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Worldwide prevalence of emerging parasite *Blastocystis* in immunocompromised patients: A systematic review and meta-analysis

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ABSTRACT

Background: Blastocystis is one of the most common pathogens of the human intestine, caused by an emerging parasite, which can lead to severe symptoms and even death in immunocompromised patients. We aimed to determine the global prevalence of Blastocystosis infection in people with immunodeficiency. A systematic literature search was conducted on Web of Science, Scopus, Google scholar, Science Direct and MEDLINE databases to select all observational studies reporting the prevalence of Blastocystosis infection in Worldwide, based on different diagnostic methods in immunocompromised patients of any age and published from inception to February 2019. Pooled estimates and 95% confidence intervals (95% CIs) were calculated using random effects models and in addition, the I^2 statistic was calculated. The geographic distribution of studies was evaluated and the diagnosis of *Blastocystis* was compared by various techniques. Electronic databases were reviewed for *Blastocystosis* infection in HIV/AIDS, cancer and other immunocompromised patients, and meta-analyses were conducted to calculate the overall estimated prevalence. Total68 eligible studies were included. The estimated pooled prevalence rate of *Blastocystosis infection* in immunocompromised patients was overall 10% (95% CI, 7–13%; I^2 96.04%) ($P < 0.001$), of whom 21% [18-25] were in Australia, 12% [4-24] in America, 11% [6-17] in Europe and 10% [5-15], 7% [3-13] in Asia and Africa, respectively. It was calculated that the estimated pooled prevalence rate of Blastocystosis infection in immunocompromised patients was overall 10% and the prevalence estimates ranged from 0.44 to 72.39. Also, overall the prevalence of parasites co-infection in immunocompromised patients was detected as 0.024%. Our finding showed that immunocompromised people show a high prevalence of Blastocystosis infection compared to the control population. Adequate information on the prevalence rate is still missing from many countries. However, current information underscore that *Blastocystis* should not be neglected.

1. Introduction

Blastocystis is a common enteric protist, belonging to the phylum *Stramenopila*, a complex and heterogeneous evolutionary assemblage of heterotrophic and photosynthetic species [1,2].It is an intestinal parasite (obligate anaerobic) that largely affects humans and animals such as mammals, reptiles, amphibians, birds and insects [1–3]. *Blastocystis* is considered as a ubiquitous protozoan with worldwide distribution, being the most prevalent parasitic eukaryote, colonizing in the human intestinal tract and infecting approximately 1 billion individuals worldwide [3,4]. The prevalence of Blastocystosis infection varied widely from country to country, according to hygienic-sanitary conditions. Early reports from industrialized countries gave lower levels of prevalence of *Blastocystis* (approximately 5%), while it seemed more prevalent (approximately 30–60%) in developing countries [5,6].

Blastocystis is a highly polymorphic organism, with four major forms such as cyst, granular, vacuolar and ameboid [7]. Although the life cycle of *Blastocystis* has not yet been fully described due to its biological complexity, but its transmission via the fecal-oral has been confirmed and most researchers believe that the transmission is via the, person-to-person, animal-person (zoonotic transmission was reported in

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Abbreviations

AIDS	Acquired immune deficiency syndrome
CI	Confidence interval
HIV	Human immunodeficiency virus
OR	Odds ratio
PRISMA	Preferred reporting items for systematic reviews and meta-analyses.

animals such as pig, horse and chicken) [8,9], or directly, through the ingestion of contaminated water or food [11].

Molecular genetic studies have been recently demonstrated that the existence of seventeen subtypes (STs) (ST1-ST17) from different mammalian, amphibian and avian species based on gene coding of partial small subunit ribosomal RNA of the *Blastocystis*. Amongst the founded subtypes in human (ST1-ST9 and ST12) [12,13], the ST1, ST2, ST3 and ST4 are common prevalent subtypes with frequency of around 90% [14,15].

Despite being firstly described more than 100 years ago, the role of *Blastocystis* as a pathogen in humans is still the focus of intense debate; several studies suggest an association between the Blastocystosis infection and gastrointestinal (GI) symptoms such as diarrhea, nausea, vomiting, abdominal pain, fatigue, flatulence, inflammatory bowel disease (IBD), and irritable bowel syndrome (IBS) [18,19], or less common dermatological complaints [20,21].

The prevalence of Blastocystosis infection may be affected by many factors, such as different geographic locations, age of hosts, eating habits, immune status of hosts and different conditions, such as HIV, hematological malignancies, use of immunosuppressive drugs, solid organ transplants at risk of opportunistic infections; this parasite is frequently found in immunocompromised patients, showing a characteristic of opportunistic pathogenesis [22].

Although several studies have been carried out in several countries but a comprehensive review study has yet done on worldwide prevalence of *Blastocystis* in immunocompromised patients. It is necessary to obtain information concerning the prevalence of Blastocystosis *infection* in special populations worldwide and understanding its epidemiology is central in formulating effective control strategies against *Blastocystis*. We conducted a systematic review and meta-analysis to assess the worldwide prevalence of Blastocystosis infection in immunocompromised individuals.

