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Review

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Amoebiasis in Iran: a systematic review and meta-analysis

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Abstract

A comprehensive meta-analysis study was performed to estimate the reliable national prevalence and molecular epidemiology of amoebiasis in Iran. Nine English and Persian databases were searched to achieve the relevant studies. Pooled estimates were generated and meta-regression was performed. We identified 71 eligible articles involving 330 930 subjects from 25 provinces to be included in the final analysis. Moreover, 17 studies compromising 462 polymerase chain reaction (PCR)-positive isolates performed molecular analysis to inter-species differentiation. The pooled prevalence of *Entamoeba* infection among Iranian population was about 1% (95% CI 0.8–2.0%). Moreover, regarding Human Development Index (HDI), a higher prevalence was observed in undeveloped provinces. Out of 462 PCR-positive isolates, 83% (95% CI 69–94%) and 12% (95% CI 3–24%) were *Entamoeba dispar*, *Entamoeba histolytica*, respectively. In subgroup analysis based on molecular results, in general, population prevalence of *Entamoeba dispar* and *E. histolytica* were 91% (95% CI 80–99%) and 7%, (95% CI 0–19%), respectively, while prevalence of these species in patients with gastrointestinal disorders were 75% (95% CI 45–96%) and 18% (95% CI 1–43%), respectively. Our findings indicate the low burden of amoebiasis in Iran. *E. dispar*, that is mostly non-pathogenic, was identified as most prevalent species. Nevertheless, we suggest more public health interventions in areas with lower HDI.

Introduction

The genus Entamoeba is constituted by cosmopolitan parasites belonging to the phylum Amoebozoa with world distribution. This genus contains seven species: *Entamoeba histolytica*, *Entamoeba dispar*, *Entamoeba moshkovskii*, *Entamoeba coli*, *Entamoeba poleki*, *Entamoeba bangladeshi* and *Entamoeba hartmanni* [1, 2]. Among these, the first three (*E. histolytica*, *E. dispar* and *E. moshkovskii*) species are morphologically identical and considered as *Entamoeba* complex. Of those, only *E. histolytica* is the causative agent of amoebiasis, a global expanded gastrointestinal disease [3, 4]. Although, some recent studies suggested that *E. moshkovskii* could have potential pathogenic effect in human [5, 6], *E. dispar* is still considered commensal organism [7].

Amebic infection is most prevalent in developing countries located in tropical and subtropical zones and its prevalence is associated with climatic conditions, sanitary and socioeconomic status [8]. It is estimated that about 50 million people were affected by invasive amoebiasis, resulting in up to 100 000 deaths per annum [3]. In a comprehensive global burden of disease study 1990–2010, Murray *et al.* (2013) reported 32 persons per 100 000 (95% confidence interval 25–41) disability-adjusted life years for amoebiasis [9].

Due to different biochemical, genetic and pathogenic features of *Entamoeba* complex, the differentiation of three aforementioned species is avery important issue in the effective clinical management of patients. For example, an infection with non-pathogenic species could mistakenly be diagnosed as *E. histolytica* infection and patient be unnecessarily treated with metronidazole that is the drug of choice for invasive amoebiasis, but not effective for the non-invasive species [10, 11].

Iran is one of the largest developing countries in Middle-East area with highly diverse geography, climatic and sociodemographic conditions. According to the Statistical Centre of Iran, the number of its total population is 80.28 million and approximately one-third of the people

live below the national poverty line [12]. During the past years, several microscopy-based studies have investigated the prevalence of amoebiasis in different population groups, although discriminating studies between species (using molecular methods) are relatively few. According to the results of these studies, amoebiasis should be considered as a public health problem in Iran. Nevertheless, there is no comprehensive study showing the reliable status of amoebiasis at the national level. In this study, we performed a systematic review and meta-analysis to achieve an overview regarding the prevalence of amoebiasis and/or *Entamoeba* complex in Iranian people and also identify the different species circulating among of them.

Methods

Search strategy and study selection

This systematic review and meta-analysis study was implemented in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [13]. To assess the prevalence of *Entamoeba* complex in Iran, relevant studies were searched from five English language databases (PubMed, Web of Science, Science Direct, Scopus and Google Scholar) and four Persian language databases (Scientific Information Database, Iran-Medex, Iran Doc and MagIran) from 1 January 1995 to 30 September 2017. This study was performed using the following keywords: 'Iran', 'Islamic Republic of Iran', 'intestinal parasite' 'amoebiasis', '*Entamoeba*', '*Entamoeba histolytica*', '*Entamoeba dispar*', '*Entamoeba moshkovskii*', '*E. histolytica*', '*E. dispar*', '*E. moshkovskii*', '*Entamoeba* complex', 'epidemiology', 'frequency', 'prevalence' 'molecular epidemiology' and 'PCR' alone or combined together with 'OR' and/or 'AND'. Reference lists of retrieved articles were explored for additional studies. We restricted our search to human subjects. After duplicate removal, the initial title and abstract screening were performed by two independent researchers (A.R. and A.T.). Only peer-reviewed original observational studies reporting the prevalence of *Entamoeba* complex using stool examination were included. Serological studies, conference papers, reviews and letters or correspondences were excluded.

Data extraction and study quality assessment

A data extraction form in an Excel sheet was designed by three investigators, A.R., A.T. and A.H. Full-text review was performed for all the selected included papers by two independent researchers (A.R. and A.T.) and information were extracted and sorted for the following variables: the first author's last name, publication year, implementation year, name of study region, design of study, type of studied population, mean age or age range of studied population, total sample size, number of infected subjects, number of *E. histolytica* or *E. dispar* or *E. moshkovskii*, in molecular studies. In cases of disagreement in data extraction, the consensus was achieved through discussion with a third researcher (A.H.). In order to evaluate the quality assessment of included studies, we used the JBI (Joanna Briggs Institute) Prevalence Critical Appraisal Tool [14]. We divided included studies to five population sub-groups based on types of participants recruited: (1) general population, (2) children, (3) immunocompromised patients, (4) patients with gastrointestinal disorders and (5) mentally retarded patients. In this classification, transplanted individuals, HIV positive patients, individuals undergoing hemodialysis or chemotherapy and individuals taking immunosuppressive drugs were considered as immunocompromised patients. Moreover, to evaluate the impact of poverty on the prevalence of infection, studied provinces were divided into developed, relatively developed and undeveloped areas according to Human Development Index (HDI) [15].

Data synthesis and statistical analysis

For the meta-analysis, we applied a random effects model to calculate pooled prevalence estimates with 95% confidence intervals using *metaprop* command in Stata software. Freeman-Tukey double arcsine transformation and score confidence intervals were applied to calculate the pooled prevalence in raw cell counts, for the individual studies [16–18]. Heterogeneity between studies was assessed using the I^2 measure and the Cochran Q-statistic and an I^2 value above 75% indicates high heterogeneity [19]. To address the sources of heterogeneity we separately performed meta-regression and subgroup analyses. Meta-regression was used for some predictors such as geographical latitude/longitude of different provinces and implementation years of studies. In addition, subgroup analysis was used for (HDI and type of participants. Assessing publication bias in prevalence studies is not routine and logical, because the main aim in these studies was only the estimate of prevalence and these studies did not examine the association between exposure an outcome (i.e. odds ratio, relative risk, etc.). Therefore, the extent of reported prevalence has no effect on the publication and in these studies, there was no publication bias. In all statistical analyses, the significance level was considered as P value < 0.05 and meta-analysis was done by using STATA version 13 (STATA Corp., College Station, Texas).

Retrieving sequence and phylogenetic tree

To show a cladistic relationship between the populations of *Entamoeba* spp. a maximum likelihood haplotype tree was drawn by MEGA 5.05 software. The sequences generated at 18S small subunit ribosomal RNA (18S rRNA) gene of *E. histolytica* (Accession nos: KX528457-KX528462) and *E. moshkoskii* (Accession no: AB520687) were directly retrieved from the GenBank database for FASTA format. The topology of the constructed tree was supported by bootstrap values of higher than 60%. *Entamoeba bovis* was considered as an out-group branch (Accession no: FN666250).

