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A Worldwide Systematic Review and Meta-Analysis of the Prevalence and Subtype Distribution of *Blastocystis* Sp. in Water Sources: A Public Health Concern

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Abstract

Contaminated water sources can result in outbreaks of parasitic infections such as *Blastocystis* sp. in communities, creating a substantial strain on healthcare systems and affecting the general health of the population. To ascertain the prevalence and subtype distribution of *Blastocystis* sp. in water sources globally, a systematic review and meta-analysis of published papers up to May 19, 2024 were carried out. A thorough search of multiple electronic databases (PubMed, Scopus, Google Scholar, and Web of Science) identified 24 studies/28 datasets meeting the inclusion criteria, encompassing 2,451 water samples from 15 countries worldwide. Water samples comprised wastewater (six datasets, 285 samples), tap/drinking water (10 datasets, 253 samples), surface water (eight datasets, 1013 samples), and uncategorized water (four datasets, 900 samples). Total estimates and 95% confidence intervals (CIs) were computed using a random-effects model. This review found that 18.8% (95% CI: 12.8–26.9%) of examined water samples contained *Blastocystis* sp. Wastewater showed the highest *Blastocystis* sp. infection rate at 35.5% (95% CI: 13.5–66.1%), followed by tap/drinking water at 19.1% (95% CI: 9.5–34.5%), surface water at 17.6% (95% CI: 7.2–36.8%), and uncategorized water at 9.9% (95% CI: 4.1–21.8%). Sensitivity analysis assessed weighted prevalence variations following the exclusion of individual studies. Subgroup analysis of *Blastocystis* sp. prevalence was performed based on publication years, countries, continents, WHO regions, sample sizes, and diagnostic methods. Water samples can be the source of infection for nine *Blastocystis* sp. subtypes (STs) (ST1-ST4, ST6, ST8, ST10, ST21, and ST24), with seven STs (ST1-ST4, ST6, ST8, and ST10) capable of infecting humans. It is important to take preventative and control measures, improve the cleanliness and quality of water sources, and promote public health awareness due to the presence of different parasites such as *Blastocystis* sp. in water sources.

Keywords: *Blastocystis* sp., prevalence, subtypes, water, water sources, systematic review

Introduction

A protozoan parasite called *Blastocystis* sp. is commonly found in fecal samples. It lives in both human and animal gastrointestinal tracts and is only anaerobic in axenic culture (Abdullah and Dyary, 2023). Recent research indicates that it has a global presence; prevalence is close to 100% in poor nations while rates are lower in industrialized nations (Kumarasamy et al., 2023). Some digestion issues in both rich and developing nations have been linked to this

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intestinal protist. *Blastocystis* sp. is found everywhere for a variety of causes (Asghari et al., 2024c). It can be acquired by a number of channels, such as person-to-person contact, zoonotic, and waterborne transmission. It spreads through the fecal-oral pathway (Asghari et al., 2024b). It is connected to socioeconomic issues in developing nations that result in inadequate sanitation (Nithyamathi et al., 2016). This protozoan may be zoonotic, which could have serious consequences for public health, given that it can move from animals to humans (Asghari et al., 2024a; Bastaminejad et al., 2024; Shams et al., 2024). In order to effectively combat zoonotic diseases like *Blastocystis* sp., the World Health Organization's "One Health" strategy promotes multidisciplinary teamwork. This approach aims to improve the best possible health for people, animals, and the environment (González-Barrio, 2022; Jinatham et al., 2021).

To date, 40–44 different subtypes (STs) and many subtype subgroups have been identified based on variations in the SSU rRNA gene between humans and animals. Nevertheless, not all strains of a certain subtype have shown clinical importance, and the relationship between distinct STs and their capacity to cause disease is still up for discussion. It has been discovered that 17 zoonotic STs (ST1-ST10, ST12-ST14, ST16, ST23, ST35, and ST41) are present in both humans and animals, with ST1–ST4 making up more than 90% of human isolates (Matovelle et al., 2024; Santin et al., 2024).

In terms of public health, polluted water sources can cause parasitic infection outbreaks, such as those caused by *Blastocystis* sp., in local communities (Efstratiou et al., 2017; Pal et al., 2018). This puts a significant burden on healthcare systems and has an impact on the population's overall health (Omarova et al., 2018). Severe parasite infections can be potentially fatal, particularly in susceptible populations such as children, expectant mothers, and immunocompromised individuals (Kurizky et al., 2020; Sappenfield et al., 2013). In order to better understand the epidemiology of this protozoan parasite in water samples, this study set out to review and summarize available data on the prevalence and STs distribution of *Blastocystis* sp. in various water sources and statistically analyze the results. These kinds of insights can help prevent parasitic infections, especially *Blastocystis* sp., by supporting the upkeep of clean water reservoirs and the execution of health measures.

