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REVIEW

Human cryptosporidiosis in Iran: a systematic review and meta-analysis

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Abstract Cryptosporidiosis caused by *Cryptosporidium* spp. is an important parasitic disease that can be life-threatening for children and immunocompromised patients. This systematic review and meta-analysis was designed to determine the prevalence rate of *Cryptosporidium* infection and related risk factors among the Iranian general population. We searched electronic databases including Google Scholar, PubMed, Science Direct, Scopus and Proquest for articles in English and SID, Magiran, IranMedex, and IranDoc for articles in Persian. Out of 4816 studies identified in the electronic search, 94 articles were eligible for inclusion in the systematic review and meta-analysis. The prevalence rate of cryptosporidiosis by using the random effect model among children, healthy people, and gastroenteritis and immunocompromised patients in Iran was estimated as 3.65, 2.94, 1.29, and 4.54%, respectively. Findings of a phylogenetic analysis inferred by gp60 and 18S ribosomal RNA markers indicated that most of the infection rate belonged to *C. parvum* (particularly subtype IIaA15G2R1) and *C. hominis* among understudied groups. The present study is the first systematic review and meta-analysis providing a comprehensive view of the prevalence of human cryptosporidiosis and its related risk factors in Iran. It seems that the awareness of *Cryptosporidium* prevalence, risk factors, and disease complications may be required for developing effective strategies to prevent infection.

Keywords *Cryptosporidium* · Cryptosporidiosis · Human · Prevalence · Iran

Introduction

Cryptosporidiosis is caused by an obligate intracellular parasite, which was first known as an opportunistic pathogen in 1907 (Tyzzer 1907). *Cryptosporidium* infection raises public health concerns in both developed and developing countries. On a global scale, its prevalence seems to be focused on the USA, Canada, Australia, Europe, particularly the UK, Germany, and Ireland (Cacciò et al. 2005; Gallas-Lindemann et al. 2013; Harp 2003; Putignani and Menichella 2010). In 1976, cryptosporidiosis first reported in a rural child and an immunocompromised man (Meisel et al. 1976; Nime et al. 1976). Now, it has been reported in over 90 countries from all continents (Fayer et al. 2000). There are various methods for transmission of this parasite including person to person, animal to animal, animal to human, waterborne, food-borne, air-borne, and sexual transmission (Fayer 2010; Fayer et al. 2000; Karanis et al. 2007; Tzipori and Ward 2002). Initial infection naturally occurs by ingestion of food or

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water contaminated with oocysts. Consequently, *Cryptosporidium* is identified as a main cause of food-borne and water-borne outbreaks (Chalmers and Davies 2010; Karanis 2006; Putignani and Menichella 2010). This protozoan parasite has several species that infect different hosts, but some of them are zoonotic (Xiao 2010). Cryptosporidiosis mainly occurs in people at risk including children, malnourished persons, elderly people, and a vast range of immunocompromised patients such as those suffering from AIDS and malignancies as well as transplant recipients (Aldeyarbi et al. 2016; Fayer et al. 2000; Shirley et al. 2012). This infection usually causes self-limiting diarrhoea in healthy people, although it could be life-threatening with a serious gastroenteritis-like syndrome in children (under 2 years of age), elderly people, and immunocompromised patients (Plutzer and Karanis 2009; Rossle and Latif 2013; Skotarczak 2010). This parasite is the main cause of acute gastroenteritis and abdominal pain with a duration of several days to weeks (Chalmers et al. 2011; Hunter and Nichols 2002; Insulander et al. 2005). Non-gastrointestinal symptoms including cholecystitis, hepatitis, and respiratory diseases also occur in immunocompromised patients (Hunter and Nichols 2002; Shirley et al. 2012). *Cryptosporidium* infection causes more economic losses to animal husbandry and livestock production. In addition, contact with animals seems to be a significant source of the infection, mainly in rural areas (Ghenghesh et al. 2012; Mahami Oskouei et al. 2014; Snelling et al. 2007). Several methods are available for laboratory diagnosis of cryptosporidiosis; they include staining and serological techniques such as the complement fixation test (CF), indirect haemagglutination test (IHA), indirect immunofluorescence assay (IFA), and the enzyme-linked immunosorbent assay (ELISA). It should be noted that recently advanced methods, such as polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), and western blot have also been used (Fayer et al. 2000; Mahmoudi et al. 2013; Skotarczak 2010; Tavares et al. 2011). Given the importance of cryptosporidiosis among human population, a summary and an analysis of the information on infection rates in a region can be helpful to understand its epidemiological aspects. In the present systematic review, we have studied papers on *Cryptosporidium* infection to more accurately estimate the prevalence rate of human cryptosporidiosis in Iran.

Materials and methods

Search strategy

We searched electronic databases including Google Scholar, PubMed, Science Direct, Scopus and Proquest for articles in English and SID, Magiran, IranMedex, and IranDoc for articles in Persian. Both English and Persian language articles were included in this study. After searching databases, another round of manual searching was conducted. The selection was made from articles written from 1990 to 2015. Our search strategy applied the following key words: cryptosporidiosis, *Cryptosporidium*, *Cryptosporidium* spp., *Cryptosporidium parvum*, *Cryptosporidium hominis*, *C. parvum*, *C. hominis*, Iran, Islamic Republic of Iran, human, cancer, transplant recipient, HIV, AIDS, immunocompromised patients, healthy people, gastroenteritis patients, intestinal parasite infections, epidemiology, and prevalence. We also used the proposed synonymous terms for our search.

Study selection

Inclusion criteria: publication of articles in 1990 to 2015, descriptive, cross-sectional, case-control, and epidemiology studies and articles published in English and Persian. We chose those studies that described the total prevalence rates for *Cryptosporidium* and cryptosporidiosis.

Exclusion criteria: articles with had different diagnostic methods, unavailable full text, and written in a language other than English or Persian. Congress articles that were not published in valuable journals were also excluded.

All searched studies from the databases were considered for suitability by three different authors. Disagreements were resolved through discussion and consensus.

Data extraction and analysis

After precise extraction of information, the extracted results were classified in a table constituted of province, year of publication, participation, gender of positive cases (male/female), diagnostic methods (serology/PCR/staining), and age. Actual estimates of prevalence were evaluated with 95% confidence intervals (CI). Entire prevalence and group-specific prevalence were considered with the help of age groups (<15, 16–30, >30 years), gender (male/female), and geographical region. A forest plot was used to indicate the heterogeneity among the studies. The statistical methods I^2 and Cochran's Q tests (P value < 0.05) were used to quantity the differences. The meta-analysis was done by using the trial version of StatsDirect statistical software and the random effects model with the assumption that the included studies were a random sample from a population of studies. In order to illustrate the taxonomic status of *Cryptosporidium* spp., sequences of glycoprotein 60 (gp60) and 18S ribosomal RNA (rRNA) markers of Iran were directly retrieved from the GenBank database (FASTA format). MEGA 5.05 software based on the maximum likelihood algorithm with the Kimura 2-parameter model was used to construct the phylogenetic tree. The accuracy of the phylogenetic tree was evaluated by 1000 bootstrap resamplings.