Results

Study characteristics

Our systematic literature search yielded 945 studies, of which 862 had not eligibility to be included in the quantitative analysis based on inclusion and exclusion criteria. A flowchart illustrating of the study selection process is depicted in Figure 1. Finally, a total of 71 studies involving 330 937 Iranian people were included in the meta-analysis. Among these, 36 studies ($n = 237\,117$) were in general population, 17 ($n = 64\,471$) in patients with gastrointestinal disorders, eight ($n = 27\,398$) in children only, six studies ($n = 910$) in immunocompromised patients and four ($n = 1041$) in mentally retarded patients. The studies were performed in all geographical area of Iran. The majority of studies have cross-sectional design and few (some studies related with immunocompromised and mentally retarded patients) have a case-control design. Main

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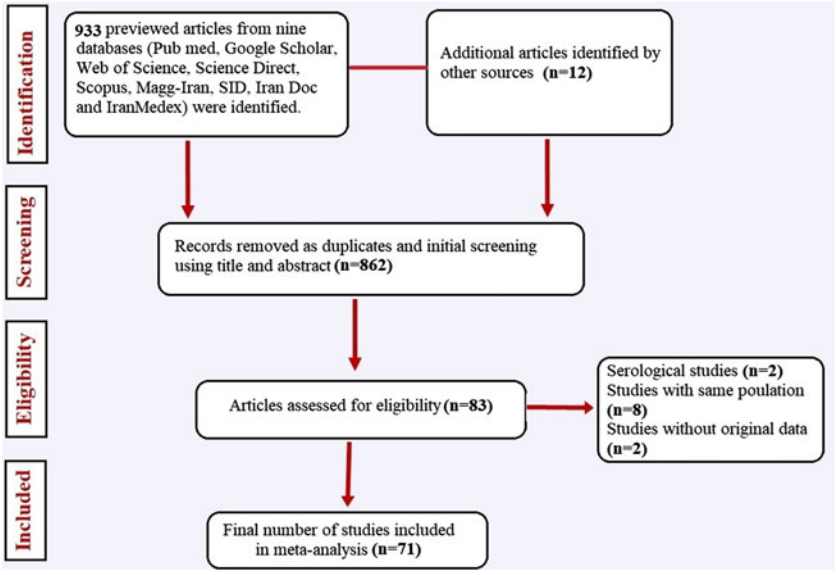

Fig. 1. Flow chart of the study selection process showing inclusion and exclusion of studies identified.

characteristics of the included studies have been embedded in Table 1. Among the included studies, 17 (containing 462 polymerase chain reaction (PCR)-positive isolates) performed molecular analysis on *Entamoeba* complex isolates for inter-species differentiation (Table 2).

Results of meta-analysis on prevalence using microscopic results

The pooled prevalence of *Entamoeba* infection among Iranian general population from 1995 to 2017 was 1% (95% CI 0.8–2.0%), however, there was significant heterogeneity in this meta-analysis ($I^2 = 98.65\%$, $P < 0.001$) (Fig. 2 and Supplementary Fig. S1). A similar prevalence was observed among patients with gastrointestinal disorders, immunocompromised patients and mentally retarded patients 1% (95% CI 0.8–2.0%). The slowly lower prevalence was observed among children 1% (95% CI 0.0–1.0%) (Fig. 2). As shown in Figure 3 and Supplementary Figure S2, meta-regression analysis on implementation year demonstrated that it is a crucial source of heterogeneity (tau2 deceased from 0.0002 to 0) and prevalence of *Entamoeba* infection reduced during the time (1995–2017) ($b = 0.009$ $P = 0.001$ $R^2 = 100\%$). Subgroup analysis regarding HDI demonstrated that prevalence of *Entamoeba* infection is higher in undeveloped provinces (2%, 95% CI 1–4%) compared with relatively developed (1%, 95% CI 1–3%) or developed provinces (1%, 95% CI 0–1%) (Supplementary Fig. S3). Meta-regression on the prevalence of *Entamoeba* infection in different provinces according to geographical latitude/longitude have not yielded any significant results (data not shown).

Results of meta-analysis on molecular epidemiology

Seventeen studies involving 44 272 human subjects and 703 *Entamoeba* complex isolates have performed molecular analysis for inter-species differentiation. Out of 703 *Entamoeba* complex isolates, 462 isolates were successfully amplified and sequenced. Among these, 396, 55 and 11 isolates were identified as *E. dispar E. histolytica* and *E. moshkovskii*, respectively (Table 2). Meta-analysis demonstrated that 83% (95% CI 69–94%; $I^2 = 87.8\%$) and 12% (95% CI 3–24%; $I^2 = 85.9\%$) of isolates were *E. dispar* and *E. histolytica*, respectively (Fig. 4a, b). In subgroup analysis based on molecular results, in general population prevalence of *E. dispar* and *E. histolytica* were 91% (95% CI 80–99%) and 7% (95% CI 0–19%), while prevalence of these species in patients with gastrointestinal disorders were 75% (95% CI 45–96%) and 18% (95% CI 1–43%), respectively (Fig. 4a, b and Table 2). Related heterogeneities are shown in Figure 4a and b. Due to a low number of *E. moshkovskii* (11 positive isolates out of only six published articles; two (0.7%) in general population and nine (6%) in patients with gastrointestinal disorders) the prevalence of this *Entamoeba* based on meta-analysis was not calculable (Table 2).

Sequence and phylogenetic analyses

The different clades of identified *Entamoeba* spp. is given in Figure 5 based on the 18S rRNA gene. Cladistic phylogenetic tree indicated the *E. dispar* clade has a sister relationship with *E. histolytica* clade in comparison with *E. moshkowskii*.

Discussion

It is critically important to understand the prevalence of amoebiasis and distributing and molecular epidemiology of *Entamoeba* complex in developing countries located at tropical and subtropical countries. This systematic review and meta-analysis study, based on approximately 331 000 human subjects, 462 PCR-positive *Entamoeba* complex isolates, resulting from 71 studies, covering 25 provinces in Iran, enables us to judge reliable the prevalence and molecular epidemiology of *Entamoeba* complex at the national level. Results of our study showed that, nationally, one percent of Iranian people were infected by *Entamoeba* complex. Prevalence reported here (1%) is much lower than those reported from Ghana (39.8%), South Africa (27%), Mexico (21%), Brazil (21%), India (19%), Malaysia (18.6%) and also lower than reports from Middle East countries, including Yemen (59%), United Arab Emirates (30%), Turkey (2.5%) and Lebanon (2.3%) [10, 91–99]. The lower prevalence of *Entamoeba* complex in Iran could be explained by prevailing of dry climate in most parts of Iran and also higher sanitary status, which had a significant improvement in the three last decades. Our results showed

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Table 1. Main characteristics of selected studies reporting the prevalence of *Entamoeba* complex in Iran