2. Methods

2.1. Data sources and search strategy

We performed this systematic review and meta-analysis in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines [23]. We searched the Web of Science, Scopus, Google scholar, Science Direct and MEDLINE databases (which are the most important international databases and index the most articles in the world, were used for search) from the inception to the February 2019 solely in English, looking for all articles that possibly contained data for *Blastocystis* prevalence in immunocompromised populations. Relevant keywords including *"Blastocystis", "Blastocystis hominis", "Immunocompromised Host", "Immunodeficiency", "Immunologic Deficiency Syndromes", "HIV", "AIDS", "Neoplasms", "cancer", "Tumor", "Malignancy", "carcinoma", "transplantation", "organ grafting"* extracted from the Mesh thesaurus were used to search the databases. In order to retrieve the maximum number of relevant articles, the appropriate strategies (Table 1) were used in each databases so that keywords are searched in important fields of documents such as title, abstract, and keywords. We systematically searched the scientific literatures for cross-sectional and case control studies that reported Blastocystosis infection in immunocompromised individuals (The protocol was registered in Ilam University of Medical Sciences No.98/2006/48).

Table 1
Search strategies in this study.

Databases	Search strategy
MEDLINE	("*Blastocystis hominis*"[MeSH Major Topic] OR "*Blastocystis hominis*" [MeSH] OR "*Blastocystis hominis*" [tiab]) AND (Immunocompromised Host [mesh] OR Immunocompromised [ti] OR immunologic deficiency syndromes [mesh] OR Immunodeficiency [tiab] OR immunodeficiency [tiab] OR HIV [mesh] OR HIV [tiab] OR AIDS [mesh] OR AIDS [tiab] OR Neoplasms [mesh] OR cancer [tiab] OR Tumor [tiab] OR Malignancy [tiab] OR carcinoma [mesh] or transplantation [mesh] OR transplantation [tiab] OR organ transplantation [mesh] OR organ grafting [tiab]) AND prevalence
Scopus and ScienceDirect	(INDEXTERMS("*Blastocystis hominis*") OR TITLE-ABS-KEY ("*Blastocystis hominis*") OR TITLE-ABS-KEY(*Blastocystis hominis*)) AND (INDEXTERMS(immunologic deficiency syndromes) OR TITLE-ABS-KEY (immunodeficiency) OR INDEXTERMS(Immunocompromised Host) OR TITLE-ABS-KEY (Immunocompromised) OR INDEXTERMS (AIDS) OR TITLE-ABS-KEY (AIDS) OR INDEXTERMS (HIV) OR TITLE-ABS-KEY (HIV) OR INDEXTERMS (Neoplasms) OR TITLE-ABS-KEY (cancer) OR TITLE-ABS-KEY (Tumor) OR TITLE-ABS-KEY (Malignancy) OR INDEXTERMS (carcinoma) OR TITLE-ABS-KEY (carcinoma) OR INDEXTERMS (transplantation) OR TITLE-ABS-KEY (transplantation) OR INDEXTERMS (organ transplantation) OR TITLE-ABS-KEY (organ grafting)) AND TITLE-ABS-KEY (prevalence)
Web of Science	(TS= *Blastocystis hominis* OR TS= *Blastocystis hominis*) AND (TS= immunologic deficiency syndromes OR TI= immunodeficiency OR TS= Immunocompromised Host OR TI= Immunocompromised OR TS= AIDS OR TI= AIDS OR TS= HIV OR TI= HIV OR TS= Neoplasms OR TI= cancer OR TI= Tumor OR TI= Malignancy OR TS= carcinoma OR TI= carcinoma OR TS= transplantation OR TI= transplantation OR TS= organ transplantation OR TI= organ grafting) AND TS= prevalence
Google Scholar	Using related keywords

2.2. Inclusion and exclusion criteria

Inclusion criteria for our systematic review and meta-analysis were: [1]: Peer-reviewed short reports and original articles, without geographical and time limitations [2], Studies conducted until February 2019 [3], Studies published with full text or abstracts in English [4], Having total sample size and positive samples in *Blastocystis* [5], Focused on HIV patients, cancer patients, transplant patients and other immunocompromised patients of all ages, regardless of their sex and occupation. Articles with incomplete information, no full text or abstracts available, repeated studies, reviews, case reported, or animal studies were also excluded, because this may limit the analyses. To get 25 articles for which neither the abstract nor the full text was available, we sent an email to the majority of authors, but unfortunately we received no response, so these articles were excluded from the study.

The titles and abstracts of all identified articles were screened by (M A, Kh Z), and irrelevant items were excluded. Abstracts and then the full text of the remaining articles were reviewed by (A MR, T H and Kh Z) to find relevant studies that met the inclusion criteria. Reasons for exclusion were recorded for all full-text articles, and data were extracted independently and in duplicate for all studies by M A, Kh Z and T H.

2.3. Data extraction

The data extraction was done by two investigators independently using Microsoft Excel with a tool. This tool included information on the author, year of publication, country of the study, sample size, positive samples in Blastocystosis, type of immunodeficiency disease, diagnostic

methods, and demographic characteristics. Two reviewers independently extracted the data. The third author was considered as arbiter to resolve any disagreements.

To allocate each paper a score, checklists of validated quality assessment for case-control and cross-sectional studies were used. The Newcastle-Ottawa scale criteria of selection, comparability, and outcome were used to evaluate all studies included in the meta-analysis. Disagreements among reviewers were resolved by discussion until consensus was reached. The agreement between the researchers was assessed using the Cohen's kappa coefficient (agreement; 98.56% - Cohen's kappa: 0.92).Studies with a score of<20% were considered 'very low', 20–40% as 'low', 40–70% as 'medium', and ≥70% as 'high' quality (Appendix 1) [24].