Study design

The current work was a global systematic review and meta-analysis of the prevalence and ST distribution of *Blastocystis* sp. in water sources. This study's reporting adhered to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) standard (Moher et al., 2015).

Search procedure

Until May 19, 2024, the researchers analyzed four global databases: Medline/PubMed, ProQuest, Scopus, and the Web of Knowledge. Medical Subject Heading (MeSH) terms, either alone or in combination, were used to conduct the search: ("Intestinal Parasites" OR "Parasitic Infections" OR "*Blastocystis* sp.") AND ("Prevalence" OR "Epidemiology" OR "Frequency" OR "Occurrence") AND ("Subtype" OR "Subtyping") AND ("Water" OR "Wastewater" OR "Drinking water" OR "Sewage"). To include more pertinent studies, more keywords were employed and the references to pertinent papers were thoroughly reviewed. After the data were imported, duplicate articles were automatically removed from the EndNote X7 software. Notably, two researchers assessed the articles independently.

Inclusion/exclusion guideline

To ascertain the frequency of *Blastocystis* sp. in water sources using genetic, serological, and microscopy approaches, this thorough worldwide review evaluated cross-sectional studies from various languages, areas, and time periods. Case reports, reviews, commentary, and studies with both human and animal subjects were excluded, as well as studies that did not disclose the overall sample size or the prevalence rate of *Blastocystis* sp.

Quality assessment

Papers were evaluated for inclusion or exclusion with the Joanna Briggs Critical Appraisal Checklist for Studies Reporting Prevalence Data (Munn et al., 2014). Papers scoring <4–6 or ≥7 were categorized as medium and high quality, respectively. Articles with a score ≤3 were not included. From the selected papers, two researchers retrieved important information, and additional researchers verified their findings. Some of the information that was extracted included the first author's last name, the type of water, the diagnostic method, the quality assessment score, the year of publication and implementation, the continent, the country, the World Health Organization (WHO) classification, the total sample size, and the number of contaminated samples.

Statistical analysis

All statistical analyses were performed using the Comprehensive Meta-Analysis v3 software package (Asghari et al., 2021). p-Values < 0.05 were considered statistically significant. The random-effects model assessed the prevalence of *Blastocystis* sp. in waters by computing pooled prevalence and 95% confidence intervals (CIs). Subgroup analysis evaluated the prevalence of infection in water sources based on the types of water, WHO regions, nations, publication years, continents, sample size, and diagnostic procedures. A forest plot diagram showed the pooled prevalence with 95% CIs. The publication bias was investigated using a funnel plot. Heterogeneity was measured using the I^2 index; values <25%, 25%–50%, and >50%, respectively, indicated low, moderate, and high heterogeneity. Sensitivity analysis evaluated weighted variations in prevalence when individual studies were excluded.

Results

Paper selection

After conducting a thorough search across four worldwide databases, 8674 initial records were found. A total of 32 articles were ultimately chosen after deduplication and a careful examination of the other publications (5214 records). Eight further studies were eliminated as a consequence of a quality assessment that was conducted using Joanna Briggs Institute (JBI) criteria. In the end, the inclusion criteria for this study were satisfied by 24 extremely relevant studies/28 datasets (Fig. 1).

Qualitative and quantitative features of the papers included

A total of 24 studies including 28 datasets were entered in this review; the datasets cover the years 2000–2024 and include six on wastewater, ten on tap/drinking water, eight on surface water, and four on uncategorized water. A total of 253 from 2451 samples were tap or drinking water, 900 were uncategorized water, 285 were wastewater, and 1013 were surface water. Six studies were conducted in Malaysia and five in Turkey, with two each in Argentina, Australia, Egypt, and Thailand, and one each from China, Iran, Nepal the Philippines, Poland, Scotland, Spain, Sweden, and Venezuela. Sample sizes varied from 2 to 480 water samples. A total of 12 publications provided comprehensive information on the distribution of *Blastocystis* sp. STs in water sources. Molecular methods were predominantly utilized for diagnosis in 16 datasets, with microscopy being employed in 12 studies (Table 1). The quality assessment using the JBI checklist revealed that 10 papers had high quality (>6 points), and the remaining 14 articles had moderate quality (4–6 points) (Supplementary Table S1).