First author	Ref	Province	Study period	Study population	Sample size	Infected (%)[a]	Quality score
Vali *et al*. (1997)	[20]	Esfahan	1996	GP	2010	104 (5.2)	6
Rouhani *et al*. (2001)	[21]	Mazandaran	1999	GP	1246	62 (4.9)	5
Razavyoon and Massoud (2002)	[22]	Mazandaran	1999	GP	2568	110 (4.2)	5
Hooshyar *et al*. (2003)	[23]	Tehran & Alborz	2002–2003	GP	12 148	87 (0.7)	7
Asgari *et al*. (2003)	[24]	Tehran	2000–2001	GP	1535	91 (9.4)	6
Hooshyar .. (2004)	[25]	Iran	1999–2002	GP	16 592	226 (1.3)	8
Sayyari *et al*. (2005)	[26]	Iran	1999–2000	GP	45 128	439 (1)	8
Rezaian and Hooshyar (2006)	[27]	Khuzestan	2004–2005	GP	782	65 (8.3)	6
Davami *et al*. (2006)	[28]	Markazi	2002–2003	GP	460	6 (1.3)	5
Solaymani-Mohammadi *et al*. (2006)	[29]	Iran	2003–2005	GP	1037	88 (8.4)	7
Nazemalhoseini Mojarad *et al*. (2007)	[30]	Golestan	2006	GP	560	23 (4)	6
Ebadi *et al*. (2008)	[31]	Yazd	2002–2004	GP	13 388	9 (0.67)	6
Shojaei Arani *et al*. (2008)	[32]	Tehran	2004–2005	GP	466	5 (1.1)	6
Ghorbani *et al*. (2008)	[33]	Hormozgan	2007	GP	1002	22 (2.2)	5
Mowlavi *et al*. (2008)	[34]	Khuzestan	2005–2007	GP	1494	20 (1.3)	7
Nasiri *et al*. (2009)	[35]	Alborz	2006–2008	GP	13 915	3 (0.02)	7
Kuzehkanani *et al*. (2011)	[36]	Hormozgan	2009–2010	GP	565	33 (5.8)	6
Kheirandish *et al*. (2011)	[37]	Lorestan	2010	GP	816	3 (0.4)	5
Rahimi Esboei *et al*. (2013)	[38]	Mazandaran	2009–2010	GP	4223	13 (0.3)	5
Abedi *et al*. (2013)	[39]	Sistan & Baluchestan	2012	GP	210	1 (0.47)	5
Asmar *et al*. (2014)	[40]	Guilan	2010	GP	700	1 (0.1)	6
Fallah *et al*. (2014)	[41]	West-Azerbaijan	2011–2012	GP	721	31 (4.2)	6
Talebimeymand *et al*. (2016)	[42]	Ilam	2014	GP	1300	69 (5.3)	5
Sharif *et al*. (2015)	[43]	Mazandaran	2012	GP	1041	9 (0.8)	7
Balarak *et al*. (2014)	[44]	Qom	2013	GP	2925	5 (0.17)	7
Hemmati *et al*. (2017)	[45]	Tehran	2013–2014	GP	19 990	46 (0.2)	6
Rahimi *et al*. (2016)	[46]	Tehran	2010–2014	GP	70 978	111 (0.16)	7
Tork *et al*. (2016)	[47]	Mazandaran	2013	GP	880	4 (0.5)	5
Norouzi and Manochehri (2016)	[48]	Kurdistan	2014	GP	3000	2 (0.06)	5
Balarak *et al*. (2016)	[49]	East-Azarbaijan	2014	GP	4612	9 (0.19)	6
Sarkari *et al*. (2016)	[50]	Kohgiluyeh & Boyer Ahmad	NA	GP	1025	9 (0.87)	6
Halakou *et al*. (2016)	[51]	Golestan	2013	GP	2139	3 (0.14)	5
Mahni *et al*. (2016)	[52]	Kerman	2013–2014	GP	1060	10 (0.9)	6
Hemmati *et al*. (2017)	[53]	Tehran	2014	GP	561	3 (0.5)	6
Jafarian and Gorgani-Firouzjaee (2017)	[54]	Mazandaran	2015	GP	4478	120 (2.5)	4
Sub-total					235 555	1842 (0.78%)	
Hazrati tappeh *et al*. (2004)	[55]	Urmia	2000–2002	GID	1788	3 (0.1)	5
Rasti *et al*. (2006)	[56]	Tehran	2005	GID	450	5 (1.1)	5
Nazemalhosseini Mojarrad *et al*. (2008)	[57]	Tehran	2005–2006	GID	1700	27 (1.6)	7
Haghighi *et al*. (2009)	[58]		2003–2005	GP	1562	8 (0.51)	4

(*Continued*)

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Table 1. (*Continued.*)

First author	Ref	Province	Study period	Study population	Sample size	Infected (%)[a]	Quality score
		Sistan & Baluchestan					
Nazemalhosseini Mojarad *et al.* (2010)	[59]	Iran	2004–2008	GID	3825	58 (1.52)	7
Rostami Nejad *et al.* (2010)	[60]	Tehran	2009	GID	912	20 (2.2)	5
Kheirandish *et al.* (2011)	[61]	Lorestan	2010	GID	862	16 (1.8)	6
Pestehchian *et al.* (2011)	[62]	Chaharmahal & Bakhtiary	2009	GID	665	11 (1.7)	6
Vahedi *et al.* (2012)	[63]	Mazandaran	2009–2010	GID	962	1 (0.1)	6
Kooshar *et al.* (2013)	[64]	Golestan	2005–2011	GID-DP	1086	69 (6.4)	7
Sharbatkhori *et al.* (2014)	[65]	Golestan	NA	GID-DP	105	25 (23.8)	5
Ayatollahi *et al.* (2014)	[66]	Yazd	2011–2012	GID	33 096	34 (0.1)	6
Zebardast *et al.* (2015)	[67]	Iran	2012–2013	GID	1520	1 (0.06)	6
Kiani *et al.* (2016)	[68]	Hamadan	2014–2015	GID	1301	4 (0.3)	7
Mohammadzadeh *et al.* (2017)	[69]	Kurdistan	2014	GID	500	18 (3.6)	7
Saki *et al.* (2017)	[70]	Khuzestan	2010–2013	GID	13 698	96 (0.7)	6
Salehi *et al.* (2017)	[71]	Khuzestan	2014–2016	GID	618	2 (0.3)	6
Bahrami *et al.* (2017)	[72]	Kurdistan	2014–2016	GID	1383	14 (1)	5
Sub-total					66 033	412 (0.62%)	
Ghorbani *et al.* (1999)	[73]	Semnan	NA	Children	359	1 (0.3)	4
Ghahramanloo *et al.* (1999)	[74]	Mazandaran	1998	Children	3429	10 (0.3)	5
Heidari and Rokni (2003)	[75]	Semnan	2002	Children	461	11 (2.4)	4
Nematian *et al.* (2004)	[76]	Tehran	1998	Children	19 209	19 (0.1)	7
Rostami *et al.* (2012)	[77]	Golestan	2010	Children	800	8 (1)	6
Ghafari *et al.* (2015)	[78]	Khuzestan	2014	Children	300	3 (1)	5
Iranikhah *et al.* (2017)	[79]	Qom	2008–9	Children	2410	18 (0.7)	6
Momen Heravi *et al.* (2013)	[80]	Esfahan	2010–2011	Children	430	5 (1.2)	6
Sub-total					27 398	75 (0.27%)	
Athari *et al.* (1998)	[81]	Tehran	1998	ICP	358	2 (0.5)	5
Togeh *et al.* (2000)	[82]	Tehran	1999	ICP	261	2 (0.8)	5
Taherkhani *et al.* (2007)	[83]	Kermanshah	NA	ICP	75	1 (1.4)	4
Daryani *et al.* (2009)	[84]	Mazandaran	2007–2018	ICP	78	1 (1.6)	6
Yosefi *et al.* (2012)	[85]	Khuzestan	NA	ICP	60	1 (1.7)	6
Fallah Omrani *et al.* (2015)	[86]	East-Azarbaijan	2013–2014	ICP	78	2 (2.5)	6
Sub-total					910	9 (0.99%)	
Mahyar *et al.* (2000)	[87]	Ghazvin	NA	MR	258	1 (0.4)	4
Sharif *et al.* (2010)	[88]	Mazandaran	2008	MR	362	6 (1.7)	6
Hazrati Tappeh *et al.* (2010)	[89]	West-Azerbaijan	2010	MR	225	1 (0.4)	5
Soosaraie *et al.* (2014)	[90]	Golestan	2008	MR	196	3 (1.5)	4
Sub-total					1041	11 (1.1%)	
Total					330 937	2349 (0.71%)	

GP, general population; MR, mentally retarded patients; ICP, immunocompromised patients; GID, patients with gastrointestinal disorders; GID-DP, diarrheic patients with gastrointestinal disorders. Studies are listed in order of year published.
[a]The percentages presented in this table are crude.