Results

Among the 4816 studies identified in the electronic search, 94 articles were eligible for inclusion in the systematic review and meta-analysis. A flowchart shows the study design process (Fig. 1). Table 1 shows the results of the literature search. A wide variation was observed in the prevalence estimates among the various studies, and the Q statistic was (Q statistic = 465.496, df = 37, $P < 0.0001$; $I^2 = 92.1\%$), (Q statistic = 414.990, df = 23, $P < 0.0001$; $I^2 = 94.5\%$), (Q statistic = 206.468, df = 13, $P < 0.0001$; $I^2 = 93.7\%$), and (Q statistic = 215.106, df = 23, $P < 0.0001$; $I^2 = 89.3\%$) in children, healthy people, and gastroenteritis and immunocompromised patients, respectively. The prevalence rate of cryptosporidiosis by using the random effect model among children, healthy people, and gastroenteritis and immunocompromised patients in Iran over the 24-year period was estimated to be 3.65% (95% CI = 2.72–4.71%), 2.94% (95% CI = 1.45–4.93%), 1.29% (95% CI = 0.58–2.29%), and 4.54% (95% CI = 2.89–6.53%), respectively. The forest plot diagrams of the current study are shown in Figs. 2, 3, 4, and 5. In this study, we could not estimate the overall prevalence rate in the other groups because there were not enough related articles to analyse and it has not been widely studied in Iran. Among the studies, three different diagnostic methods were utilized to evaluate *Cryptosporidium* infection in general population. They were staining (mZN and auramine phenol), serology (ELISA, IFA, and direct immunofluorescence), and PCR. The most commonly used diagnostic methods for cryptosporidiosis in the general population of Iran were mZN (89 studies), followed by PCR (17 studies), and serology (nine studies). Results of the meta-analysis showed a significant difference between groups of stool appearance ($P < 0.001$) and also season ($P = 0.001$). The prevalence of *Cryptosporidium* infection was significantly higher in autumn and patients with diarrhoea (Table 2). Results of the heterogeneity of the meta-analysis for other factors (gender, age, residency, and contact with animals) revealed that they were homogeneous ($P > 0.05$). The prevalence rate of cryptosporidiosis in the general population of several provinces of Iran is shown in Fig. 6. The prevalence range of human cryptosporidiosis in various regions of Iran was between 0.83 and 24% in Guilan and Yazd provinces, respectively. The phylogenetic analysis inferred by gp60 and 18S rRNA markers indicated that the majority infection rate belonged to *C. parvum* (especially subtype IIaA15G2R1) and *C. hominis* among understudied groups (Fig. 7).

Discussion

Cryptosporidium is one of the important causes of diarrhoea occurring mostly in developing countries (El Kader et al. 2012; Leav et al. 2003; Shirley et al. 2012). Different epidemiological studies on the prevalence of cryptosporidiosis are available nowadays. The present systematic review and meta-analysis is the most comprehensive and first estimate of human cryptosporidiosis in Iran. On the other hand, previous studies done on this subject were more limited to specific groups and restricted areas. These data can be used to evaluate prevalence of cryptosporidium in various parts of Iran and target groups. This study is designed by using nine databases and 94 records published between 1991 and 2015. The prevalence of human cryptosporidiosis varies in different population groups of Iran. However, the rate of infection is higher among immunocompromised patients (95% CI = 2–6%).

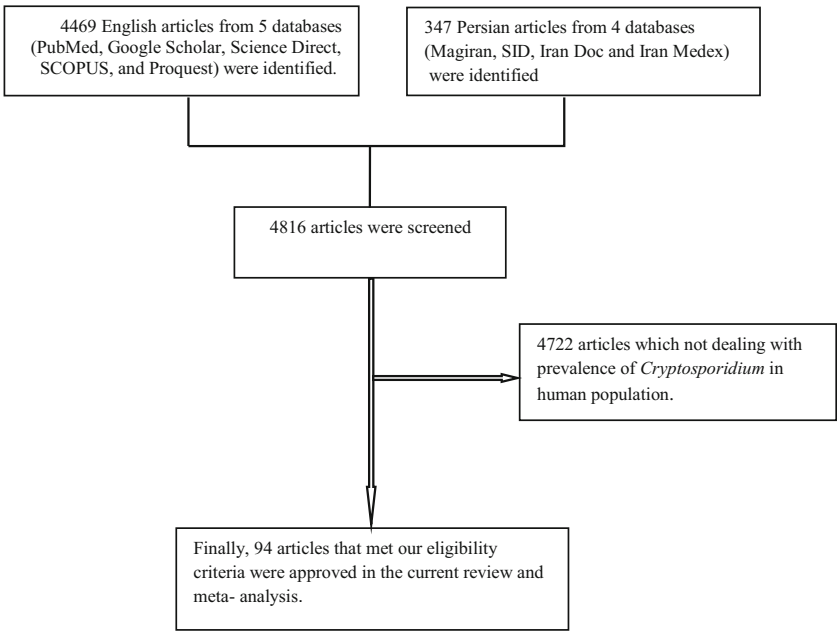

Fig. 1 Flowchart describing the study design process

Table 1 Baseline characteristics of included studies

Province	Year	Participation			Positive cases			Method			Age	Ref
		All	Male	Female	All (%)	Male (%)	Female (%)	Serology	PCR	Staining		
Healthy people												
West Azerbaijan	1991	115	85	30	0 (0)	0 (0)	0 (0)	–	–	0	NA	(Nouri et al. 1991)
Tehran	2000	322	165	157	1 (0.31)	NA	NA	–	–	1	20– > 51 years	(Athari et al. 2000)
Tehran	2001	340	NA	NA	3 (0.88)	NA	NA	–	–	3	NA	(Shekarabi et al. 2001)
Tehran	2002	23	NA	NA	1 (4.34)	NA	NA	–	–	1	NA	(Mokhber Dezfouli and Meshgi 2002)
Tehran	2005	250	92	158	1 (0.4)	NA	NA	–	–	1	NA	(Akhlaghi et al. 2005)
Isfahan	2006	140	60	80	5 (3.57)	1 (1.6)	4 (5)	–	–	5	NA	(Seyrafian et al. 2006)
Isfahan	2006	91	35	56	4 (4.39)	0 (0)	4 (7.14)	–	–	4	NA	(Seyrafian et al. 2006)
Fars	2007	400	NA	NA	43 (10.75)	NA	NA	–	–	43	0– > 51 years	(Mirzaei 2007)
Isfahan	2008	349	NA	NA	19 (5.44)	12	7	–	–	19	NA	(Azami and Dorostkar Moghadam 2008)
East Azerbaijan	2008	86	NA	NA	5 (5.81)	NA	NA	–	–	5	1–52 years	(Hassanpour 2008)
Tehran	2008	105	NA	NA	0 (0)	0 (0)	0 (0)	–	–	0	11–79 years, (Mean 40)	(Jahani et al. 2008)
Mazandaran	2011	105	NA	NA	0 (0)	0 (0)	0 (0)	–	–	0	11– > 21 years	(Nahrevanian et al. 2011)
Hamadan	2012	300	NA	NA	16 (5.33)	NA	NA	–	–	16	NA	(Heidari and Gharakhani 2012)
Khuzestan	2012	62	NA	NA	9 (14.51)	NA	NA	–	–	9	0– > 10 years	(Heidarnegadi et al. 2012)
Isfahan	2012	250	NA	NA	0 (0)	NA	NA	–	–	0	0– > 50 years	(Azizi et al. 2012)
Yazd	2012	100	NA	NA	24 (24)	NA	NA	–	–	24	NA	(Sazmand et al. 2012)
East Azerbaijan	2012	1825	NA	NA	3 (0.16)	NA	NA	–	2	3	NA	(Shahbazi et al. 2012)
Hamadan	2013	228	NA	NA	2 (0.87)	NA	NA	–	–	2	NA	(Jafari et al. 2013)
Chaharmahal and Bakhtiari	2013	65	47	18	4 (6.15)	NA	NA	–	–	4	17–60 years	(Khalili et al. 2013)
Isfahan	2014	422	NA	NA	63 (14.92)	NA	NA	–	36	63	0– > 5 years	(Izadi et al. 2014)
Isfahan	2015	100	20	80	0 (0)	0 (0)	0 (0)	–	–	0	NA	(Mohtashamipour et al. 2015)
Hamadan	2015	371	189	182	3 (0.80)	NA	NA	–	–	3	15– > 60 years	(Haghighi et al. 2015)
Hamadan	2015	228	135	93	3 (1.31)	3 (2.22)	0 (0)	8 (ELISA)	–	3	1–79 years	(Jafari et al. 2015)
Mazandaran	2015	1041	620	421	5 (0.48)	NA	NA	–	–	5	18–63 years	(Sharif et al. 2015)
Gastroenteritis												
West Azerbaijan	1991	248	NA	NA	19 (7.66)	7	12	–	–	19	NA	(Nouri et al. 1991)
Mazandaran	2004	100	58	42	6 (6)	4 (6.89)	2 (4.76)	–	–	6	0–14 years	(Sharif et al. 2004)
Tehran	2007	104	NA	NA	3 (2.88)	NA	NA	–	–	3	NA	(Nahrevanian et al. 2007)
Mazandaran	2008	802	456	346	1 (0.12)	NA	NA	–	–	1	NA	(Ghorban nia delavar et al. 2008)
Guilan	2009	617	350	267	7 (1.13)	NA	NA	–	–	7	0– > 30 years	(Vahabzadeh et al. 2009)

