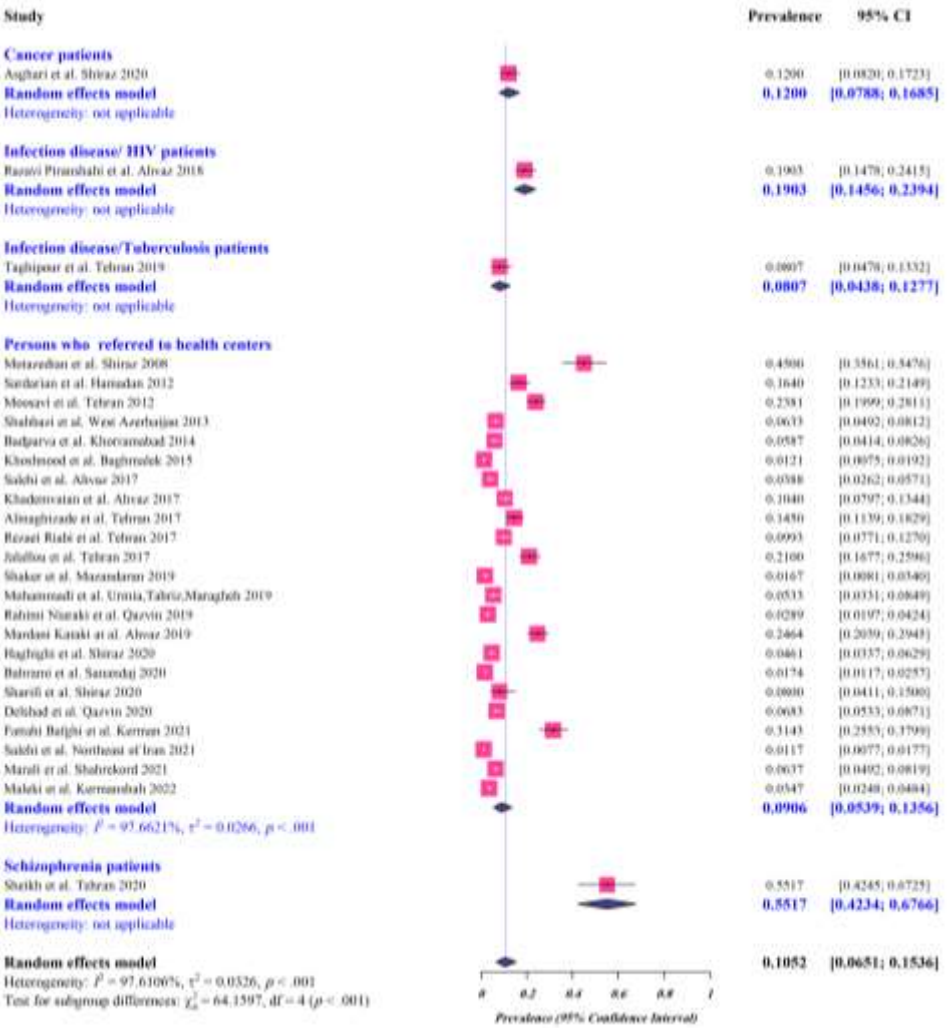

Figure 6. Forest plot for a random effect meta-analysis of subtyped *Blastocystis* frequency isolated from human in Iran according to participants

subtyped *Blastocystis* sp. infection in the general Iranian population.

The distribution of subtypes in the study population revealed subtypes ST1 to ST7 and ST9; to date, human subtypes ST8 and ST12 have not been reported in Iran. According to our meta-analysis, ST3 and ST1 were the most prevalent subtypes in Iran, respectively. According to the review of Jiménez et al. (2019) and in line with our results, the most frequent subtypes identified in humans and animals in North and South America were ST1, ST2, and ST3 (60). According to the present findings, ST3 (0.04; 95% CI: 0.01-0.07) had the highest prevalence in various parts of Iran. In a systematic review and meta-analysis concluded in Brazil, ST1 was the most frequent subtype, and the overall percentage of infection with subtypes ST1, ST2, and ST3 was reported as 86.2% (61). Although it has been proposed that ST3 is a subtype of human origin only, it has been reported in several hosts (e.g., non-human primates, cattle, and pigs) (62, 63).

It can be concluded that transmission in these countries occurs more frequently through direct human-to-human transmission. According to the forest plot for a random-effects meta-analysis of *Blastocystis* sp., ST1 was common in the north-central (0.04; 0.02-0.06), south (0.04; 0.00-0.20), and southwest (0.02; 0.00-0.03) of Iran. This subtype has low specificity, and some studies have pointed to its zoonotic transmission.

In addition to humans, ST1 has been detected in dogs, chickens, cattle, pigs, and non-human primates (64, 65).