2.4. Statistical analysis

A procedure for pooling the prevalence, in the meta-analysis of multiple studies was used and the results were displayed in a forest plot. The heterogeneity of results across studies was checked, using Cochran's Q test (with P-value <0.10), and quantified by the I^2 statistic. The I^2 statistic greater than 75% was considered as high heterogeneity. Tau-squared (t^2 or Tau^2) statistic was used to explore the between-study variance. Potential influences on the prevalence estimates were investigated, using the subgroup analyses, including different immunocompromised populations and geographical distribution.

In order to determine a weighted-mean estimate of prevalence for the indicated infections across included studies, the prevalence estimates of each study were pooled, using a random-effects meta-analysis model at a confidence level of 95%. All meta-analysis was performed, using Stata software version 14 (Stata Corp, College Station, TX, USA).

3. Results

Our searches identified 621 records on Feb 2019. After removal of duplicates and initial screening, we reviewed 351 papers in full. Total270 potentially relevant articles were excluded from this meta-analysis after consulting the full text. Finally, total 68 studies were used for quantitative and meta-analysis (Fig. 1).We assessed the overall quality of the data in the included studies to be low to moderate (Appendix).

Characteristics of the included studies are listed in Table 2. In brief, 45 articles investigated the Blastocystosis infection in HIV/AIDS patients, 8 publications described the Blastocystosis infection in cancer patients, whereas 15 studies reported the Blastocystosis infection in others immunocompromised patients (e.g., transplant and hemodialysis patients). In terms of epidemiological design, 37 of the included publications were cross-sectional and 31 were case-control studies (Table 2). The oldest study was conducted in 1986(25).

In this study, the overall population consisted of 7642 HIV infected patients and 4227 control population. The estimated pooled prevalence of Blastocystosis infection in the HIV/AIDS patients and control population was 9% (95% CI, 5–13%) and 6% (95%CI, 1–14%), respectively ($p<0.001$). In ten studies, including 1415 cancer patients with infection and 405 control population, the prevalence of Blastocystosis infection in cancer patients and control was 9% (95% CI, 4–15%) and 6% (95% CI, 0–19%), respectively ($p< 0.001$). In 2013, the prevalence of Blastocystosis infection in patients and control was 13% (95% CI, 8–18%) and 8% (95% CI, 3–15%), respectively ($p< 0.001$), in other

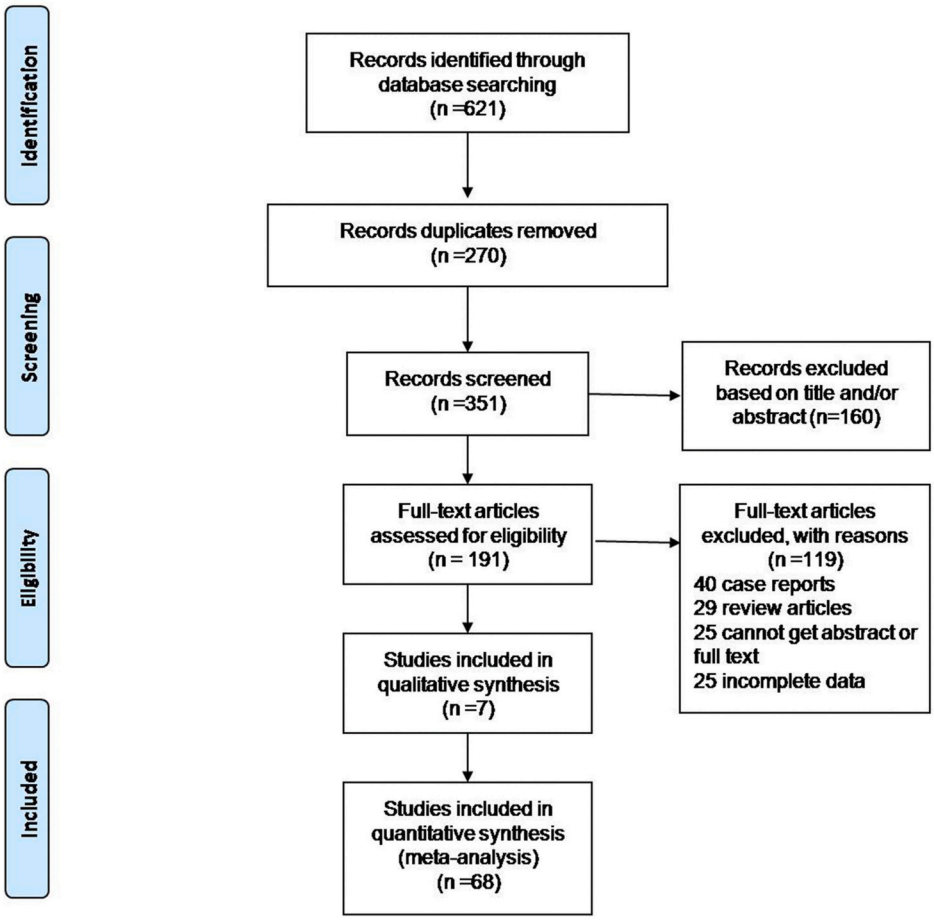

Fig. 1. PRISMA flow diagram for the selection of included studies.