Table 1 (continued)

Province	Year	Participation			Positive cases			Method			Age	Ref
		All	Male	Female	All (%)	Male (%)	Female (%)	Serology	PCR	Staining		
Tehran	2010	867	NA	NA	24 (2.76)	NA	NA	–	–	24	NA	(Pirestani et al. 2010)
Tehran	2011	850	NA	NA	29 (3.41)	NA	NA	–	29	29	NA	(Kuzehkanan et al. 2011)
Mazandaran	2011	420	NA	NA	0 (0)	0 (0)	0 (0)	–	–	0	11– > 21 years	(Nahrevanian et al. 2011)
Chaharmahal and Bakhtiari	2012	156	88	68	5 (3.20)	3 (3.40)	2 (2.94)	5 (ELISA)	–	–	16–85 years	(Khalili et al. 2012)
Mazandaran	2012	962	565	397	1 (0.10)	1 (0.17)	0 (0)	–	–	1	NA	(Vahedi et al. 2012)
Mazandaran	2014	348	185	163	8 (2.29)	NA	NA	–	8	8	NA	(Gholami et al. 2014)
Guilan, …	2014	4200	NA	NA	5 (0.11)	NA	NA	–	–	5	20– > 51 years	(Mafi et al. 2014)
Guilan	2015	177	NA	NA	0 (0)	0 (0)	0 (0)	–	–	0	NA	(Majidi-Shad et al. 2015)
Hamadan	2015	1301	683	618	17 (1.30)	NA	NA	–	–	17	0– > 12 years	(Kiani et al. 2015)
Tehran, …	2015	1520	782	738	1 (0.06)	NA	NA	–	1	1	1–92 years	(Zebardast et al. 2015)
Immunocompromised												
Tehran	2000	385	230	155	3 (0.77)	NA	NA	–	–	3	20– > 51 years	(Athari et al. 2000)
Tehran	2001	185	NA	NA	1 (0.54)	1	0	–	–	1	NA	(Shekarabi et al. 2001)
Mazandaran	2004	100	38	62	5 (5)	2 (5.26)	3 (4.83)	–	–	5	0–14 years	(Sharif et al. 2004)
Tehran, …	2004	206	176	30	3 (1.45)	NA	NA	–	–	3	NA	(Zali et al. 2004)
Tehran	2004	214	138	76	3 (1.40)	NA	NA	–	–	3	1– > 46 years	(Nahrevanian et al. 2004)
Isfahan	2006	642	NA	NA	30 (4.67)	NA	NA	–	30	30	NA	(Dorostcar Moghaddam et al. 2006)
West Azerbaijan	2006	72	NA	NA	3 (4.16)	NA	NA	–	–	3	(Mean 9)	(Hazrati Tappeh et al. 2006b)
West Azerbaijan	2006	87	25	32	10 (11.49)	NA	NA	–	–	10	NA	(Hazrati Tappeh et al. 2006a)
Razavi Khorasan	2007	100	67	33	22 (22)	16 (23.88)	6 (18.18)	22 (ELISA)	–	22	0–17 years, (Mean 7/6)	(Berenji et al. 2007)
Lorestan	2007	306	295	11	6 (1.96)	NA	NA	–	–	6	20–50 years	(Fallahi et al. 2007)
Kermanshah	2007	75	70	5	20 (26.66)	NA	NA	–	–	20	20– > 50 years	(Taherkhani et al. 2007)
Hamadan	2007	190	94	96	1 (0.52)	NA	NA	–	–	1	12–88 years, (Mean 48/5 ± 18.7)	(Monsef et al. 2007)
Isfahan	2008	228	170	58	8 (3.50)	5 (2.94)	3 (5.17)	–	–	8	NA	(Azami and Dorostkar Moghadam 2008)
Khuzestan	2010	176	120	56	9 (5.11)	7 (5.83)	2 (3.57)	9 (ELISA)	–	–	1–76 years	(Dehkordy et al. 2010)
Tehran	2012	71	NA	NA	9 (12.67)	NA	NA	–	–	9	NA	(Ghorbanzadeh et al. 2012)
Isfahan	2012	250	NA	NA	0 (0)	0 (0)	0 (0)	–	–	0	NA	(Azizi et al. 2012)
Isfahan	2012	183	151	32	11 (6.01)	9 (5.96)	2 (6.25)	–	11	11	NA	(Izadi et al. 2012)
Kurdistan	2013	74	67	7	6 (8.10)	NA	NA	–	–	6	5–50 years, (Mean 36)	(Ghobadi et al. 2013)
Khuzestan	2013	100	3	97	2 (2)	NA	NA	–	–	2	(Mean 28/6 ± 9.2)	(Rahdar et al. 2013)
Fars	2013	44	23	21	5 (11.36)	5 (21.73)	0 (0)	–	5	5	1.8–10 years	(Agholi et al. 2013b)

Table 1 (continued)

Province	Year	Participation			Positive cases			Method			Age	Ref
		All	Male	Female	All (%)	Male (%)	Female (%)	Serology	PCR	Staining		
Fars	2013	356	273	83	34 (9.55)	NA	NA	–	34	34	10–69 years	(Agholi et al. 2013a)
Khuzestan	2014	371	NA	NA	15 (4.04)	NA	NA	–	15	15	NA	(Rafiei et al. 2014)
Khuzestan	2014	200	119	81	9 (4.5)	NA	NA	–	–	9	3– > 51 years	(Kazemi et al. 2014)
Hamadan	2014	180	94	86	1 (0.55)	0 (0)	1 (1.16)	–	–	1	14–70 years, (Mean 42)	(Jafari et al. 2014)
Tehran	2015	350	195	155	3 (0.85)	NA	NA	–	–	3	NA	(Salehi Sangani et al. 2015)
Hemodialysis												
West Azerbaijan	2006	103	55	48	4 (3.88)	3 (5.45)	1 (2.08)	–	–	4	NA (Mean age 50)	(Hazrati Tappeh et al. 2006a)
Isfahan	2006	104	65	39	12 (11.53)	5 (7.69)	7 (17.94)	–	–	12	NA	(Seyrafian et al. 2006)
East Azerbaijan	2015	78	50	28	9 (11.53)	NA	NA	–	–	9	20– > 65 years	(Omrani et al. 2015)
Diabetes												
Tehran	2005	250	91	159	6 (2.4)	NA	NA	–	–	6	NA	(Akhlaghi et al. 2005)
Isfahan	2015	100	20	80	2 (2)	NA	NA	–	–	2	NA	(Mohtashamipour et al. 2015)
Children												
Zanjan	1994	1000	NA	NA	26 (2.6)	12	14	–	–	26	0–12 years	(Haniloo 1994)
Hamadan	1996	554	NA	NA	30 (5.41)	13	17	–	–	30	0– > 10 years	(Fallah and Haghighi 1996)
Kermanshah	2000	400	NA	NA	13 (3.25)	NA	NA	–	–	13	NA	(Hamzavi 2000)
Tehran	2001	170	NA	NA	7 (4.11)	NA	NA	7 (DF)	–	7	0–10 years	(Shekarabi et al. 2001)
Markazi	2001	405	NA	NA	31 (7.65)	NA	NA	–	–	31	0–5 years	(Mosayebi and Islami rad 2001)
Ilam	2001	979	NA	NA	29 (2.96)	NA	NA	–	–	29	NA	(Naserifar and Khosravi 2001)
Isfahan	2002	240	NA	NA	9 (3.75)	NA	NA	–	–	9	NA	(Talari et al. 2002)
Tehran	2003	500	351	149	5 (1)	4 (1.13)	1 (0.67)	–	–	5	6–12 years	(Maleki and Sadegh Hasani 2003)
Sistan and Baluchestan	2003	528	311	217	25 (4.73)	17 (5.46)	8 (3.68)	–	–	25	NA	(Dabirzadeh et al. 2003)
Semnan	2004	153	88	65	5 (3.26)	4 (4.54)	1 (1.53)	–	–	5	0–12 years, (Mean 5/4)	(Akbari-Eidigahi et al. 2004)
Lorestan	2005	400	200	200	19 (4.75)	11 (5.5)	8 (4)	–	–	19	0–10 years	(Maleki et al. 2005)
Isfahan	2005	180	NA	NA	41 (22.77)	NA	NA	41 (IFA)	–	41	0–3 years	(Dorostcar Moghaddam and Azami 2005)
Hormozgan	2005	245	NA	NA	17 (6.93)	9	8	–	–	17	0–7 years, (Mean 2/9)	(Hamedi et al. 2005)
Chaharmahal and Bakhtiari	2006	618	341	277	12 (1.94)	NA	NA	12 (ELISA)	–	–	0–5 years	(Khalili et al. 2006)
Ardabil	2006	371	159	212	15 (4.04)	7 (4.40)	8 (3.77)	–	–	15	0–6 years	(Mohammadi ghalehbin et al. 2006)
West Azerbaijan	2006	30	NA	NA	0 (0)	0 (0)	0 (0)	–	–	0	NA	(Hazrati Tappeh et al. 2006b)
Kermanshah	2007	616	373	243	64 (10.38)	39 (10.45)	25 (10.28)	–	–	64	0–3 years	(Moghaddam 2007)
Tehran	2007	420	238	182	10 (2.38)	4 (1.68)	6 (3.29)	–	–	10	0–10 years	(Nikmanesh et al. 2007)
	2007	171	97	74	8 (4.67)	7 (7.21)	1 (1.35)	8 (ELISA)	–	–	0–5 years	(Khalili et al. 2007)