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Table 2. Main characteristics of studies reporting molecular distinguish of *Entamoeba* complex

First author, Year	Ref	Sample size *N*	Positive microscopy *N*	Positive-PCR *N*	*Entamoeba histolytica* *N* (%)[a]	*Entamoeba dispar* *N* (%)[a]	*Entamoeba moshkovskii* *N* (%)[a]	Study population
Hooshyar *et al.* (2003)	[23]	12 148	87	48	2	46	0	GP
Hooshyar *et al.* (2004)	[25]	16 592	226	101	8	93	0	GP
Solaymani-Mohammadi *et al.* (2006)	[29]	1037	88	88	0	87	1	GP
Rezaian and Hooshyar (2006)	[27]	782	65	21	2	19	0	GP
Nazemalhoseini Mojarad *et al.* (2007)	[30]	560	23	23	7	16	0	GP
Kheirandish *et al.* (2011)	[61]	816	3	3	0	3	0	GP
Fallah *et al.* (2014)	[41]	724	31	25	8	17	0	GP
Hemmati *et al.* (2017)	[53]	561	3	3	0	2	1	GP
Sub-Total		33 220	526	312	27 (8.6%)	283 (90.7%)	2 (0.7%)	
Rasti *et al.* (2006)	[56]	450	5	4	0	4	0	GID
Nazemalhosseini Mojarrad *et al.* (2008)	[57]	1700	22	22	1	21	0	GID
Haghighi *et al.* (2009)	[58]	1562	8	6	0	6	0	GID
Nazemalhosseini Mojarad *et al.* (2010)	[59]	3825	58	57	2	53	2	GID
Kheirandish *et al.* (2011)	[61]	862	16	16	0	15	1	GID
Pestehchian *et al.* (2011)	[62]	665	11	11	10	1	0	GID
Sharbatkhori *et al.* (2014)	[65]	105	25	5	2	3	0	GID
Mohammadzadeh *et al.* (2017)	[69]	500	18	18	11	2	5	GID
Bahrami *et al.* (2017)	[72]	1383	14	11	2	8	1	GID
Sub-Total		11 052	177	150	28 (18.6%)	113 (75.4%)	9 (6%)	
TOTAL		44 272	703	462	55 (11.9%)	396 (85.7)	11 (2.4)	

[a]The percentages presented in this table are crude.

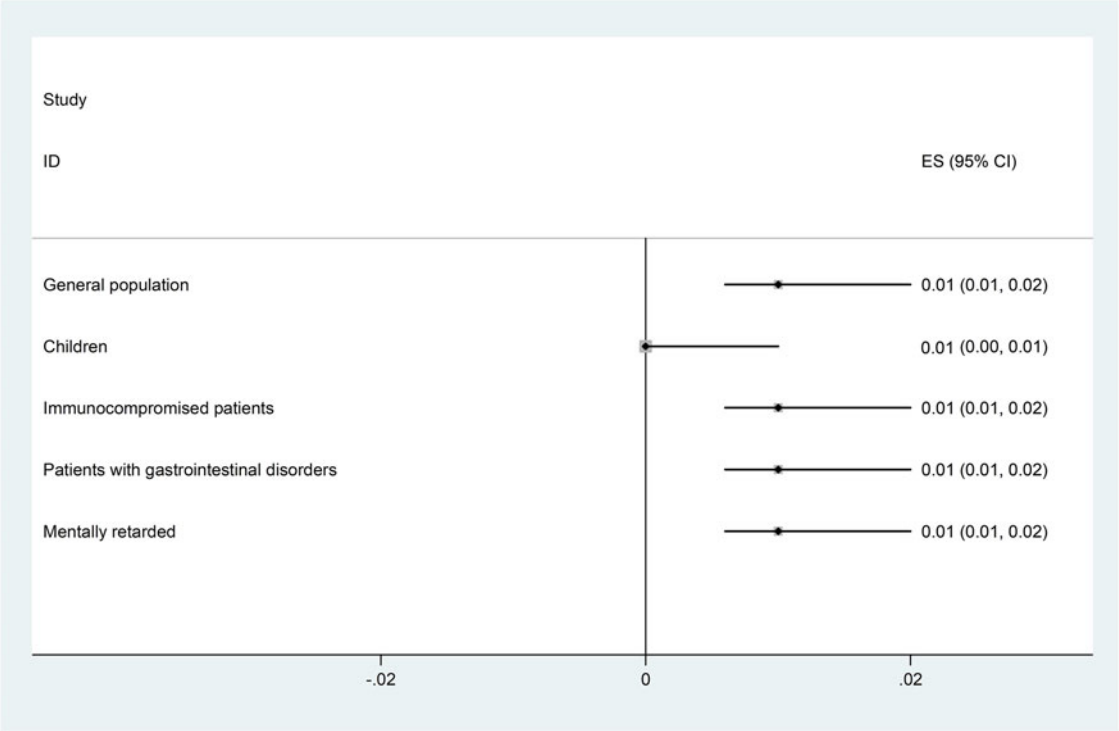

Fig. 2. Forest plot for random-effects meta-analysis on prevalence *Entamoeba* infection in different groups of Iranian population (using microscopic results).

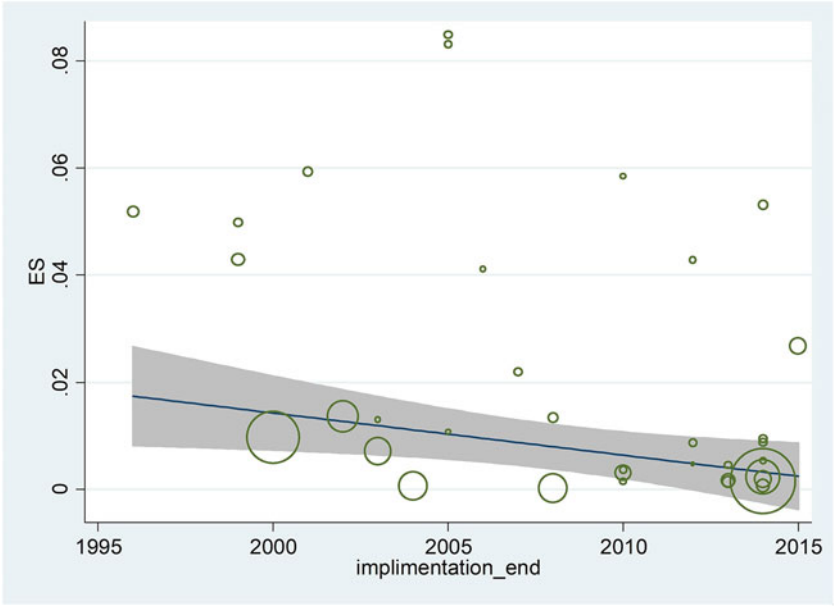

Fig. 3. Meta-regression regarding the effects of during time on the prevalence of *Entamoeba* infection in Iranian population.

a decrease in the prevalence of *Entamoeba* complex during the time, indicating that improvement of sanitary status and implementation of health educational programs in the three last decades in Iran was effective to reduce the intestinal parasites including *Entamoeba* complex. In this present study, we observed the similar prevalence of *Entamoeba* complex in different population groups. Although in contrast with our results, some previous studies demonstrated that *Entamoeba* infection is more prevalent in immunocompromised or mentally retarded patients and one of the major health problems in this individuals [100–104]. This could be explained by the fact that such individuals are suppressed in their immune responses and unable to provide adequate personal hygiene, poor environmental sanitations existing in mentally retarded institutions that is due to lack of toilet training and also direct person-to-person transmission of *Entamoeba* complex [88, 101]. Another result from the present study was a higher prevalence of *Entamoeba* infection in undeveloped provinces. In agreement with our results, it is well known that poverty, overcrowding, poor socioeconomic conditions, impoverished sanitation and hygiene conditions, as well as illiteracy and malnutrition, are