In the present study, after ST3 and ST1, one of the most common subtypes was ST2, the most prevalent subtype in north-central Iran (0.04; 0.01-0.08). This finding is in agreement with those obtained by Alinaghizade et al. and Jalallou et al., who referred to ST2 as the predominant subtype (38, 42). After these three subtypes (ST1, ST2, and ST3), ST5 was the most prevalent subtype in southeast Iran (0.03; 0.01-0.06); this subtype is often isolated from pigs and cattle and rarely reported in humans (66).

ST4, which was reported among the Iranian population in four studies, is more prevalent in southeast Iran (0.03; 0.01-0.06). Despite fewer reports of this subtype in Iran, it is common among Nepalese, USA, European, and African populations; moreover, it has been associated with acute diarrhea (1, 11). In several studies, ST4 has been observed in wild rodents (reservoir hosts) as well as marsupials, ratites, and primates. Other subtypes, including ST6 and ST7, which are considered avian subtypes and occasionally found in some mammals, such as pigs, cattle, goats,

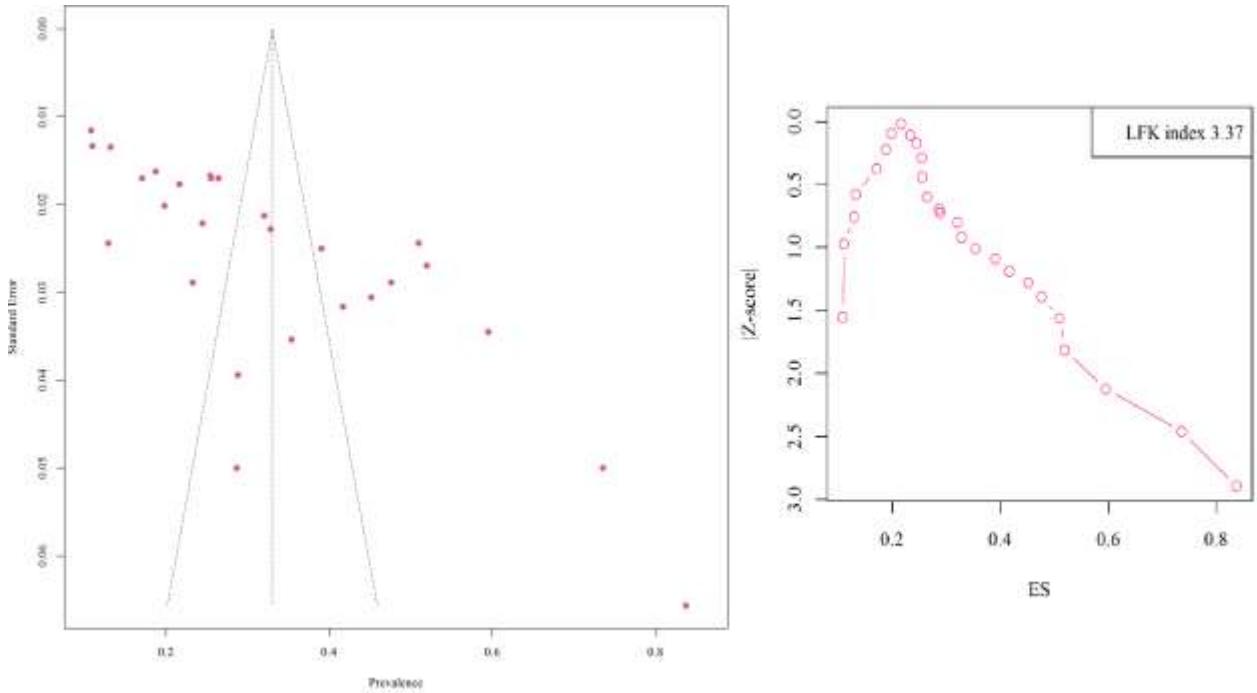

Figure 7. Publication bias calculated for cross-sectional studies using Funnel plot and dioplot

dogs, cynomolgus monkeys, and ruffed lemurs, and are distributed around the world, constitute a small share of cases of *Blastocystis* sp. (1, 8). Nevertheless, the highest prevalence of ST7 was reported in Brazil (13, 67). The ST6 subtype is more prevalent in north-central Iran (0.02; 0.00-0.86) and the ST7 subtype is more prevalent in south (0.01; 0.00-0.08) and southeast (0.01; 0.00-0.03) Iran. Seven studies in Iran that reported *Blastocystis* sp. mixed infection used the subtype-specific sequence-tagged-site (STS) primers. A mixed infection rate ranging from 1.1-14.3 has been observed in different studies across the globe (7, 68-70). In addition, selected DNA extraction methods (direct stool sample or culture medium) are other factors that affect the reporting of mixed infections, because the use of culture medium may ignore some mixed subtypes due to different growth rates in various subtypes of *Blastocystis* sp. (34).