Table 1 (continued)

Province	Year	Participation			Positive cases			Method			Age	Ref
		All	Male	Female	All (%)	Male (%)	Female (%)	Serology	PCR	Staining		
Chaharmahal and Bakhtiari												
Tehran	2008	1020	NA	NA	12 (1.17)	NA	NA	–	12	12	0–8 years	(Tahvildar Bidrooni et al. 2008)
Qazvin	2008	1000	600	400	3 (0.3)	NA	NA	–	–	3	0–12 years	(Ghoreishi et al. 2008)
Tehran, …	2008	1263	584	678	31 (2.45)	NA	NA	–	31	31	0–12 years	(Keshavarz et al. 2008)
Isfahan	2008	65	47	18	3 (4.61)	2 (4.25)	1 (5.55)	–	–	3	0–5 years	(Azami and Dorostkar Moghadam 2008)
East Azerbaijan	2010	100	NA	NA	12 (12)	NA	NA	–	–	12	0–15 years	(Shirazi et al. 2010)
Tehran	2010	424	NA	NA	7 (1.65)	NA	NA	–	–	7	0–12 years	(Kermani et al. 2010)
Isfahan	2010	606	254	352	28 (4.62)	16 (6.29)	12 (3.40)	–	–	28	0–10 years	(Saneian et al. 2010)
East Azerbaijan	2011	900	539	361	70 (7.77)	NA	NA	–	–	70	6–12 years	(Hakimi et al. 2011)
Mazandaran	2011	150	NA	NA	16 (10.66)	NA	NA	–	–	16*	0–6 years	(Ranjbar-Bahadori et al. 2011)
Tehran	2011	794	NA	NA	19 (2.39)	NA	NA	–	19	19	NA	(Taghipour et al. 2011)
Qazvin	2011	469	NA	NA	12 (2.55)	NA	NA	–	12	12	0–12 years	(Nazemalhosseini Mojarad et al. 2011)
Bushehr	2012	374	217	157	49 (13.10)	29 (13.36)	20 (12.73)	49 (ELISA)	–	–	0–5 years	(Fouladvand et al. 2012)
Tehran	2013	2500	1353	1157	30 (1.2)	NA	NA	–	32	30	0–12 years	(Salehi et al. 2013)
Khuzestan	2014	19	NA	NA	1 (5.26)	NA	NA	–	1	1	0–5 years	(Rafiei et al. 2014)
Kermanshah	2014	700	NA	NA	15 (2.14)	6	9	–	–	15	0–15 years	(Hamzavi et al. 2014)
Hamadan	2014	420	222	198	2 (0.47)	1 (0.45)	1 (0.50)	–	–	2	0–10 years, (Mean 5)	(Asadi et al. 2014)
Markazi	2015	150	NA	NA	4 (2.66)	NA	NA	–	–	4	0–8 years	(Ghorbanzadeh et al. 2015)
Guilan	2015	42	NA	NA	0 (0)	0 (0)	0 (0)	–	–	0	NA	(Mahmudi et al. 2015)
Fars	2015	541	NA	NA	0 (0)	0 (0)	0 (0)	–	–	0	NA	(Foroutani 2015)
Golestan	2015	547	328	219	27 (4.93)	16 (4.87)	11 (5.02)	–	15	27	0–6 years	(Sharbatkhori et al. 2015)
Fars	2015	106	61	45	2 (1.88)	1 (1.63)	1 (2.22)	–	–	2	0–12 years	(Kargar jahromi et al. 2015)
East Azerbaijan	2015	113	NA	NA	2 (1.76)	NA	NA	–	2	2	0–12 years	(Mahdavi Poor et al. 2015)

*In this study, 24 positive cases identified with auramine phenol method

DF direct immunofluorescence, *IFA* indirect immunofluorescence assay, *ELISA* enzyme-linked immunosorbent assay, *NA* not available