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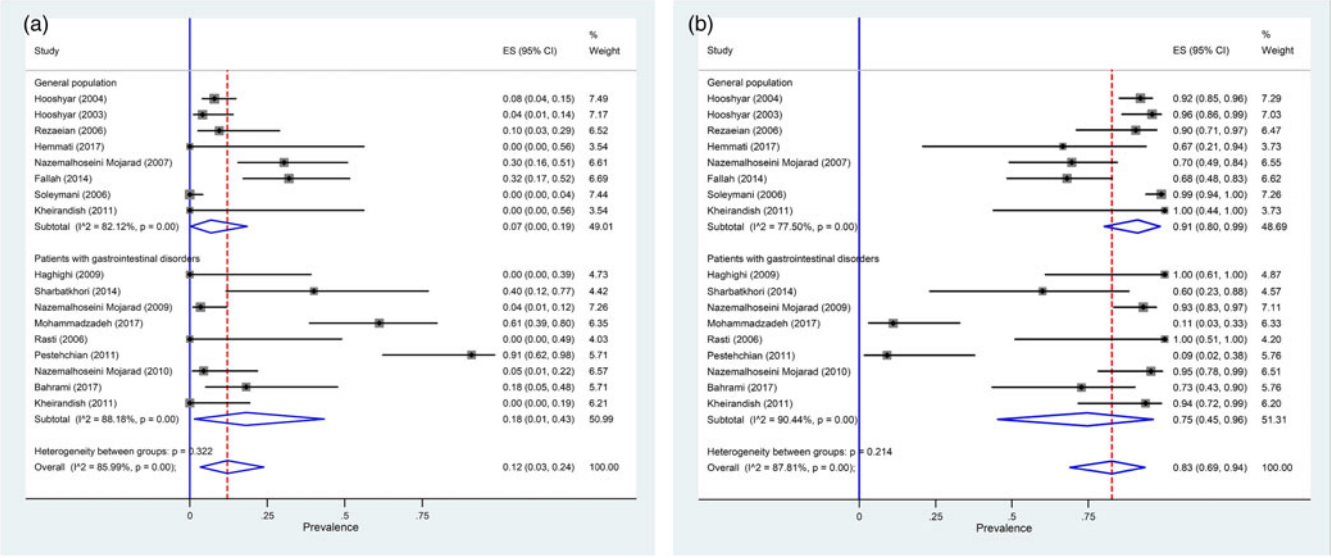

Fig. 4. Forest plot for random-effects meta-analysis on molecular prevalence *Entamoeba histolytica* (a) and *Entamoeba dispar* (b) among Iranian general population and patients with gastrointestinal disorders.

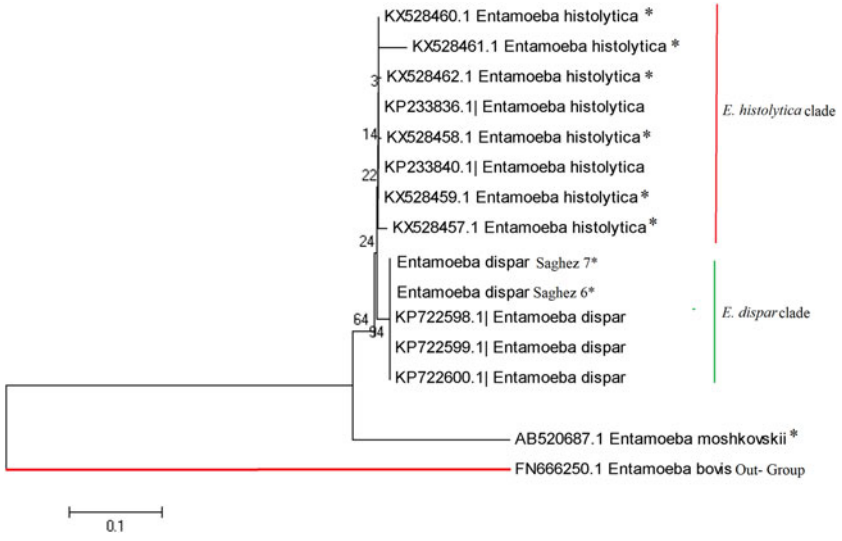

Fig. 5. Phylogenetic analysis of 5S nucleotide sequences of *Entamoeba* complex isolates recovered from different part of Iran.

main factors contributing to the high prevalence of *Entamoeba* infection [10, 105, 106].

Regarding the molecular epidemiology of *Entamoeba* complex in Iran, our result indicated that 396 (83%), 55 (12%) and 11 (2.4%) of the 480 PCR-positive isolates were *E. dispar*, *E. histolytica* and *E. moshkovskii*, respectively. In Yemen and United Arab Emirates, two southern neighbours of Iran, *E. histolytica*, *E. dispar* and *E. moshkovskii* were identified in 44.2%, 34.4% and 39.9% of the 276 PCR-positive products [10] and 13.3%, 6.7% and 3.3% of the 120 samples, respectively [92]. Results from these studies suggest that in southern neighbour countries of Iran, where hot-humid climate prevails, *E. histolytica* is more prevalent than *E. dispar*. While in Turkey, a northwestern neighbour of Iran, study by Kurt *et al.*, 2008 reported that *E. histolytica* and *E. dispar* were identified in 23.7% and 52.5% of the 59 PCR-positive products and in a study by Dagci *et al.*, 2007 all obtained isolates were *E. dispar*, suggesting that *E. dispar* is the predominant species in Turkey, where hot- or cold-dry climate prevails [93, 107]. In our study, *E. dispar* and *E. moshkovskii* have highest and lowest prevalence in Iranian people. Similar to our results, Anuar *et al.* [91] in Malaysia reported that *E. dispar* was the most prevalent species (13.4%), followed by *E. histolytica* (3.2%) and *E. moshkovskii* (1.0%). While in Australia and Colombia *E. moshkovskii* had a similar prevalence to *E. dispar* [108, 109]. The prevalence *E. histolytica*, *E. dispar* and *E. moshkovskii* were 5.6%, 70.8% and 61.8% in Australia and 0.55%, 23.2% and 25.4% in Colombia, respectively [108, 109]. Molecular results observed in the present study highlight higher prevalence of *E. histolytica* in patients with gastrointestinal disorders (18%) compared with general population (7%) and contrariwise pattern for *E. dispar* that had a higher prevalence in general population (91%) than patients with gastrointestinal disorders (75%). This result is corroborated by previous studies in different part of world, indicating that *E. dispar* is responsible for asymptomatic amoebic infection and higher *E. histolytica* burden is associated with diarrhoeal or gastrointestinal symptoms [91, 95, 110–112].

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An interesting result in our study is a higher prevalence of *E. moshkovskii* in patients with gastrointestinal disorders (6%) than the general population (0.7%). This result is in agreement with some recent studies suggesting that *E. moshkovskii* could have potential pathogenic effect in humans [5, 113, 114]. In line with these studies, Shimokawa *et al.* [6], demonstrated that *E. moshkovskii* is associated with diarrhoea in infants and causes diarrhea and colitis in mice.

The Cladistic phylogenetic tree disclosed that the *E. moshkowskii* has a greater genetic variability (a distinct branch with distance scale 20%) than *E. histolytica/E. dispar* clades (Fig. 5). Mohammadzadeh *et al.*[69] have recently identified a new mutant of *E. moshkovskii* in a dysentery fecal sample from Saghez city, Kurdistan province, Northwest Iran. This indicates that occurrence of single nucleotide polymorphism can potentially play a pivotal role on pathogenicity rate of *E. moshkovskii* in clinical isolates. However, more studies with a higher case number are required on ethnic population from different geographic regions of Iran, in order to verify this assumption.

The strengths of this study included the large number of included studies, very large and diverse baseline population, covering different provinces and geographical areas of Iran, rigorous methodology, presentation of pooled data to highlight differences within and between different population groups, determination of molecular epidemiology regarding to high number of *Entamoeba* isolates and genetic characterisation of *Entamoeba* species. Moreover, this study is likely limited by significant heterogeneity existing between studies, lacking data for some few provinces and also underestimation the true prevalence, due to the different proficiency of the experimenter in included studies.