Global Prevalence of Blastocystis sp. in Water Sources

According to this study, *Blastocystis* sp. infected 18.8% (95% CI: 12.8–26.9%) of water samples collected worldwide (Fig. 2). The present systematic review and meta-analysis revealed significant heterogeneity among the included studies, as indicated by the statistical analysis ($Q = 268.7$, $I^2 = 89.9\%$, $p < 0.001$).

Weighted prevalence of Blastocystis sp. based on water types

The greatest *Blastocystis* sp. infection rate was found in wastewater, which was found to be 35.5% (95% CI: 13.5–66.1%). Tap/drinking water came in second at 19.1% (95% CI: 9.5–34.5%), surface water came in third at 17.6% (95% CI: 7.2–36.8%), and uncategorized water came in last at 9.9% (95% CI: 4.1–21.8%) (Table 2 and Fig. 3).

ST distribution of Blastocystis sp. in water samples

Nine *Blastocystis* sp. STs (ST1–ST4, ST6, ST8, ST10, ST21, and ST24) have been shown to be present in water samples, with seven of these STs (ST1–ST4, ST6, ST8, and ST10) having the ability to infect people (Table 1).

Pooled prevalence of Blastocystis sp. in water sources based on examined subgroups

Table 2 displays the subgroup-specific prevalence of *Blastocystis* sp. in water sources according to publication year, continent, WHO region, nation, water type, sample size, and diagnostic technique (Supplementary Figs. S1, S2, S3, S4, S5, and S6).

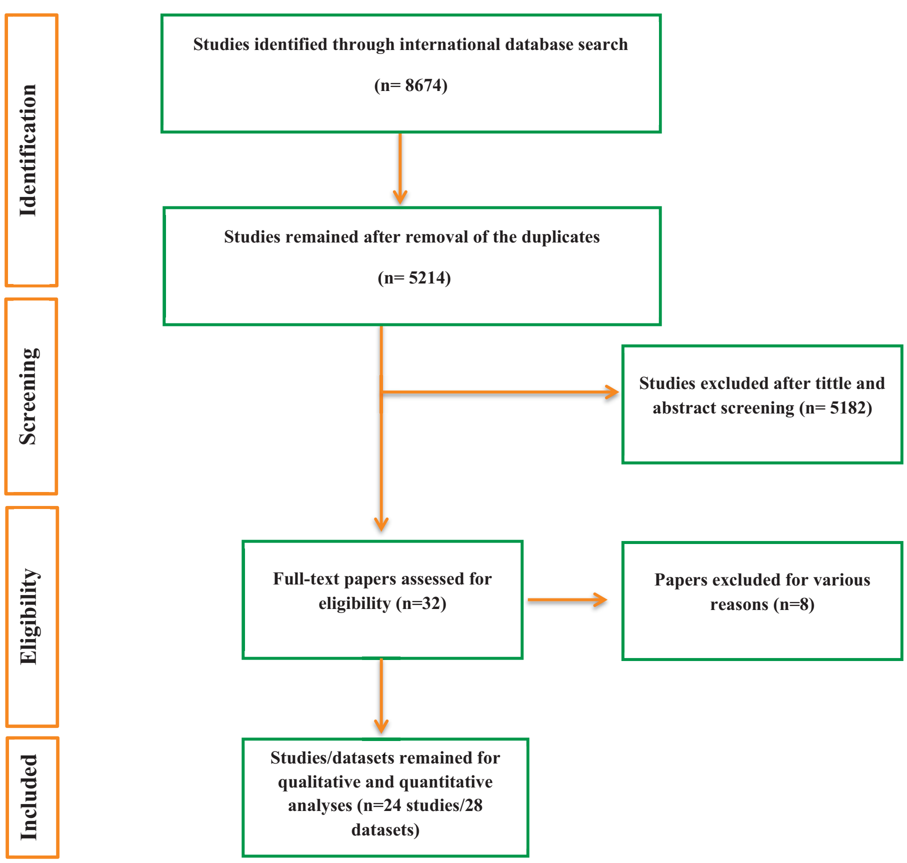

FIG. 1. Flowchart depicting the process of included studies in the present review.