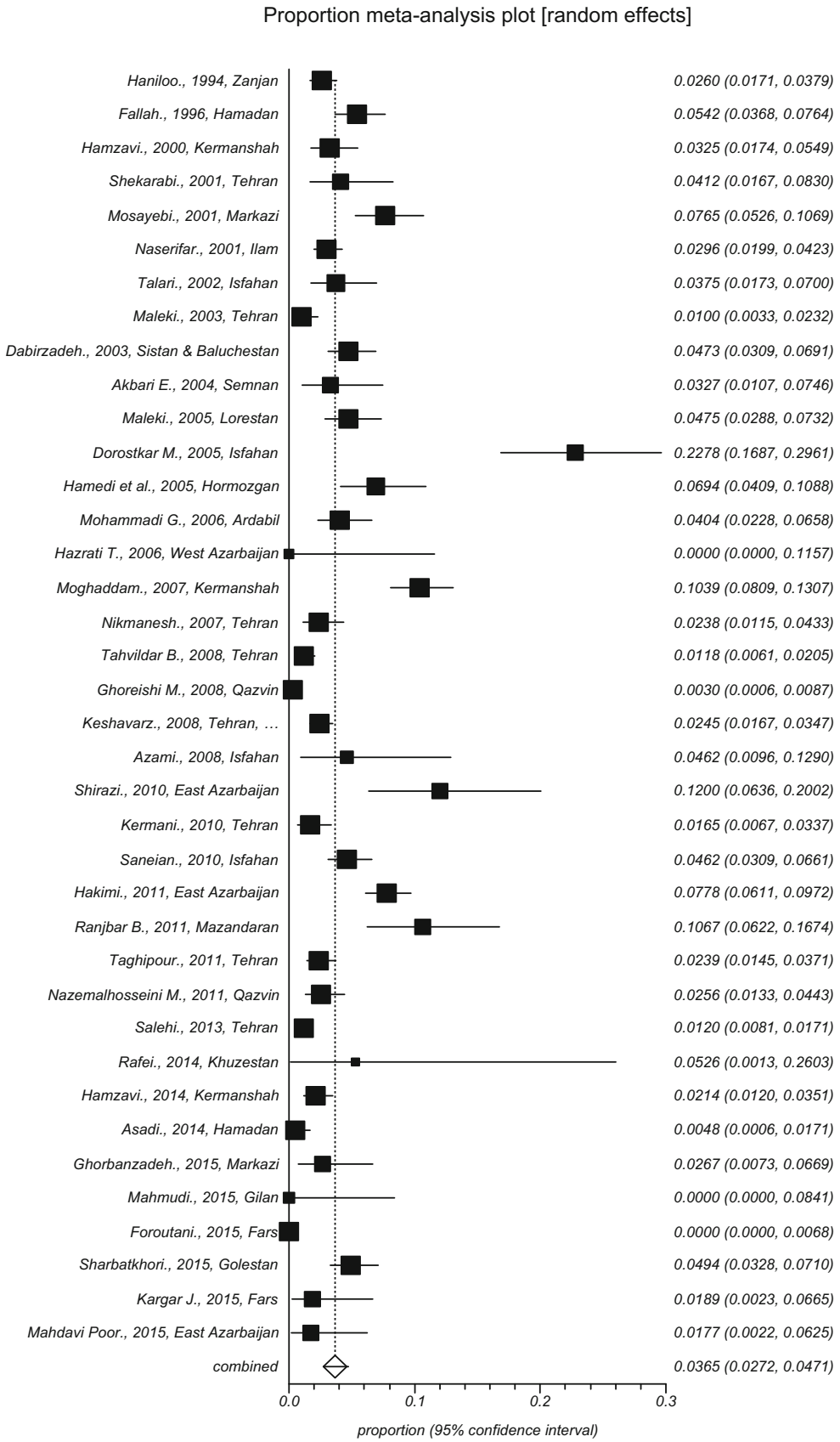

Fig. 2 Forest plot diagram of 38 studies showing positivity rates of *Cryptosporidium* infection in the Iranian children population with staining method (first author, year and province of study)

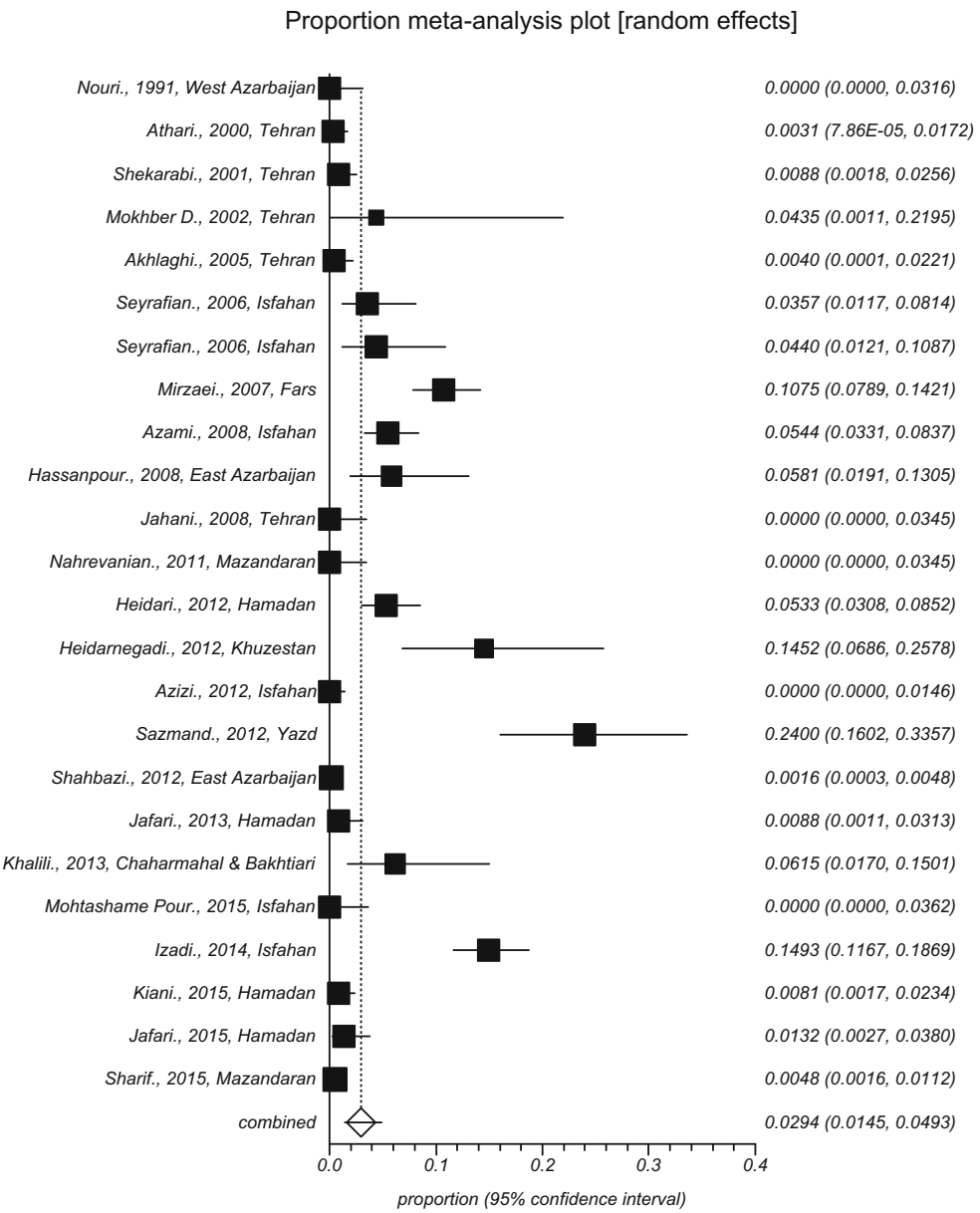

Fig. 3 Forest plot diagram of 24 studies showing positivity rates of *Cryptosporidium* infection in the Iranian healthy population with staining method (first author, year and province of study)

Therefore, immunocompromised patients are a particularly susceptible group with high prevalence rates of infection and should be placed under surveillance. Many reports from different parts of the world have investigated the prevalence of *Cryptosporidium* infection. Compared to various regions throughout the world, particularly in developing countries, cryptosporidium prevalence in Iran is moderate. African countries, Central and South American countries, Asian countries, and others in the Pacific and Caribbean areas have the highest prevalence rate of this infection (1.3–31.5%), while North America and Europe report low prevalence rates of cryptosporidiosis (0.1–14.1%) (Cardona et al. 2011; Davies et al. 2009; Fayer 2004; Gatei et al. 2006). The prevalence rate of cryptosporidiosis among immunocompetent individuals was reported to be 0.6–20% and 4–20% in Western and developing countries, respectively (Chacin-Bonilla et al. 1991; Davies et al. 2009; Snelling et al. 2007). In contrast, *Cryptosporidium* spp. infection among AIDS patients is 3 and 50% in developed and developing countries, respectively (Kumurya and Gwarzo 2013). The results showed that *C. hominis* is more prevalent in North and South America, Australia, and Africa, while *C. parvum* is common in Europe, especially in the UK (Aldeyarbi et al. 2016; Putignani and Menichella 2010). The prevalence rate of cryptosporidiosis in the Middle East countries were as follows: in Iraq, the recorded prevalence of *Cryptosporidium* infection among children with severe diarrhoea and dehydration ranged within 8.6–9.7% (Latif and Rossle 2015). However, a similar investigation using both direct wet mount and modified Ziehl-Neelsen staining has indicated that the highest and lowest