In conclusion, despite these limitations, this systematic review and meta-analysis study provides a comprehensive overview of the prevalence and molecular epidemiology of amoebiasis in Iran. We have found that there is the low burden of *Entamoeba* complex infection (1%) in Iran, although lower developed areas were more influenced. Moreover, our results have shown that less- or non-phatogenic *Entamoeba* isolate (*E. dispar*) is the predominant specie in Iran. The decline in prevalence of *Entamoeba* complex observed across time is likely due to growing standards of living and improved hygiene status in Iran in last years. These data should be taken into consideration by the health authorities of the country and, given the very low incidence of amoebiasis, proper diagnosis and treatment of patients should be done correctly. We suggest additional investigations to further clarify the prevalence of amoebiasis in Iran based on both epidemiological and molecular studies, to guide the development of appropriate public health interventions.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0950268818001863

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References

1. **Fotedar R *et al.*** (2007) Laboratory diagnostic techniques for Entamoeba species. *Clinical Microbiology Reviews* **20**, 511–532.
2. **Royer TL *et al.*** (2012) *Entamoeba bangladeshi* nov. sp., Bangladesh. *Emerging Infectious Diseases* **18**, 1543.
3. **Haque R *et al.*** (2003) Amebiasis. *New England Journal of Medicine* **348**, 1565–1573.
4. **Ximénez C *et al.*** (2009) Reassessment of the epidemiology of amebiasis: state of the art. *Infection, Genetics and Evolution* **9**, 1023–1032.
5. **Fotedar R *et al.*** (2008) *Entamoeba moshkovskii* infections in Sydney, Australia. *European Journal of Clinical Microbiology & Infectious Diseases* **27**, 133–137.
6. **Shimokawa C *et al.*** (2012) *Entamoeba moshkovskii* is associated with diarrhea in infants and causes diarrhea and colitis in mice. *The Journal of Infectious Diseases* **206**, 744–751.
7. **Ali IKM, Clark CG and Petri WA** (2008) Molecular epidemiology of amebiasis. *Infection, Genetics and Evolution* **8**, 698–707.
8. **Nowak P, Mastalska K and Loster J** (2015) *Entamoeba histolytica*-pathogenic protozoan of the large intestine in humans. *Journal of Clinical Microbiology and Biochemical Technology* **1**, 010–017.
9. **Murray CJ *et al.*** (2013) Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *The Lancet* **380**, 2197–2223.
10. **Al-Areeqi MA *et al.*** (2017) First molecular epidemiology of *Entamoeba histolytica*, *E. dispar* and *E. moshkovskii* infections in Yemen: different species-specific associated risk factors. *Tropical Medicine & International Health* **22**, 493–504.
11. **Wolfe MS** (1973) Nondysenteric intestinal amebiasis: treatment with diloxanide furoate. *JAMA* **224**, 1601–1604.
12. **SCI. Statistical Centre of Iran** (2012) *Summary and Statistical Report of the 2012 Population and Housing Census*. Tehran: Population Census Commission Mazandaran Province: Population Census Commission Amol County.
13. **Moher D *et al.*** (2015) Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* **4**, 1.
14. **Munn Z *et al.*** (2014) The development of a critical appraisal tool for use in systematic reviews addressing questions of prevalence. *International Journal of Health Policy and Management* **3**, 123.
15. **Sabermahani A *et al.*** (2013) Provincial human development index, a guide for efficiency level analysis: the case of Iran. *Iranian Journal of Public Health* **42**, 149.
16. **Freeman MF and Tukey JW** (1950) Transformations related to the angular and the square root. *The Annals of Mathematical Statistics* **21**, 607–611.
17. **Harris R *et al.*** (2008) Metan: fixed-and random-effects meta-analysis. *Stata Journal* **8**, 3.
18. **Nyaga VN, Arbyn M and Aerts M** (2014) Metaprop: a Stata command to perform meta-analysis of binomial data. *Archives of Public Health* **72**, 39.
19. **Riahi SM and Mokhayeri Y** (2017) Methodological issues in a meta-analysis. *Current Medical Research and Opinion* **33**, 1813–1813.
20. **Vali GR *et al.*** (1997) Prevalence of parasitic infection among food distributors in Kashan, 1996 . *Feyz* **1**, 45–52.
21. **Rouhani S, Kianian H and Athari A** (2001) Prevalence of intestinal parasites in rural area of Sari, city 1999. *Journal of Zanjan University of Medical Sciences* **9**, 33–40.
22. **Razavyoon T and Massoud J** (2002) Intestinal parasitic infections in Fraydoon Kenar, Mazandaran. *Journal of School of Public Health and Institute of Public Health Research* **1**, 39–49.
23. **Hooshyar H, Rezaian M and Kazemi B** (2003) Distribution and differential diagnosis of *Entamoeba histolytica* from *Entamoeba dispar* by the PCR-RFLP method in Central Iran. *Annals of Saudi Medicine* **23**, 363–366.
24. **Asgari G, Nateghpour M and Rezaian M** (2003) Prevalence of intestinal parasites in the inhabitants of islam – shahr district. *Journal of School of Public Health and Institute of Public Health Research* **1**, 67–74.
25. **Hooshyar H *et al.*** (2004) The distribution of *Entamoeba histolytica* and *Entamoeba dispar* in northern, central, and southern Iran. *Parasitology Research* **94**, 96–100.