Table 1. The Main Details of 24 Articles About the Occurrence and Subtype Distribution of *Blastocystis* sp. in Water Samples

Author, Year	*Water Type*	*Time Tested*	*Country*	*Total No.*	*Infected No.*	*Prevalence (%)*	*Method*	*STs*[h]
Basualdo et al., 2000	Tap/drinking water	UC[d]	Argentina	4	2	50	MIC[e]	—
Suresh et al., 2005a	Wastewater[a]	UC	Malaysia	50	34	68	MIC and CL[f]	—
Suresh et al., 2005b	Wastewater	UC	Scotland	73	13	17.8	MIC and CL	—
Basualdo et al., 2007	Tap/drinking water	2002–2003	Argentina	19	1	5.3	MIC	—
Leelayoova et al., 2008	Tap/drinking water	2005	Thailand	5	1	20	MOL[g]	ST1
Eroglu and Koltas, 2010	Tap/drinking water	UC	Turkey	25	3	12	MOL	ST1
Flores-Carrero et al., 2011	Tap/drinking water	2008–2010	Venezuela	36	0	0	MIC and CL	—
Banaticla and Rivera, 2011	Wastewater	2009–2010	Philippines	62	9	14.5	MOL	ST1, ST2
Ithoi et al., 2011	Surface water[b]	2004–2005	Malaysia	480	133	27.7	CL	—
Lee et al., 2012	Surface water	2009	Nepal	4	4	100	MOL	ST4, ST1
Khalifa et al., 2014	Various water sources[c]	2009–2013	Egypt	336	20	5.9	MIC	—
Richard et al., 2016	Tap/drinking water	UC	Malaysia	85	22	25.9	MIC	—
Karaman et al., 2017	Various water sources	2012–2013	Turkey	228	47	20.6	MIC	—
Noradilah et al., 2017	Surface water	2014	Malaysia	13	4	30.8	MIC	—
Noradilah et al., 2017	Tap/drinking water	2014	Malaysia	8	0	0	MIC	—
Noradilah et al., 2017	Various water sources	2014	Malaysia	2	0	0	MIC	—
Moreno et al., 2018	Surface water	UC	Spain	3	3	100	MOL	ST2, ST4
Koloren et al., 2018	Surface water	2011–2014	Turkey	268	10	3.7	MOL	ST1, ST3
Zahedi et al., 2019	Tap/drinking water	UC	Australia	26	16	61.5	MOL	ST1-ST4, ST6, ST8
Waters et al., 2019	Surface water	2015	Australia	134	7	5.2	MOL	—
Javanmard et al., 2019	Wastewater	2018–2019	Iran	12	5	41.7	MOL	ST2, ST6, ST8
Koloren and Karaman, 2019	Tap/drinking water	UC	Turkey	25	0	0	MOL	—
Koloren and Karaman, 2019	Surface water	UC	Turkey	75	4	5.3	MOL	ST1, ST3
Stensvold et al., 2020	Wastewater	2014	Sweden	26	26	100	MOL	ST1-ST4, ST8, ST10
Adamska, 2022	Surface water	2009–2012	Poland	36	5	13.9	MOL	ST1, ST3
Jinatham et al., 2022	Tap/drinking water	UC	Thailand	20	6	30	MOL	ST3
Elseadawy et al., 2023	Wastewater	UC	Egypt	62	4	6.4	MOL	ST2, UI[i]
Wang et al., 2024	Various water sources	2020–2022	China	334	21	6.3	MOL	ST1, ST2, ST10, ST21, ST24

[a]Both treated and untreated wastewater samples fall under this classification.
[b]Surface water includes saltwater in the ocean and freshwater in rivers, streams, and lakes.
[c]This includes unclassified water samples and groundwater samples.
[d]Unclear.
[e]Microscopic detection.
[f]Culture method.
[g]Molecular detection.
[h]Subtypes.
[i]Unidentified subtypes.