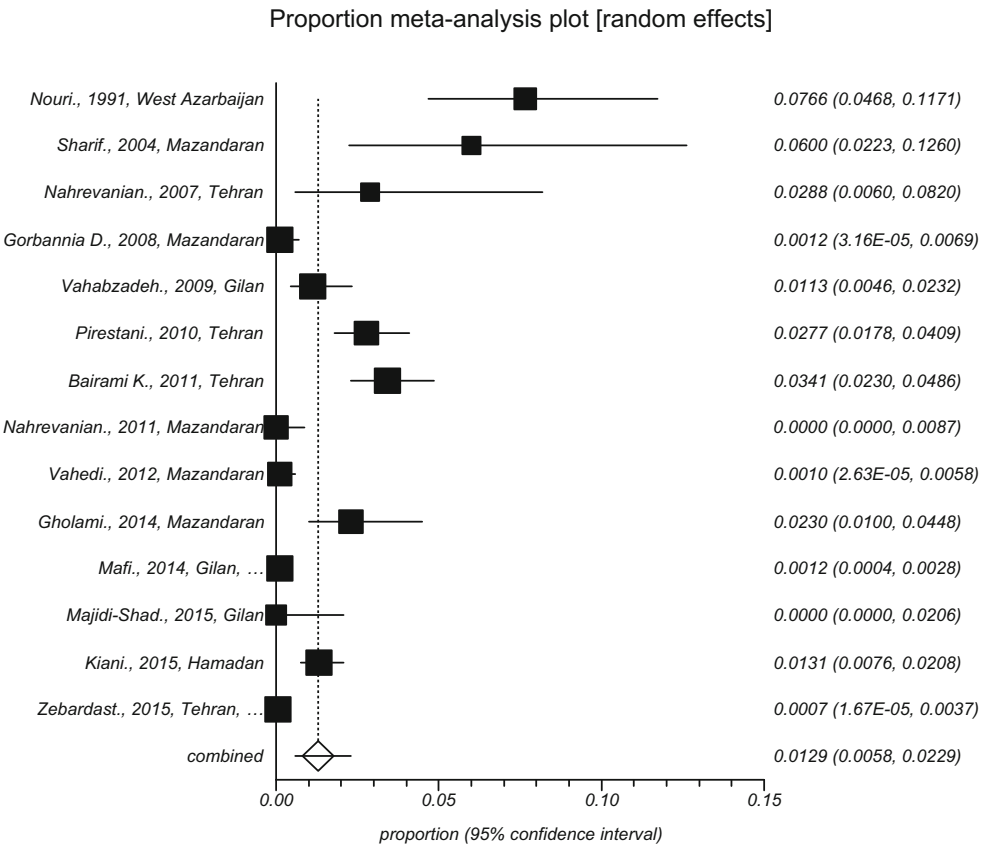

Fig. 4 Forest plot diagram of 14 studies showing positivity rates of *Cryptosporidium* infection in the Iranian gastroenteritis population with staining method (first author, year and province of study)

rates were found in Baghdad (14.6%) and Babylon (2.2%), respectively (Latif and Rossle 2015). In another study at Erbil City, Kurdistan region, Iraq, 14% of all samples were detected positive by direct wet mount and modified Ziehl-Neelsen methods (Koyee and Faraj 2015). A study on children with diarrhoea, which uses modified safranin-methylene blue staining, in Kuwait indicated that 10% of cases were positive for *Cryptosporidium* spp. (Iqbal et al. 2001). In addition, Iqbal et al. showed that 3.4% of children with diarrhoea aged between 6 months and 16 years in Kuwait were found to be infected by *C. parvum* (Iqbal et al. 2011). It should be noted that owing to common borders, similar climatic and demographic conditions, and proximity of Iraq and Kuwait, the infection rate of *Cryptosporidium* was almost the same. The prevalence rate of cryptosporidiosis among diarrhoeal patients in Saudi Arabia was determined to be 9.4% by using wet mount stained with the modified mZN method (Hawash et al. 2014). In another study from Saudi Arabia, *Cryptosporidium* infection has been reported in 4.7 and 32% of asymptomatic and symptomatic children under 5 years old, respectively (Al Braiken et al. 2003). Although the overall prevalence of *Cryptosporidium* infection in Iraq, Kuwait, and Saudi Arabia is almost the same with Iran, but it seems that the infection rate is lower in the Iranian children. In Yemen, during 2006–2007, among a total of 712 faecal samples of children with different ages, 34.7% were found positive for this parasite (Al-Shamiri et al. 2010). In another study from Yemen, the prevalence of *Cryptosporidium* infection was reported to be 1–50% (Alyousefi et al. 2013). In contrast to the results of studies in Iran, cryptosporidiosis is higher in Yemen. The infection rate among children under 5 years in Peshawar, Northwest Pakistan, was reported to be 9% (Mumtaz et al. 2010). In addition, the infection rate in immunocompetent adults with acute diarrhoea was determined as 55% in Karachi, Pakistan, by using the modified acid fast-staining method (Ali et al. 2014). In Turkey, out of 707 faecal samples obtained from elementary school students, four (0.6%) were tested positive for *Cryptosporidium* spp. (Otağ et al. 2007). In the another study in Turkey, *Cryptosporidium* oocysts were found in 7.1% (161 of 2281) from patients who were admitted with the gastrointestinal complaints (Karaman et al. 2015). Although the prevalence rate of human cryptosporidiosis among the Iranian population is low compared to neighbouring countries, it seems that the epidemiology of this parasitic infection in Iran is somewhat similar to its western neighbours, which may be due to the similarities in socio-economic status, health policy, and the climate conditions.

The phylogenetic analysis demonstrates that *C. parvum* (especially subtype IIaA15G2R1) and *C. hominis* are unequivocally circulating among children and immunocompromised populations in Iran. Moreover, the prevalence of *C.*

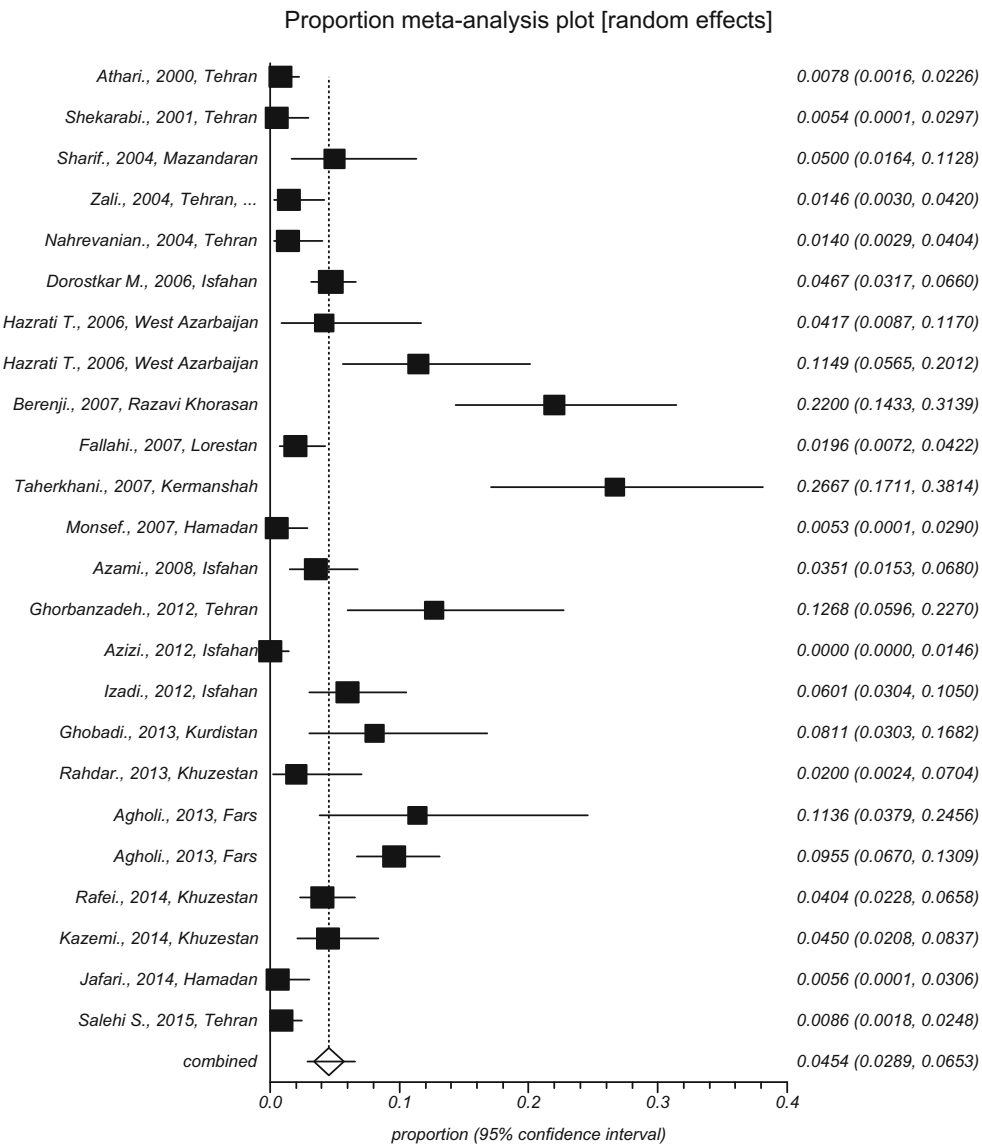

Fig. 5 Forest plot diagram of 24 studies showing positivity rates of *Cryptosporidium* infection in the Iranian immunocompromised population with staining method (first author, year and province of study)