Table 2
Characteristics of the included studies of *Blastocystosis* infection in people immunocompromised.

Author	Year	Sample	Infected Sample	Diarrhea	Male	Female	Average age	Quality score
Henry et al. [25]	1986	46	1	46	-	-	-	Low
Chacin-Bonilla et al. [26]	1992	27	3	27	21	6	46	Low
Cotte et al. [27]	1993	81	11	81	-	-	-	Low
Albrecht et al. [28]	1995	262	99	262	-	-	-	Low
Ok et al. [29]	1997	111	32	30	-	-	34	High
Escobedo et al. [30]	1997	67	17	42	51	16	-	High
Mathewson et al. [31]	1998	83	2	83	-	-	-	High
Germani et al. [32]	1998	430	4	290	-	-	-	High
Amenta et al. [33]	1999	48	3	48	21	26	28	High
Brandonisio et al. [34]	1999	154	16	65	109	45	-	High
Cimerman et al. [35]	1999	200	1	45	139	61	34	High
Koltas et al. [36]	1999	413	32	-	-	-	-	High
Menon et al. [37]	1999	50	2	12	31	19	5	Low
Tasova et al. [38]	2000	406	25	33	228	178	-	High
Prasad et al. [39]	2000	59	2	26	-	-	-	Low
Gassama et al. [40]	2001	594	5	279	364	230	38	High
Lebbad et al. [41]	2001	52	8	52	26	26	35	High
Mohandas et al. [42]	2002	120	4	53	-	-	-	High
Flórez et al. [43]	2003	115	29	90	-	-	-	Medium
Büyükbaba Boral et al. [44]	2004	38	1	23	-	-	-	Medium
Zali et al. [45]	2004	206	9	28	203	3	-	High
Oguntibeju et al. [46]	2006	90	4	34	50	40	3	High
Arslan et al. [47]	2007	95	4	94	55	40	33	High
Stark et al. [48]	2007	1868	315	-	1868	-	-	High
Chincha et al. [49]	2009	2056	727	-	1138	926	50	High
Eksi et al. [50]	2009	219	1	89	115	104	53	High
Adamu et al. [51]	2009	200	3	75	110	90	41.5	High
Kurniawan et al. [52]	2009	268	194	268	253	15	30	High
Ramana et al. [53]	2009	452	2	-	223	229	40	Medium
Saksirisampant et al. [54]	2009	90	2	71	73	17	39.5	High
Viriyavejakul et al. [55]	2009	64	7	20	49	15	-	High
Al-Magrine et al. [56]	2010	136	7	57	84	52	35.5	High
Idris et al. [57]	2010	42	23	42	18	24	33	High
Ramana et al. [58]	2010	452	13	110	223	229	-	High
Berenji et al. [59]	2010	51	9	-	38	13	38	High
Alemu et al. [60]	2011	248	20	-	132	116	26	High
Poirier et al. [61]	2011	186	27	-	102	84	57	High
Noor et al. [62]	2012	140	51	82	82	58	40	High
Abdel-Hafeez et al. [63]	2012	450	30	450	-	-	-	High
Al-Qobati et al. [64]	2012	206	10	-	115	91	14.17	Low
Amancio et al. [65]	2012	105	3	-	54	51	40.19	High
Sherchan et al. [66]	2012	146	9	78	61	85	32.5	High
Yosefi et al. [67]	2012	60	10	-	53	7	34	High
Zabolinejad et al. [68]	2013	89	5	15	53	36	7.5	High
Gil et al. [69]	2013	196	63	29	112	84	46.5	High
Gupta et al. [70]	2013	200	3	19	127	73	32	High
Roka et al. [71]	2013	333	17	50	82	251	35	High
Tian et al. [72]	2013	590	75	-	278	312	-	High
Feyisayo Jegede et al. [73]	2014	155	3	20	83	72	32.5	High
Kumarasamy et al. [74]	2014	425	65	-	-	-	-	High
Mtapuri-Zinyowera et al. [75]	2014	113	3	30	34	79	44	Low
Pala et al. [76]	2014	201	11	201	131	70	-	High
Barazesh et al. [77]	2015	88	8	34	46	42	-	High
Khalil et al. [78]	2015	400	30	-	264	136	36	High
Omrani et al. [79]	2015	218	16	24	120	98	51	High
Rossen et al. [80]	2015	168	19	-	89	79	27	High
Anvari-Tafti et al. [81]	2016	220	2	-	-	-	-	High
Ghimire et al. [82]	2016	112	1	-	65	47	-	High
Yadav et al. [83]	2016	88	2	30	55	33	22.5	High
Yersal et al. [84]	2016	232	25	39	79	153	61	High
Zhang et al. [85]	2017	381	27	110	220	161	56	High
Piranshahi et al. [86]	2017	286	51	-	-	-	-	High
Berenji et al. [87]	2017	142	4		82	60	42	High
Mohamed et al. [88]	2017	218	50	12	-	-	-	High
Rasti et al. [89]	2017	385	11	-	170	215	-	High
Bednarska et al. [90]	2018	283	3	-	121	164	-	High
Jupsa-Mbiandou et al. [91]	2018	528	36	28	189	339	-	High
Seid et al. [92]	2018	86	53	86	37	49	35	High

immunocompromised patients from 15 studies. The results are shown in Figs. 2 and 3.