Study name	Event rate	Lower limit	Upper limit	p-Value
Basualdo, 2000	0.500	0.123	0.877	1.000
Suresh, 2005a	0.680	0.540	0.794	0.013
Suresh, 2005b	0.178	0.106	0.283	0.000
Basualdo, 2007	0.053	0.007	0.294	0.005
Leelayoova, 2008	0.200	0.027	0.691	0.215
Eroglu and Koltas, 2010	0.120	0.039	0.313	0.001
Flores-Carrero, 2011	0.014	0.001	0.182	0.003
Banaticla and Rivera, 2011	0.145	0.077	0.256	0.000
Ithoi, 2011	0.277	0.239	0.319	0.000
Lee, 2012	0.900	0.326	0.994	0.140
Khalifa, 2014	0.060	0.039	0.090	0.000
Richard, 2016	0.259	0.177	0.362	0.000
Karaman, 2017	0.206	0.159	0.264	0.000
Noradilah, 2017a	0.308	0.120	0.591	0.177
Noradilah, 2017b	0.056	0.003	0.505	0.052
Noradilah, 2017c	0.167	0.010	0.806	0.299
Moreno, 2018	0.875	0.266	0.993	0.198
Koloren, 2018	0.037	0.020	0.068	0.000
Zahedi, 2019	0.615	0.421	0.779	0.244
Waters, 2019	0.052	0.025	0.106	0.000
Javanmard, 2019	0.417	0.185	0.692	0.566
Koloren and Karaman, 2019a	0.019	0.001	0.244	0.006
Koloren and Karaman, 2019b	0.053	0.020	0.134	0.000
Stensvold, 2020	0.981	0.764	0.999	0.005
Adamska, 2022	0.139	0.059	0.293	0.000
Jinatham, 2022	0.300	0.141	0.527	0.082
Elseadawy, 2023	0.065	0.024	0.160	0.000
Wang, 2024	0.063	0.041	0.095	0.000
	0.188	0.128	0.269	0.000

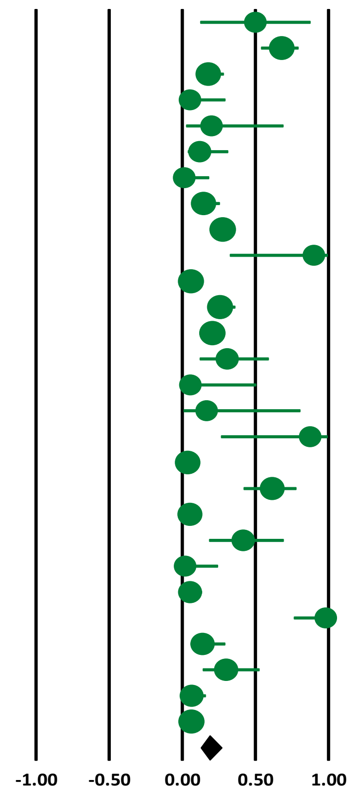

FIG. 2. The pooled prevalence of *Blastocystis* sp. in water sources, based on data from the included studies, using a random-effects model and 95% confidence intervals. *Green colors indicate the event rate/prevalence reported in each study, while the black color represents the final weighted prevalence.

Sensitivity analysis

Based on the sensitivity analysis, excluding particular datasets on *Blastocystis* sp. in water samples did not notably alter the overall frequency (Supplementary Fig. S7).

Publication bias

There was no significant publication bias in the current systematic review and meta-analysis (Egger's regression: intercept = −0.358, 95% lower limit = −2.305, 95% upper limit = 1.588, t-value = 0.378, $p = 0.354$) (Fig. 4).

Discussion

Paying attention to all water sources, especially drinking and treated water, is crucial due to the risk of contamination by parasites such as *Blastocystis* sp. These parasites can lead to health problems when consumed. Ensuring water safety and quality is vital in preventing the spread of such parasites and safeguarding public health. Monitoring and managing contaminants in water sources are key to protecting individuals who depend on these sources for drinking water (Baldursson and Karanis, 2011; Speich et al., 2016). Previous review studies have examined *Blastocystis* sp.'s prevalence and STs distribution in water samples (Attah et al., 2023; Barati et al., 2022). However, the information they assessed was either from limited studies or studies lacking total/infected sample sizes and *Blastocystis* sp. prevalence rates. This study is the first systematic review and meta-analysis to address this matter with greater thoroughness and specificity.

A previous systematic review and meta-analysis found that *Blastocystis* sp. prevalence in water samples was 10% (95% CI: 6–15%) from studies up to 2022 (10 studies) (Barati et al., 2022). In the current study, based on 24 publications, the prevalence of this enigmatic protozoa in water sources is reported as 18.8% (95% CI: 12.8–26.9%) up to 2024. Sensitivity analysis, considering the exclusion of individual studies reporting *Blastocystis* sp. prevalence, indicated that no outliers exist among the included studies capable of significantly impacting *Blastocystis* sp. prevalence (17%–20.1%) in water sources. Besides water sources, *Blastocystis* sp. has been found in edible plants and marine animals, such as fish and shellfish, highlighting its foodborne risks (Gantois et al., 2020; Jinatham et al., 2023; Ryckman et al., 2024). Given *Blastocystis* sp.'s possible pathogenicity, these findings indicate that it may reach humans via contaminated drinking water or raw/undercooked food. Thus, raising public awareness, practicing personal hygiene, and ensuring the safety of water and food intake can avert the risks