parvum compared to *C. hominis* has been reported to a large extent. Furthermore, findings showed that gp60 has more potential than 18S rRNA for identification of subtypes of *Cryptosporidium* spp. The results of a study conducted in Kuwait showed that *C. parvum* is the most commonly identified species in children. Furthermore, the majority of the *C. parvum* isolates belonged to subtypes IIa in that study (Iqbal et al. 2011), and its results are consistent with our results. Also, similar results have been reported by Mahgoub et al. from Jordan and Mahdi et al. from Iraq. In these studies, *C. parvum* is the most common zoonotic species among humans (Mahdi and Ali 1999; Mahgoub et al. 2004). Other studies have been conducted with similar findings in other areas of the developing and developed world such as South Africa, India, Netherlands, the UK, and the USA (Feltus et al. 2006; Gatei et al. 2007; Leav et al. 2002; Leoni et al. 2006; Wielinga et al. 2008).

There are many various risk factors that could play a role in the development of cryptosporidiosis among different populations. These include foreign travel, especially from endemic countries, season, geographic location, contact with infected individual or animals (particularly calves), and accidental ingestion of contaminated water during swimming (Cacciò and Putignani 2014). It is also noteworthy that there is a relationship between risk factors and *Cryptosporidium* species. For instance, the most known risk factor for *C. parvum* is to be in contact with animals, while the major risk factor for *C. hominis* is diaper changing in diaper-aged children (even those with no diarrhoea) that spreads the parasite to others (Bouzid et al. 2013; Cacciò and Pozio 2006). Given these risk factors, a substantial number of individuals are exposed to the risk of being infected with *Cryptosporidium*. These groups include members of the medical staff at children's medical centres, childcare workers, parents of infected children, farmers, and people

Table 2 Demographic factors associated to positivity for *Cryptosporidium* in the Iranian general population

Factor	Total individuals	Positive cases	Overall prevalence (95% CI)	*P* value	References
Gender					(Agholi et al. 2013b; Akbari-Eidigahi et al. 2004; Asadi et al. 2014; Azami and Dorostkar Moghadam 2008; Berenji et al. 2007; Dabirzadeh et al. 2003; Dehkordy et al. 2010; Fouladvand et al. 2012; Hazrati Tappeh et al. 2006b; Izadi et al. 2012; Jafari et al. 2014; Jafari et al. 2015; Kargar jahromi et al. 2015; Khalili et al. 2012; Khalili et al. 2007; Maleki and Sadegh Hasani 2003; Maleki et al. 2005; Moghaddam 2007; Mohammadi ghalehbin et al. 2006; Mohtashamipour et al. 2015; Nikmanesh et al. 2007; Nouri et al. 1991; Saneian et al. 2010; Seyrafian et al. 2006; Sharbatkhori et al. 2015; Sharif et al. 2004; Vahedi et al. 2012)
Male	4775	222	4.6 (4.1–5.3)	0.11	
Female	3612	141	3.9 (3.3–4.6)		
Age					(Akbari-Eidigahi et al. 2004; Asadi et al. 2014; Dehkordy et al. 2010; Fouladvand et al. 2012; Gholami et al. 2014; Ghoreishi et al. 2008; Hamedi et al. 2005; Hamzavi 2000; Hamzavi et al. 2014; Haniloo 1994; Heidari and Gharakhani 2012; Izadi et al. 2012; Kargar jahromi et al. 2015; Khalili et al. 2006; Khalili et al. 2007; Maleki and Sadegh Hasani 2003; Maleki et al. 2005; Mirzaei 2007; Moghaddam 2007; Mohammadi ghalehbin et al. 2006; Nikmanesh et al. 2007; Ranjbar-Bahadori et al. 2011; Seyrafian et al. 2006; Sharif et al. 2004)
<15	7273	337	4.6 (4.2–5.1)	0.08	
16–30	281	20	7.1 (4.6–10.7)		
>30	432	27	6.3 (4.3–8.9)		
Residency					(Asadi et al. 2014; Gholami et al. 2014; Ghoreishi et al. 2008; Haniloo 1994; Izadi et al. 2012; Kargar jahromi et al. 2015; Khalili et al. 2012; Maleki et al. 2005; Moghaddam 2007; Mohammadi ghalehbin et al. 2006)
Urban	2558	84	3.3 (2.7–4.0)	0.7	
Rural	2032	71	1.5 (1.1–2.2)		
Contact with animals					(Akbari-Eidigahi et al. 2004; Hazrati Tappeh et al. 2006b; Izadi et al. 2012; Jafari et al. 2013; Jafari et al. 2015; Kargar jahromi et al. 2015; Khalili et al. 2012; Nikmanesh et al. 2007)
Yes	576	14	2.4 (1.5–4.0)	0.7	
No	960	27	2.8 (1.9–4.1)		
Stool appearance					(Fouladvand et al. 2012; Hamzavi 2000; Hamzavi et al. 2014; Haniloo 1994; Izadi et al. 2012; Jafari et al. 2015; Khalili et al. 2012; Khalili et al. 2006; Moghaddam 2007; Naserifar and Khosravi 2001; Zali et al. 2004)
diarrhoea	3250	212	6.5 (5.7–7.4)	<0.001	
Non-diarrhoea	2148	18	0.8 (0.5–1.3)		
Season					(Dabirzadeh et al. 2003; Fouladvand et al. 2012; Gholami et al. 2014; Haniloo 1994; Maleki et al. 2005; Sazmand et al. 2012)
Spring	917	38	3.1 (3.0–5.6)	0.001	
Summer	729	44	6.0 (4.5–8.0)		
Autumn	359	36	10.0 (7.3–13.6)		
Winter	696	33	4.7 (3.4–6.6)		

travelling abroad to endemic countries. On the other hand, in most cases, immunocompromised patients and children are more at risk than other groups. Results of the present systematic review indicate that the prevalence rate of *Cryptosporidium* infection is different among various populations. Data analysis disclosed that immunocompromised patients and children are two groups with the highest prevalence rate of cryptosporidiosis in Iran. In this regard, our findings are consistent with most studies conducted around the world, which indicates a high prevalence in these high-risk groups. In line with the study conducted in Iraq (Rahi et al. 2013), our results indicate that the infection rate is a slightly higher in men (4.6%) than women (3.9%), but this difference is not statistically significant. This difference is probably the result of greater exposure to risk factors, such as occupational reasons in the Iranian males. Although the infection rate is relatively high in the young age group (16–30 years old) than the age groups of >30 and <15 years, we did not find any statistically significant relationship between age and the rate of infection. Similarly, in Nicaragua, no reported correlation between age groups and the prevalence rate of cryptosporidiosis. (Muñoz-Antoli et al. 2011). In contrast, results of an epidemiological study in Ireland showed that younger age groups (82%) had significantly higher prevalence than older age groups (18%) (Garvey and McKeown 2009). Residency is another factor related with cryptosporidiosis, which should be taken into consideration. Our results show that *Cryptosporidium* prevalence in urbanites was higher than those who live in rural areas. It should be noted that Iran is a tropical country with a long summer, and thus the swimming season increases in recreational centres such as swimming pools, beaches, lakes, and rivers. Of course, migration of villagers to cities and keeping pets, such as dogs and cats, may also contribute to the high prevalence of cryptosporidiosis in urban areas. Similar results have been reported in Tunisia (Rym et al. 2007). Based on the results of a study in North Cumbria, England, no relationship was found between the prevalence of cryptosporidiosis and contact with animals (Goh et al. 2004). An analysis of the present review also