The identified studies were done in 32 countries distributed worldwide. The regions were chosen based on the Global Burden of disease; the included studies were from 10 countries in Asia, 7 countries in Europe, 6 countries in America, 8 countries in Africa, and one country in Australia.

The prevalence estimates of Blastocystosis infection ranged from

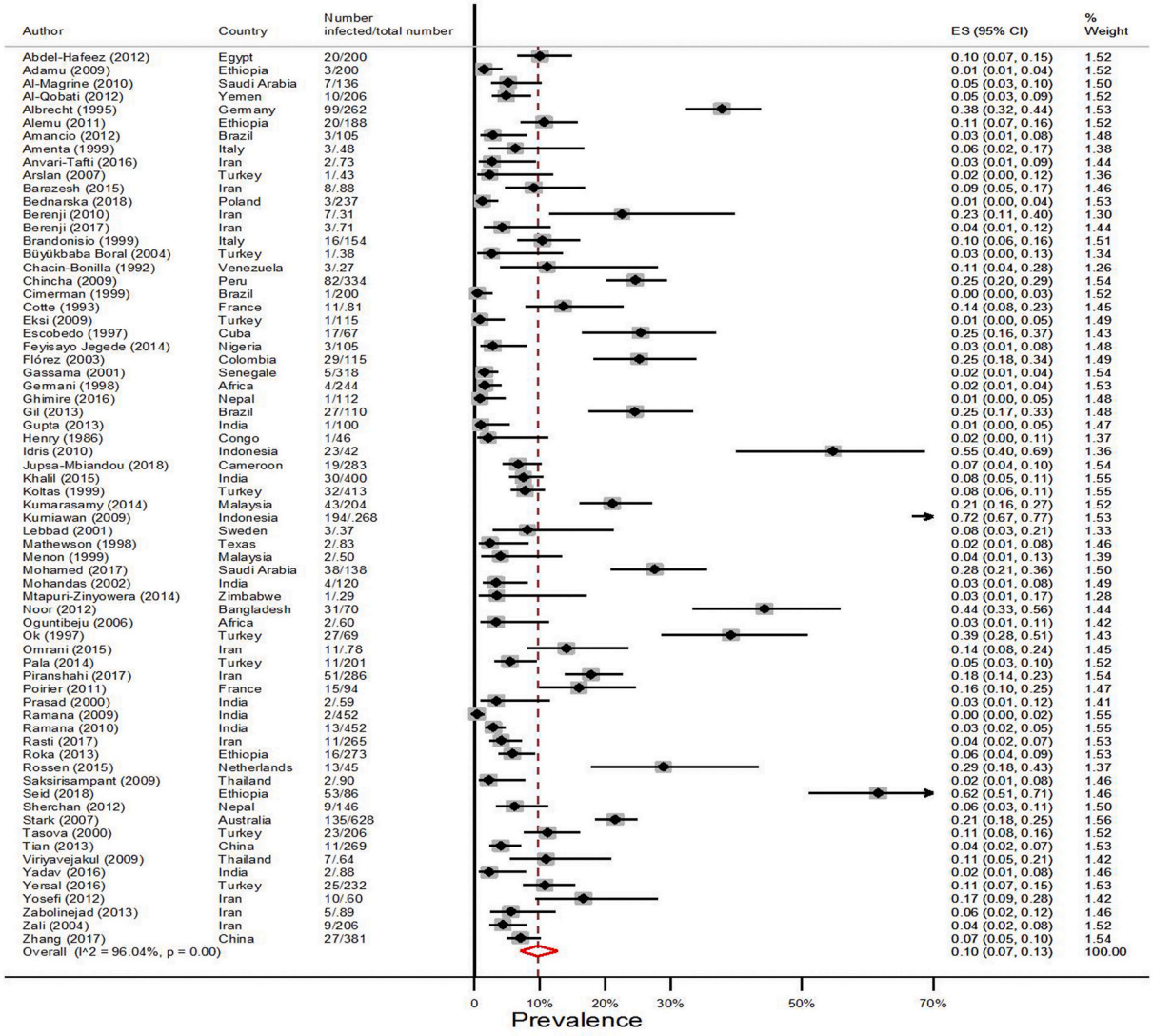

Fig. 2. Forest plot of Blastocystis infection prevalence in immunocompromised people.

0.44 to 72.39.

The estimated pooled prevalence of Blastocystosis infection in immunocompromised people was overall 10% (95% CI, 7–13%), of whom 10% [5–15] were in Asia, 11% [6–17] in Europe, 12% [4–24] in America,7% [3–13] and 21% [18–25] in Africa and Australia, respectively (Fig. 4). In Australia, information about *Blastocystis* was missing: only one study was done in Australia.

The technique that led to the diagnosis of *Blastocystis* was described in 9997 patients from 58 different articles. *Blastocystis* was detected in 8% (95% CI, 5–12%) of the samples by microscopy techniques, whereas 16% (95% CI, 12–20%) and 15% (95% CI, 7–25%) were positive by culture and molecular methods, respectively (Table 3).