26. **Sayyari A *et al.*** (2005) Prevalence of intestinal parasitic infections in the Islamic Republic of Iran. *Eastern Mediterranean Health Journal* **11**, 377–383.
27. **Rezaian M and Hooshyar H** (2006) Differential diagnosis of *Entamoeba histolytica* from *Entamoeba dispar* and a study on the intestinal parasites in rural areas of Ahwaz and Hamidieh. *Journal of School of Public Health and Institute of Public Health Research* **4**, 33–38.
28. **Davami MH *et al.*** (2006) An investigation on the prevalence of intestinal Parasitic infections in food handlers in Arak. *Pars of Jahrom University of Medical Sciences* **3**, 8–15.
29. **Solaymani-Mohammadi S *et al.*** (2006) Comparison of a stool antigen detection kit and PCR for diagnosis of *Entamoeba histolytica* and *Entamoeba dispar* infections in asymptomatic cyst passers in Iran. *Journal of Clinical Microbiology* **44**, 2258–2261.
30. **Nazemalhoseini Mojarad E *et al.*** (2007) Prevalence of *Entamoeba histolytica* and *Entamoeba dispar* in Gonbad City, 2006, Iran. *Iranian Journal of Parasitology* **2**, 48–52.
31. **Ebadi M *et al.*** (2008) Parasitic infections (Helminth and Protozoa) in cases referring to Yazd Central Laboratory, 2002–2004. *The Journal of Shahid Sadoughi University of Medical Sciences* **15**, 53–58.
32. **Shojaei Arani A *et al.*** (2008) Prevalence of intestinal parasites in a population in south of Tehran, Iran. *Revista do Instituto de Medicina Tropical de São Paulo* **50**, 145–149.
33. **Ghorbani GhA, Izadi M and Esfahani AA** (2008) Association of drinking water and prevalence of intestinal parasites in military persons. *Journal of Military Medicine* **10**, 159–166.
34. **Mowlavi G *et al.*** (2008) Prevalence of intestinal parasites in tribal parts of Khuzestan province during 2005–07. *Govaresh* **12**, 219–228.
35. **Nasiri V *et al.*** (2009) Intestinal parasitic infections among inhabitants of Karaj City, Tehran province, Iran in 2006–2008. *The Korean Journal of Parasitology* **47**, 265.
36. **Kuzehkanani AB *et al.*** (2011) Enteric protozoan parasites in rural areas of Bandar-Abbas, southern Iran: comparison of past and present situation. *Iranian Journal of Public Health* **40**, 80.
37. **Kheirandish F *et al.*** (2011) Differential diagnosis of *Entamoeba* spp. in gastrointestinal disorder patients in Khorramabad, Iran. *African Journal of Microbiology Research* **5**, 2863–2866.
38. **Rahimi Esboei B *et al.*** (2013) Laboratories performance after outsourcing in the hospitals of Shahid Beheshti University of Medical Sciences. *Medical Laboratory Journal* **7**, 37–41.
39. **Abedi M *et al.*** (2013) Prevalence study of intestinal parasitic infections among Health Card applicants Zabol city in 2012. *Journal of Zabol University of Medical Sciences and Health Services* **5**, 53–59.
40. **Asmar M *et al.*** (2014) Prevalence of intestinal Parasitic infections in the Urban Areas of Bandar Anzali, Northern Iran. *Journal of Guilan University of Medical Sciences* **22**, 18–25.
41. **Fallah E *et al.*** (2014) Differential detection of *Entamoeba histolytica* from *Entamoeba dispar* by parasitological and nested multiplex polymerase chain reaction methods. *Journal of Analytical Research in Clinical Medicine* **2**, 27–32.
42. **Talebimeymand F, Abasian L and Rashnavadi M** (2016) Investigating the prevalence of intestinal parasites in Ilam City in 2014. *Scientific Journal of Ilam University of Medical Sciences* **24**, 1–7.
43. **Sharif M *et al.*** (2015) Prevalence of intestinal parasites among food handlers of Sari, Northern Iran. *Revista do Instituto de Medicina Tropical de São Paulo* **57**, 139–144.
44. **Balarak D, Jaffari Modrek M and Ansari H** (2014) Prevalence of intestinal parasites among the food handlers in the City of Qom, 2014. *Community Health Journal* **8**, 20–28.
45. **Hemmati A *et al.*** (2017) Molecular epidemiology and drug resistance study of *Entamoeba histolytica* in clinical isolates from Tehran, Iran. *Journal of Applied Biotechnology Reports* **3**, 513–517.
46. **Rahimi M *et al.*** (2016) The prevalence of intestinal parasites in the patients referred to the laboratories of Baqiyatallah hospital during 2010–2014. *Journal of Ardabil University of Medical Sciences* **15**, 414–422.
47. **Tork M *et al.*** (2016) Prevalence of intestinal parasitic infections and associated risk factors in West of Mazandaran province, Iran. *Journal of Mazandaran University of Medical Sciences* **25**, 81–88.
48. **Norouzi R and Manochehri A** (2016) Prevalence of intestinal parasites in refereed patients to Shahid Ghazi hospital of Sanandaj province in the year of 2014. *Journal of Jiroft University of Medical Sciences* **2**, 126–131.
49. **Balarak D *et al.*** (2016) Prevalence of intestinal parasitic infection among food handlers in Northwest Iran. *Journal of Parasitology Research* **2016**, 8461965.
50. **Sarkari B *et al.*** (2016) Prevalence and risk factors of intestinal protozoan infections: a population-based study in rural areas of Boyer-Ahmad district, Southwestern Iran. *BMC Infectious Diseases* **16**, 703.
51. **Halakou A *et al.*** (2016) Investigating the intestinal parasitic infections in Gonbad e Kavous in 2013. *Experimental Animal Biology* **4**, 75–81.
52. **Mahni MB *et al.*** (2016) Prevalence of intestinal parasitic infections in Jiroft, Kerman Province, Iran. *Iranian Journal of Parasitology* **11**, 232.
53. **Hemmati N *et al.*** (2017) Prevalence and risk factors of human intestinal parasites in Roudehen, Tehran province, Iran. *Iranian Journal of Parasitology* **12**, 364.
54. **Jafarian F and Gorgani-Firouzjaee T** (2017) Frequency of parasitic infections among patients referred to Ayatollah Rohani Hospital in Babol, Northern of Iran (2015). *Novin Health Journal* **1**, 34–40.
55. **Hazrati tappeh K *et al.*** (2004) A study on frequency the intestinal parasite infections in patients referring to Ghods Clinic of Urmia Medical Sciences University during 78–81. *Journal of Nursing and Midwifery Urmia University of Medical Sciences* **2**, 11–20.
56. **Rasti S, Haghighi A and Hatami M** (2006) Differential diagnosis of *Entamoeba histolytica* from *Entamoeba dispar* by PCR. *Feyz Journal of Kashan University of Medical Sciences* **10**, 30–34.
57. **Nazemalhosseini Mojarrad E *et al.*** (2008) Genetic diversity among *Entamoeba histolytica* and *Entamoeba dispar* strains in gastrointestinal disorder patients in Tehran, Iran. *Research in Medicine* **32**, 213–220.
58. **Haghighi A *et al.*** (2009) Frequency of enteric protozoan parasites among patients with gastrointestinal complaints in medical centers of Zahedan, Iran. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **103**, 452–454.
59. **Nazemalhosseini Mojarad E *et al.*** (2010) Discrimination of *Entamoeba moshkovskii* in patients with gastrointestinal disorders by single-round PCR. *Japanese Journal of Infectious Diseases* **63**, 136–138.
60. **Rostami Nejad M *et al.*** (2010) Prevalence of intestinal parasites in patients with gastrointestinal symptoms by focus on soil-transmitted helminthes infection. *Gastroenterology and Hepatology from Bed to Bench* **3**, 190–194.
61. **Kheirandish F *et al.*** (2011) Prevalence of intestinal parasites in bakery workers in Khorramabad, Lorestan Iran. *Iranian Journal of Parasitology* **6**, 76.
62. **Pestehchian N *et al.*** (2011) Frequency of *Entamoeba histolytica* and *Entamoeba dispar* prevalence among patients with gastrointestinal complaints in Chelgerd city, southwest of Iran. *Journal of Research in Medical Sciences* **16**, 1436.
63. **Vahedi M *et al.*** (2012) Prevalence of parasites in patients with gastroenteritis at East of Mazandaran province, Northern Iran. *Tropical Biomedicine* **29**, 568–574.
64. **Kooshar F *et al.*** (2013) Frequency of intestinal parasites in diarrhea patients in Gorgan, 2005–2011. *Medical Laboratory Journal* **4**, 54–60.
65. **Sharbatkhori M *et al.*** (2014) Discrimination of *Entamoeba* spp. in children with dysentery. *Gastroenterology and Hepatology from Bed to Bench* **7**, 164.
66. **Ayatollahi J *et al.*** (2014) Frequency of intestinal parasites in the patients referred to the central and Shahid Sadoughi Laboratories of Yazd (2011–2012). *Tolooebehdasht* **13**, 48–57.
67. **Zebardast N *et al.*** (2015) Frequency of intestinal parasites in patients with gastrointestinal disorders, in different parts of Iran during 2012–2013. *International Journal of Enteric Pathogens* **3**, e22682.
68. **Kiani H *et al.*** (2016) Prevalence, risk factors and symptoms associated to intestinal parasite infections among patients with gastrointestinal disorders in nahavand, western Iran. *Revista do Instituto de Medicina Tropical de São Paulo* **58**, 42.
69. **Mohammadzadeh A *et al.*** (2017) Gene migration for re-emerging amebiasis in Iran's northwest–Iraq borders: a microevolutionary scale for reflecting epidemiological drift of *Entamoeba histolytica* metapopulations. *Parasitology Research* **116**, 217–224.