TABLE 2. SUBGROUP ANALYSIS OF *BLASTOCYSTIS* SP. IN EXAMINED WATER SAMPLES ACCORDING TO PUBLICATION YEAR, CONTINENT, WHO REGION, COUNTRY, WATER TYPE, SAMPLE SIZE, AND DIAGNOSTIC METHOD

Subgroup Variable	*Prevalence % (95% CI)*	*Heterogeneity (Q)*	*df (Q)*	I^2 *(%)*	p *value*
Publication year					
<2010	28.7 (8.7–63)	34.7	4	88.5	$p < 0.05$
2010–2014	15 (5.9–33)	64.2	5	92.2	$p < 0.05$
2015–2019	17.4 (9.1–30.8)	91.8	11	88	$p < 0.05$
2020–2024	20 (6.9–45.7)	32.7	4	87.8	$p < 0.05$
Continent					
Africa	6 (4.1–8.8)	0.1	1	0	$p > 0.05$
Asia	27.1 (16.2–41.6)	102	11	89.2	$p < 0.05$
Europe	15.8 (7.7–29.8)	57.5	8	86.1	$p < 0.05$
Oceania	22.9 (1.1–88.9)	36.2	1	97.2	$p < 0.05$
South America	9.4 (0.9–54.9)	7.3	2	72.7	$p < 0.05$
WHO region					
AMR	9.4 (0.9–54.9)	7.3	2	72.7	$p < 0.05$
EMR	12 (3.4–34.5)	15	2	86.7	$p < 0.05$
EUR	15.8 (7.7–29.8)	57.5	8	86.1	$p < 0.05$
SEAR	40.5 (12–77.3)	4.2	2	52.8	$p > 0.05$
WPR	22.7 (12.2–38.2)	131.5	9	93.1	$p < 0.05$
Country					
Argentina	19.2 (1.4–80.2)	4.1	1	75.4	$p < 0.05$
Australia	22.9 (1.1–88.9)	36.2	1	97.2	$p < 0.05$
China	6.3 (4.1–9.5)	0	0	0	$p > 0.05$
Egypt	6 (4.1–8.8)	0.2	1	0	$p > 0.05$
Iran	41.7 (18.5–69.2)	0	0	0	$p > 0.05$
Malaysia	33.3 (19.2–51.1)	31.8	5	84.3	$p < 0.05$
Nepal	9 (32.6–99.4)	0	0	0	$p > 0.05$
Philippines	14.5 (7.7–25.6)	0	0	0	$p > 0.05$
Poland	13.9 (5.9–29.3)	0	0	0	$p > 0.05$
Scotland	17.8 (10.6–28.6)	0	0	0	$p > 0.05$
Spain	87.5 (26.6–99.3)	0	0	0	$p > 0.05$
Sweden	98.1 (76.4–99.9)	0	0	0	$p > 0.05$
Thailand	28.2 (14.1–48.6)	0.2	1	0	$p > 0.05$
Turkey	7.6 (2.7–19.5)	34.4	4	88.4	$p < 0.05$
Venezuela	1.4 (0.1–18.2)	0	0	0	$p > 0.05$
Sample size					
≤100	22.5 (13.4–35.3)	164.7	22	86.6	$p < 0.05$
>100	10.9 (5.1–21.9)	100.2	4	96	$p < 0.05$
Diagnostic method					
MIC	21 (12.9–32.2)	105.6	11	89.6	$p < 0.05$
MOL	18.9 (10.2–32.4)	120.5	15	87.5	$p < 0.05$
Water type					
Surface water	17.6 (7.2–36.8)	86.1	7	91.9	$p < 0.05$
Tap/drinking water	19.1 (9.5–34.5)	31.6	9	71.5	$p < 0.05$
Uncategorized waters	9.9 (4.1–21.8)	36.3	3	91.7	$p < 0.05$
Wastewater	35.5 (13.5–66.1)	64.1	5	92.2	$p < 0.05$

associated with this protozoan parasite. The molecular analysis of the included studies revealed that water samples, particularly wastewater and drinking water, can serve as a viable source for *Blastocystis* sp. infection. Of the 17 zoonotic *Blastocystis* STs (ST1-ST10, ST12-ST14, ST16, ST23, ST35, and ST41), 7 (ST1-ST4, ST6, ST8, and ST10) have been detected in water sources, suggesting a potential for transmission of infection to humans and various animals.