4. Discussion

Opportunistic infections are one of the major factors in mortality of immunocompromised patients, and the clinical symptoms of the infection with *Blastocystis* were more severe in patients with immunodeficiency. There is increasing evidence showing that *Blastocystis* may cause opportunistic infections in immunocompromised populations, such as cancer patients, transplant recipients and AIDS patients [93].

Parasitic infections remain as a principal reason of morbidity and mortality in immunocompromised patients, with high prevalence around the world. To date, most of the investigations in *Blastocystis* field have aimed at explaining the impact and roles of this parasite in the formation or progression of the disease. However, obscure and inconsistent results have eventuated from these researches. On the other hand, as the immunocompromised and immune dysregulation disorders are emerging, the importance of defining the relationship between Blastocystosis infection prevalence and immunocompromised patients is being highlighted more than ever.

The potency to effectively struggle parasitic infections requires an active inflammatory response from host. There are not enough studies about the immune response again *Blastocystis*, however, based on some in vitro and in vivo investigations, cellular immune response and anti-inflammatory cytokine production has been affected by *Blastocystis* infection. Therefore, the prevalence of this parasite among immunocompromised patients such as cancer patients, transplant recipients and AIDS patients is not unexpected. In all three mentionedimmunocompromised disorders, T cells responses is not strong enough. Cellular immunity in patients receiving immunosuppressive drugs such as corticosteroids and in aggressive immunosuppressive transplant recipients 'patients, acquired immunodeficiency, as well as cancer patients is dysregulated, hence, these patients are expected to being high prevalence population with several opportunities' infection as well as *Blastocystis*.

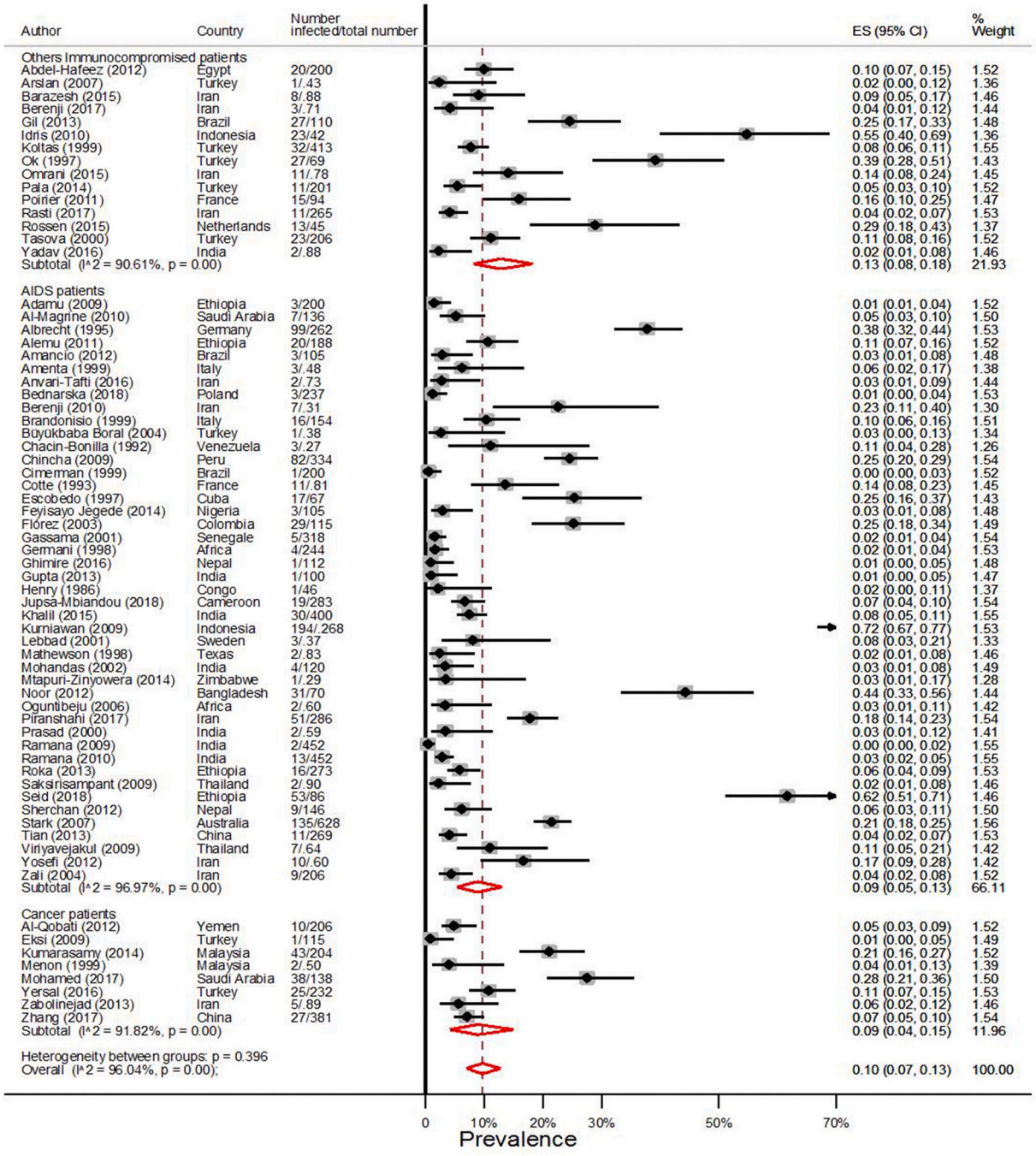

Fig. 3. Meta-Analysis of the prevalence of Blastocystis infection in immunocompromised people using random effects model.