A mixed infection rate ranging from 1.1-14.3 has been observed in different studies across the globe (7, 68-70). In addition, selected DNA extraction methods (direct stool sample or culture medium) are other factors that affect the reporting of mixed infections, because the use of culture medium may ignore some mixed subtypes due to different growth rates in various subtypes of *Blastocystis* sp. (34). According to this meta-analysis, mixed infection with ST1 and ST3 (0.01; 0.00-0.03) as well as mixed 3 and 4 (0.01; 0.00-0.04) was more prevalent in Iran.

It has been proposed that differences in the prevalence of subtypes in humans around the world may be due to differences in cyst numbers, cyst survival, infectious dose, climate, geography, eating habits, participants, mode of transmission, and travel to tropical countries (4, 71-73).

The present study found that of the eight geographic regions in Iran, the largest amount of published data was related to regions in north-central and southwest Iran. A total of 12 articles representing about 44.44% of the analyzed samples in the present study were related to these two regions. One study only was conducted for each of the north, southeast and northeast regions of the country. Moreover, the molecular epidemiology of *Blastocystis* sp. has been incompletely known in other parts of Iran, such as east of the country. In fact, more studies with larger sample sizes are needed to determine the true distribution of *Blastocystis* sp. across Iran.

In north-central Iran, we observed a high prevalence of subtyped *Blastocystis* sp. (20%; 95% CI 6 to 38) in contrast to north and northeastern Iran (1%; 95% CI 0 to 3). The significant number of samples in the north-central (total sample: 1,893), west (total sample: 3,094), and southeast (total sample: 3,986) of the country decreased the probability of sampling error. The prevalence of *Blastocystis* sp. is seemingly influenced by climate conditions; therefore, in this study, the prevalence of subtyped *Blastocystis* sp. was sub-group analyzed based on climate conditions. In the present study, the highest prevalence of the subtyped *Blastocystis* sp. was observed in geographic regions with hot, desert, and semi-desert weather. In addition, we should not ignore the population under study. In other words, suitable humidity improves environmental conditions for the distribution of parasitic intestinal infections, increasing the chance of transmission of such infections as *Blastocystis* sp. among a population. In this study, the highest pooled prevalence of subtyped *Blastocystis* sp. was found in areas with relative humidity between 20-40% (15%; 95% CI 9 to 24) (Fig. 5).

In fact, more studies have been done in areas with low humidity, and they have revealed a higher prevalence, while coastal areas in northern Iran which have high humidity, have been the subject of only one study conducted in Mazandaran province (Table 1).

Various studies suggest a higher prevalence of *Blastocystis* sp. in tropical areas with high humidity, but these findings were not supported by our research. This may be due to the lack of studies in areas of Iran which have high humidity. Moreover, factors such as technical weakness in correct diagnosis, migration and travel factors, and the studied populations should not be ignored in the studies.

In Iran, 23 studies have examined *Blastocystis* sp. subtyping among individuals attending health centers (9%; 95% CI 5 to 13), and the highest prevalence was related to patients with schizophrenia (55%; 95% CI 42 to 67).

Importantly in Iran, only one study performed by Sheikh et al. in Tehran reported ST9 in schizophrenia patients; this subtype has not yet been isolated from non-human sources (52).

ST3 has been reported as the most common subtype of *Blastocystis* sp. in patients with schizophrenia, HIV,

and cancer, whereas ST1 has been reported as the most predominant subtype of *Blastocystis* sp. in patients with tuberculosis and HIV (referred to hospital). According to a systematic review conducted by Deng et al. in China, most studies have investigated *Blastocystis* sp. infection in patients with different degrees of diarrhea (66). In addition, some case-control studies in Iran have surveyed the association between *Blastocystis* sp. infection and skin disorders and irritable bowel syndrome (IBS) (29, 31). In the meta-analysis results of the present study, no significant difference was observed between the two groups (case and control). However, ST1 (0.39; 95% CI, 0.16 -98) was reported as lower in case groups than in control groups. Nonetheless, the OR of infection by ST3 (0.98; 95% CI, 0.30-3.20) was higher in case groups. Stensvold et al. (2016) reported that the genetic diversity of *Blastocystis* sp. is possibly associated with its pathogenicity (59, 74).

Rostami et al. conducted a meta-analysis study about the role of *Blastocystis* sp. in IBS in the world and demonstrated a significant positive impact of *Blastocystis* sp. subtypes ST1 and ST3 on IBS (75). Bahrami et al. conducted a review study, and, they reported ST1, ST2, and ST3 as the most frequent subtypes in case groups of urticaria and skin disorders, which was similar to the results of our meta-analysis, which indicated that these three subtypes were more prevalent (76).

Further case-control studies can be effective in obtaining better and generalizable results in Iran.

Conclusion

The present systematic review pointed out that all human *Blastocystis* sp. subtypes reported worldwide, except ST8 and ST12, were found in different parts of Iran. Although the most common subtype in Iran was ST3, the existence of other subtypes may raise the issue of zoonosis.

Conflict of interests

The authors declare no conflict of interest.

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