Besides assessing the overall prevalence of *Blastocystis* sp. in water sources, its prevalence was also analyzed across various subgroups including publication years, countries, continents, diagnostic methods, WHO regions, sample sizes, and water types. Prevalence assessment based on publication year indicated a high occurrence of *Blastocystis* sp. before 2010 (<2010) at 28.7% (95% CI: 8.7–63%). However, due to the limited and varying number of studies and diagnostic methods used across different publication years, establishing a clear trend in *Blastocystis* sp. prevalence based on publication year is challenging. The evaluation of *Blastocystis* sp.'s prevalence in continents and countries revealed higher contamination rates in Asian (27.1%; 95% CI: 16.2–41.6%) and Oceania (22.9%; 95% CI: 1.1–88.9%) water samples, as well as in Sweden (98.1%; 95% CI: 76.4–99.9%), Spain (87.5%; 95% CI: 26.6–99.3%), Iran (41.7%; 95% CI: 18.5–69.2%), and Thailand (28.2%; 95% CI: 14.1–48.6%). While unequal and limited studies can impact results, enhancing control and prevention measures and raising public awareness in these regions should not be ignored. The highest prevalence of

Group by Water sources	Study name	Event rate	Lower limit	Upper limit	p-Value
Surface water	Ithoi, 2011	0.277	0.239	0.319	0.000
Surface water	Lee, 2012	0.900	0.326	0.994	0.140
Surface water	Noradilah, 2017a	0.308	0.120	0.591	0.177
Surface water	Moreno, 2018	0.875	0.266	0.993	0.198
Surface water	Koloren, 2018	0.037	0.020	0.068	0.000
Surface water	Waters, 2019	0.052	0.025	0.106	0.000
Surface water	Koloren and Karaman, 2019b	0.053	0.020	0.134	0.000
Surface water	Adamska, 2022	0.139	0.059	0.293	0.000
Surface water		0.176	0.072	0.368	0.003
Tap/drinking water	Basualdo, 2000	0.500	0.123	0.877	1.000
Tap/drinking water	Basualdo, 2007	0.053	0.007	0.294	0.005
Tap/drinking water	Leelayoova, 2008	0.200	0.027	0.691	0.215
Tap/drinking water	Eroglu and Koltas, 2010	0.120	0.039	0.313	0.001
Tap/drinking water	Flores-Carrero, 2011	0.014	0.001	0.182	0.003
Tap/drinking water	Richard, 2016	0.259	0.177	0.362	0.000
Tap/drinking water	Noradilah, 2017b	0.056	0.003	0.505	0.052
Tap/drinking water	Zahedi, 2019	0.615	0.421	0.779	0.244
Tap/drinking water	Koloren and Karaman, 2019a	0.019	0.001	0.244	0.006
Tap/drinking water	Jinatham, 2022	0.300	0.141	0.527	0.082
Tap/drinking water		0.191	0.095	0.345	0.000
Uncategorized waters	Khalifa, 2014	0.060	0.039	0.090	0.000
Uncategorized waters	Karaman, 2017	0.206	0.159	0.264	0.000
Uncategorized waters	Noradilah, 2017c	0.167	0.010	0.806	0.299
Uncategorized waters	Wang, 2024	0.063	0.041	0.095	0.000
Uncategorized waters		0.099	0.041	0.218	0.000
Wastewater	Suresh, 2005a	0.680	0.540	0.794	0.013
Wastewater	Suresh, 2005b	0.178	0.106	0.283	0.000
Wastewater	Banaticla and Rivera, 2011	0.145	0.077	0.256	0.000
Wastewater	Javanmard, 2019	0.417	0.185	0.692	0.566
Wastewater	Stensvold, 2020	0.981	0.764	0.999	0.005
Wastewater	Elseadawy, 2023	0.065	0.024	0.160	0.000
Wastewater		0.355	0.135	0.661	0.355