Our finding corroborates evidence of a high prevalence of Blastocystosis infection in Australia, America, Europe and Asia, and low prevalence in Africa. There are significant differences between different countries for Blastocystosis infection in the Asia (0.4–72.39%), Africa (1.5–61.63%), America (0.5–25.37%) and Europe (0.87–39.13%) in immunocompromised individuals. The geographic distribution of studies significantly affected the pooled estimate (Fig. 4). Regarding individual countries, the estimate was the highest in the Indonesia (72.39%, 54.76%) [52–57], Ethiopia (61.63%) [92], Bangladesh (44.29%) [62], Turkey (39.13%) [29], Germany (37.79%) [28], Netherlands (28.89%) [80], Saudi Arabia (27.54%) [88], Cuba (25.37%) [30], Colombia (25.22%) [43], Peru (24.55%) [49], Brazil (24.55%) [69], Iran (22.58%) [59], Australia (21.50%) [48] and Malaysia (21.08%) [74]. In contrast, a low prevalence has been shown in India (0.44%, 1%) [53–70], Brazil (0.5%) [35], Turkey (0.87%) [50], Nepal (0.89%) [82] and Poland (1.27%) [90].

In immunocompromised patients a high prevalence has been reported in Australia (21%) (Few data were available from Australia, as only one study was conducted in Australia) [48].

Numerous factors, such as genetics, personal hygiene, type of health care which are applied to different patients, age, as well as geographical and occupational conditions of each individuals, may affect the prevalence in different countries. Another important factor which can affect the rate of prevalence is the quality and sensitivity of detection tests. The results obtained from this systematic review showed that the prevalence rate of Blastocystosis infection inimmunocompromised patients, using culture method(16%) was higher than other methods (15% molecular and 8% microscopy assays). The techniques based on microscopy are not

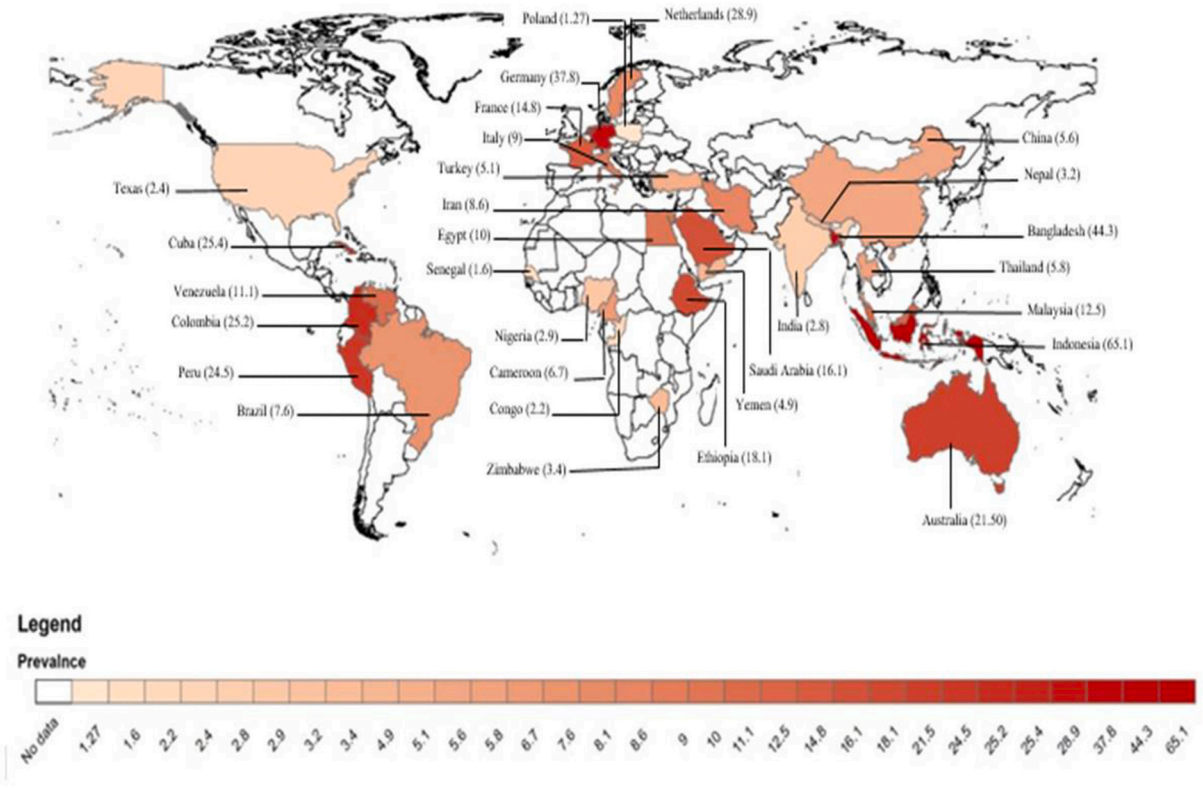

Fig. 4. Geographical distribution of the included studies.

Table 3
Characteristics of the included studies of *Blastocystosis* infection in people immunocompromised.