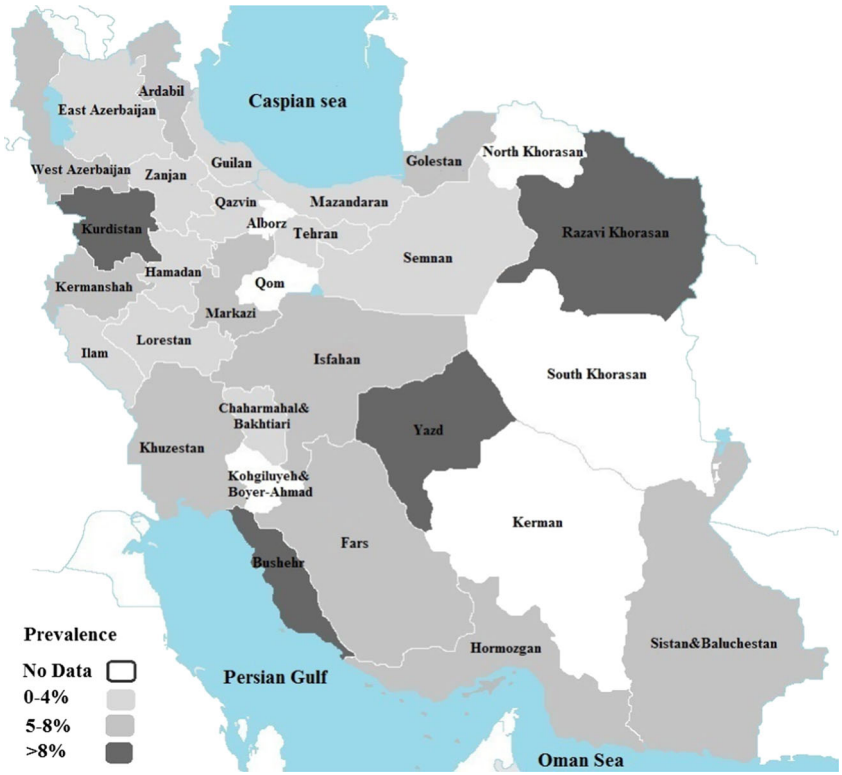

Fig. 6 Prevalence of *Cryptosporidium* infection in the Iranian general population in different provinces according to staining method positivity

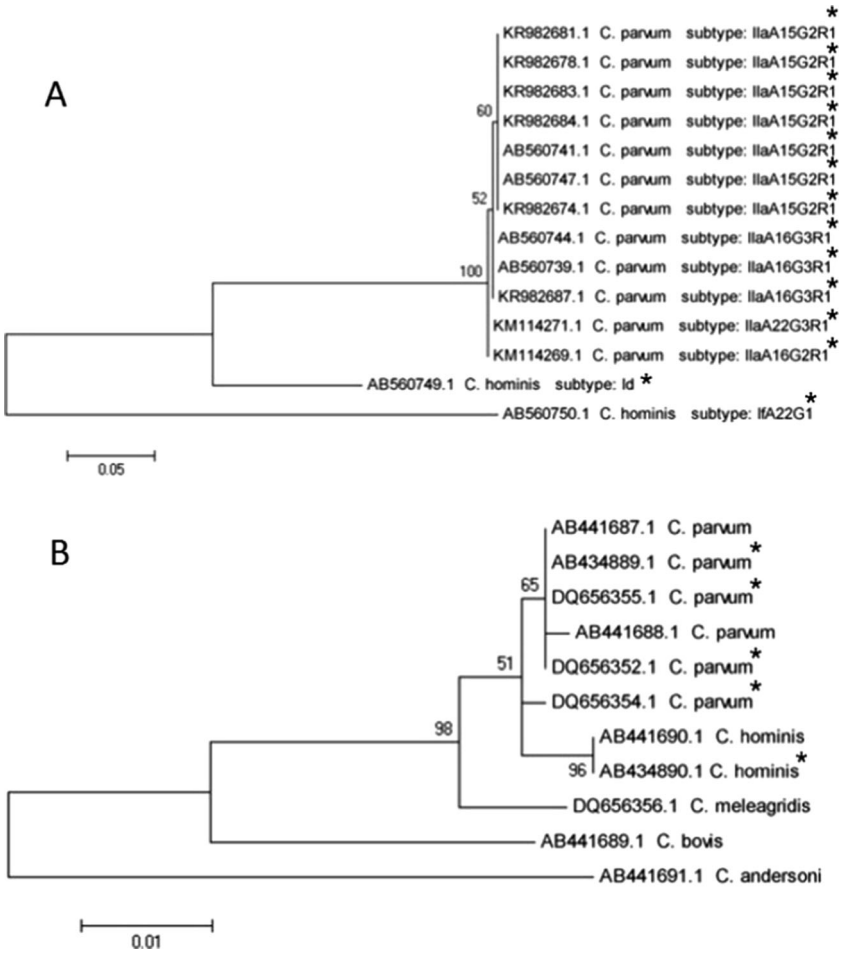

Fig. 7 Phylogenetic analysis of gp60 (**a**) and 18S rRNA (**b**) nucleotide sequences of *Cryptosporidium* spp. isolates recovered from different samples in Iran. (* = isolated from human)

indicates the lack of such relationship. Studies conducted in Iran showed that diarrhoea is the most common clinical sign of cryptosporidiosis in both healthy people and immunocompromised patients. They are similar to the study done in Turkey and are different from the ones conducted in Nicaragua (Muñoz-Antoli et al. 2011; Yilmaz et al. 2008) although it should be noted that diarrhoea is self limited in most immunocompetent individuals. Previous investigations reported a correlation between seasons and cryptosporidiosis (Jagai et al. 2009; Lake et al. 2005). According to our analysis, there was such a relationship and so the high prevalence of infection was observed in autumn (10%) and summer (6%). Climate change is a significant challenge to global health in this century (Lal et al. 2013). Concurrent with rainfall and the subsequent water flow, *Cryptosporidium* oocysts in animal manures can easily get transferred to surface water (Lake et al. 2005). On the other hand, warm temperature is one of the most critical parameters to increase the prevalence of cryptosporidiosis. Temperature can be one of the most important triggers of excystation (Cacciò and Putignani 2014). Our results revealed that the rate of *Cryptosporidium* infection in Iran have a wide range between 0.83–24%. This could be due to climatic variation in different geographical areas of Iran. Based on our analysis in Iran, the maximum prevalence of *Cryptosporidium* infection has been observed in Razavi Khorasan, Yazd, Bushehr, and Kurdistan provinces. The high prevalence rate of cryptosporidiosis in the southern provinces, particularly Bushehr, is probably related to several factors such as the hot and humid climate. But the high infection rate in Kurdistan and Razavi Khorasan provinces may be due to population density and commuting of infected people from neighbouring countries. However, other factors, such as public health level and access to safe drinking water, should also be considered.

It should be noted that this systematic review has a few limitations. Some of these limitations include: (1) heterogeneous epidemiological findings, (2) not paying attention to some of the related risk factors by most studies, and (3) the lack of similar studies in some provinces. These limitations may affect the overall prevalence rate in the Iranian general population.

Conclusions

The present study is the first systematic review and meta-analysis providing a comprehensive view of the prevalence of human cryptosporidiosis and related risk factors in Iran. More than two thirds of Iran's provinces have experienced relatively high prevalence (>4%) of this infection among the general population. In addition, infection in high-risk groups, such as immunocompromised patients and children, is highly prevalent. It seems that awareness of *Cryptosporidium* prevalence, risk factors, and disease complications may be required for developing effective strategies to prevent such infection.

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Conflict of interest The authors declare that they have no conflicts of interest.

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