70. **Saki J *et al.*** (2017) Prevalence of intestinal parasitic infections in Haftkel County, Southwest of Iran. *International Journal of Infection* **4**, e15593.
71. **Salehi R *et al.*** (2017) Prevalence and subtype identification of *Blastocystis* isolated from humans in Ahvaz, Southwestern Iran. *Gastroenterology and Hepatology from Bed to Bench* **10**, 235.
72. **Bahrami F *et al.*** (2017) Differential detection of Entamoeba histolytica, *Entamoeba dispar* and *Entamoeba moshkovskii* in fecal samples by a nested multiplex PCR in the west of Iran. *Shahid Beheshti University of Medical Science* **2017**, 25–33.
73. **Ghorbani R, Pazooki R and Ahmadian AR** (1999) The prevalence of intestinal parasites and relative factors in children below 2 years old in the urban areas of Semnan in 1996–97. *Journal of Gorgan University of Medical Sciences* **1**, 39–45.
74. **Ghahramanloo M, Hassanjani Roshan M and Haji Ahmadi M** (1999) Prevalence of intestinal parasites in primary school children, Eastern Bandpay, Babol ,1999. *Journal of Babol University of Medical Science* **3**, 47–51.
75. **Heidari A and Rokni M** (2003) Prevalence of intestinal parasites among children in day-care centers in Damghan-Iran. *Iranian Journal of Public Health* **32**, 31–34.
76. **Nematian J *et al.*** (2004) Prevalence of intestinal parasitic infections and their relation with socio-economic factors and hygienic habits in Tehran primary school students. *Acta Tropica* **92**, 179–186.
77. **Rostami M *et al.*** (2012) The prevalence of intestinal parasitic infections in primary school students in Gorgan, Iran. *Medical Laboratory Journal* **6**, 42–46.
78. **Ghafari R, Rafiei A and Tavalla M** (2015) Prevalence of intestinal parasites among children referred to Abozar Hospital in Ahvaz. *Jundishapur Scientific Medical Journal* **13**, 627–633.
79. **Irankhah A, Aghaali M and Damanpak Moghaddam V** (2017) Prevalence of intestinal parasitic diseases in the students of Qom City and hygiene status of their schools, Iran. *QOM University of Medical Sciences Journal* **10**: 61–70.
80. **Momen Heravi M *et al.*** (2013) Prevalence of intestinal parasites infections among Afghan children of primary and junior high schools residing Kashan city, Iran, 2009–2010. *Iranian Journal of Medical Microbiology* **7**, 46–52.
81. **Athari A, Sadafi H and Touke G** (2000) Prevalence of intestinal parasites among patients consumed of immunosuppressive drugs in Tehran, Iran. *Journal of Zanjan University of Medical Sciences* **8**, 61–68.
82. **Togeh GR *et al.*** (2000) Parasitic infestation in cancer patients chemotherapy. *Tehran University Medical Journal* **58**, 52–58.
83. **Taherkhani H *et al.*** (2007) The frequency of intestinal parasites in HIV positive patients admitted to the disease consultation center in Kermanshah province. *Medical Laboratory Journal* **1**, 41–44.
84. **Daryani A *et al.*** (2009) Prevalence of intestinal parasites and profile of CD4+ counts in HIV+/AIDS people in north of Iran, 2007–2008. *Pakistan Journal of Biological Sciences: PJBS* **12**, 1277–1281.
85. **Yosefi F *et al.*** (2012) A study on prevalence of gastrointestinal parasitic infections in HIV (+) patients referred to Ahvaz Razi Hospital in 2008–2009. *Jundishapur Journal of Microbiology* **5**, 424–426.
86. **Fallah Omrani V *et al.*** (2015) Prevalence of intestinal parasite infections and associated clinical symptoms among patients with end-stage renal disease undergoing hemodialysis. *Infection* **43**, 537–544.
87. **Mahyar A *et al.*** (2000) Intestinal parasitic in mentally retarded children of Qazvin. *The Journal of Qazvin University of Medical Sciences* **4**, 64–70.
88. **Sharif M *et al.*** (2010) Intestinal parasitic infections Among intellectual disability children in rehabilitation centers of northern Iran. *Research in Developmental Disabilities* **31**, 924–928.
89. **Hazrati Tappeh K *et al.*** (2010) Prevalence of intestinal parasitic infections among mentally disabled children and adults of Urmia, Iran. *Iranian Journal of Parasitology* **5**, 60.
90. **Soosaraie M, Pagheh A and Gholami S** (2014) Prevalence of intestinal parasitic infections in rehabilitation centers in Golestan province, Iran. *Medical Laboratory Journal* **8**, 42–47.
91. **Anuar TS *et al.*** (2012) Molecular epidemiology of amoebiasis in Malaysia: highlighting the different risk factors of *Entamoeba histolytica* and *Entamoeba dispar* infections among Orang Asli communities. *International Journal for Parasitology* **42**, 1165–1175.
92. **ElBakri A *et al.*** (2013) Differential detection of *Entamoeba histolytica, Entamoeba dispar* and *Entamoeba moshkovskii* in fecal samples by nested PCR in the United Arab Emirates (UAE). *Acta Parasitologica* **58**, 185–190.
93. **Kurt O *et al.*** (2008) Investigation of the prevalence of amoebiasis in Izmir province and determination of *Entamoeba* spp. using PCR and enzyme immunoassay. *The New Microbiologica* **31**, 393.
94. **Nath J *et al.*** (2015) Molecular epidemiology of amoebiasis: a cross-sectional study among North East Indian population. *PLoS Neglected Tropical Diseases* **9**, e0004225.
95. **Ngobeni R *et al.*** (2017) Entamoeba in South Africa: correlations with the host microbiome, parasite burden and first description of *E. bangladeshi* outside of Asia. *The Journal of Infectious Diseases* **19**, 1592–1600.
96. **Ramos F *et al.*** (2005) *Entamoeba histolytica* and *Entamoeba dispar*: prevalence infection in a rural Mexican community. *Experimental Parasitology* **110**, 327–330.
97. **Saab BR *et al.*** (2004) Intestinal parasites among presumably healthy individuals in Lebanon. *Saudi Medical Journal* **25**, 34–37.
98. **Santos HLC *et al.*** (2007) Comparison of multiplex-PCR and antigen detection for differential diagnosis of *Entamoeba histolytica*. *Brazilian Journal of Infectious Diseases* **11**, 365–370.
99. **Verweij JJ *et al.*** (2003) Prevalence of *Entamoeba histolytica* and *Entamoeba dispar* in northern Ghana. *Tropical Medicine & International Health* **8**, 1153–1156.
100. **Hung C-C *et al.*** (2008) Increased risk for *Entamoeba histolytica* infection and invasive amebiasis in HIV seropositive men who have sex with men in Taiwan. *PLoS Neglected Tropical Diseases* **2**, e175.
101. **Moran P *et al.*** (2005) *Entamoeba histolytica* and/or *Entamoeba dispar*: infection frequency in HIV+/AIDS patients in Mexico City. *Experimental Parasitology* **110**, 331–334.
102. **Ramakrishnan K *et al.*** (2007) Prevalence of intestinal parasitic infestation in HIV/AIDS patients with diarrhea in Madurai City, South India. *Japanese Journal of Infectious Disease* **60**, 209–210.
103. **Rivera WL, Santos SR and Kanbara H** (2006) Prevalence and genetic diversity of *Entamoeba histolytica* in an institution for the mentally retarded in the Philippines. *Parasitology Research* **98**, 106–110.
104. **Tachibana H *et al.*** (2000) Asymptomatic cyst passers of *Entamoeba histolytica* but not *Entamoeba dispar* in institutions for the mentally retarded in Japan. *Parasitology International* **49**, 31–35.
105. **Lim Y *et al.*** (2009) Intestinal parasitic infections amongst Orang Asli (indigenous) in Malaysia: has socioeconomic development alleviated the problem? *Tropical Biomedicine* **26**, 110–122.
106. **Ngui R *et al.*** (2011) Prevalence and risk factors of intestinal parasitism in rural and remote West Malaysia. *PLoS Neglected Tropical Diseases* **5**, e974.
107. **Dagci H *et al.*** (2007) Differentiation of *Entamoeba histolytica* and *Entamoeba dispar* by PCR: a preliminary study in Izmir, Turkey. *The New Microbiologica* **30**, 45.
108. **Fotedar R *et al.*** (2007) PCR detection of *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* in stool samples from Sydney, Australia. *Journal of Clinical Microbiology* **45**, 1035–1037.
109. **López MC *et al.*** (2015) Molecular epidemiology of *Entamoeba*: first description of *Entamoeba moshkovskii* in a rural area from Central Colombia. *PLoS ONE* **10**, e0140302.
110. **Gilchrist CA *et al.*** (2016) Role of the gut microbiota of children in diarrhea due to the protozoan parasite *Entamoeba histolytica*. *The Journal of Infectious Diseases* **213**, 1579–1585.
111. **Haghighi A *et al.*** (2003) Geographic diversity among genotypes of *Entamoeba histolytica* field isolates. *Journal of Clinical Microbiology* **41**, 3748–3756.
112. **Taniuchi M *et al.*** (2013) Etiology of diarrhea in Bangladeshi infants in the first year of life analyzed using molecular methods. *The Journal of Infectious Diseases* **208**, 1794–1802.
113. **Ali IKM *et al.*** (2003) *Entamoeba moshkovskii* infections in children in Bangladesh. *Emerging Infectious Diseases* **9**, 580.
114. **Yakoob J *et al.*** (2012) *Entamoeba* species associated with chronic diarrhoea in Pakistan. *Epidemiology & Infection* **140**, 323–328.