Category		N of studies	Prevalence rate	95% CI	I^2	Heterogeneity statistic	P.Value
Study type	Cross-sectional	37	9	5, 14	96.88%	1153.66	<0.001
	Case-control	31	11	7, 15	94.4%	535.65	<0.001
Laboratory method	Culture	2	16	12, 20	-	-	-
	Direct wet	47	8	5, 12	96.12%	1185.63	<0.001
	Molecular	9	15	7, 25	96.55%	231.57	<0.001
	Unknown	10	9	2, 19	95.5%	200.13	<0.001
Overall		68	10	7, 13	96.04%	1690.83	<0.001

sensitive enough, particularly for chronic infections. Molecular detection technique was employed as it has a high specificity and sensitivity amongst different techniques. As per literature and by statistical analysis, the molecular detection technique was considered as the gold standard detection method, and in this systematic review 15% were positive by molecular methods. One can hypothesize that the type of diagnostic tests influences on the results of this meta-analysis. The fact that the prevalence rate in developed societies in Europe and the United States is higher than that of in Africa may be attributed to the high accuracy methods used in these developed societies. However, this claim requires deeper investigations based on the used diagnostic methods.

In this study, the highest prevalence of the Blastocystosis infection was observed in Australia research. It should be kept in mind that only one article considering Australia's data, was included in this analysis, and the reason of the high prevalence of the Blastocystis could be assigned to the fact that HIV positive subjects in that study were homosexual men (gay). It has been shown that the rate of intestinal parasitic infections in homosexual men community is significantly higher than that of others due to oral-anal 1 contact. Nevertheless, this interpretation requires further studies to make a correct judgment.

The overall prevalence of parasites co-infection in immunocompromised patients was 0.024%. The most common parasite was *Dientamoeba fragilis* (0.14%) and the other parasites were identified as follows: *Cryptosporidium parvum* (0.11%), *Trichuris trichiuria* (0.09%), *Enteromonas hominis* (0.08%), *Giardia lamblia* (0.07%), *Isospora belli* (0.06%), *Microsporidia* sp (0.06%), *Schistosoma* sp (0.06%), *Cyclospora cayetanensis* (0.05%), *Entamoeba histolytica* (0.05%), *Endolimax nana* (0.05%), *Entamoeba coli* (0.05%), *Entamoeba hartmanni* (0.03%), *Ascaris Lumbricoides* (0.03%), *Entrobious vermicularis* (0.03%), *Opisthorchis* sp (0.03%), *Hymenolepis nana* (0.03%), *Iodamoeba butschlii* (0.02%), *Chilomastics mesnili* (0.02%), *Strongyloides stercoralis* (0.02%), *Hookworm* (0.02%)and*Teania* sp (0.01%). Regarding the results obtained in this systematic review, Blastocystosis infection was reported in 1202 immunocompromised individuals, and analysis of co-infection parasites revealed that 63 positive samples of *Blastocystis* had co-infection with the following parasites: *Cryptosporidium parvum* [22], *Giardia lamblia* [13], *Cyclospora cayetanensis* [10], *Microsporidia* sp [5], *Entamoeba histolytica* [3], *Strongyloides stercoralis* [1], *Hymenolepis nana* [1], *Endolimax nana* [6], *Iodamoeba butschlii* [1] and *Entamoeba coli* [1].

Our systematic review and meta-analysis has several limitations: First, a number of potentially relevant studies were identified through our systematic review and meta-analysis, but not all data were available. As most of these studies might not have relevant data, there is a risk that we missed some data, because if the articles might not contain relevant data, and the exclusion criteria were well conducted, there is no risk of missing data. Second, most of the data resulted from the conventional

diagnostic microscopy techniques ($n = 47$), which have a lower sensitivity, compared to the polymerase chain reaction (molecular diagnostic techniques). Therefore, most studies examined a single stool specimen, potentially leading to a false negative result. Third, insufficient data were collected about further relevant factors on Blastocystosis infection (e.g., age and $CD4^+$ lymphocyte count (cell/mm^3)). Fourth, the included studies were from Asia ($n = 31$), Africa ($n = 12$), America ($n = 8$), Europe ($n = 16$) and Australia ($n = 1$), and the study quality was variable; thus more robust surveillance of Blastocystosis infection in immunocompromised people is required in these regions. Fifth, different subtypes of *Blastocystis* may cause different clinical manifestations in immunocompromised people. However, we did not analyze their distribution characteristics, as the diagnostic microscopy techniques in most of the selected studies, could not identify the species within the subtypes of *Blastocystis*.

5. Conclusion

It was calculated that the estimated pooled prevalence rate of Blastocystosis infection in immunocompromised patients was overall 10% and the prevalence estimates ranged from 0.44 to 72.39. Also, overall prevalence of parasites co-infection in immunocompromised patients was detected as 0.024%. It is generally concluded that the prevalence of Blastocystosis infection, in a variety of levels is evident in different developing countries. Because of high probability of intestinal parasites infections, especially *Blastocystis* opportunistic parasite, in the immunocompromised people, it is necessary to pay more attention to controlling and preventing these infections. We hope that similar investigations will be expanded in the future to collect more useful information to help health policy makers in different countries to better understand the prevalence and pattern of the disease.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.micpath.2020.104615.

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