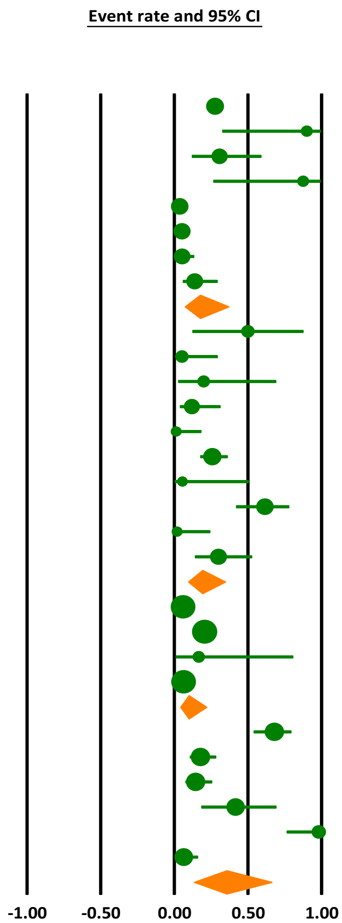

FIG. 3. The global prevalence of *Blastocystis* sp. in various water samples.

Blastocystis sp. was reported in the waters of the SEAR WHO region (40.5%; 95% CI: 12–77.3%) and in sample sizes of ≤100 (22.5%; 95% CI: 13.4–35.3%). This highlights the importance of avoiding small sample sizes in epidemiological studies to minimize significant calculation errors in determining infection prevalence within specific groups. The

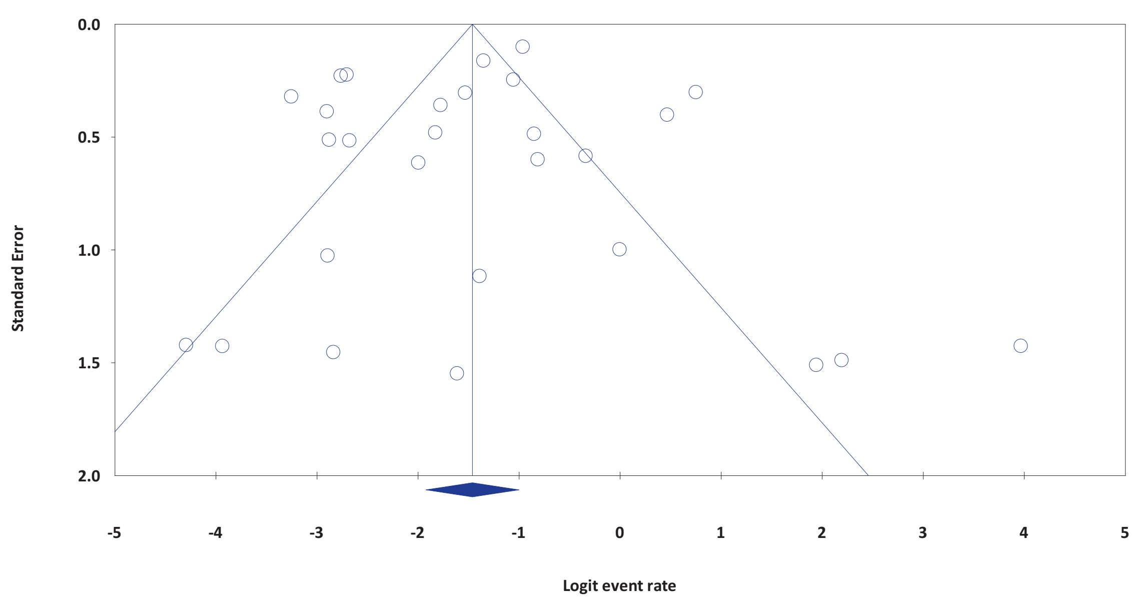

FIG. 4. The funnel plot illustrates the publication bias within this study.

pooled prevalence reported by molecular and microscopic methods was 18.9% (95% CI: 10.2 – 32.4%) and 21% (95% CI: 12.9 – 32.2%), respectively. Despite differences in sample size and number of studies, this suggests a potential error in microscopic methods when detecting various forms of *Blastocystis* sp. in water samples. Among the four groups of surface water, tap/drinking water, wastewater, and unclassified water samples, the highest prevalence of *Blastocystis* sp. was reported with 35.5% (95% CI: 13.5–66.1%) and 19.1% (95% CI: 9.5–34.5%) in wastewater and tap/drinking water, respectively. While wastewater may justify the high prevalence of parasitic infections like *Blastocystis* sp. due to some contamination, drinking water should generally be free of infectious and pathogenic agents. These findings underscore the critical need for the proper treatment and purification of drinking water to ensure it is safe and accessible for public consumption.

In the present study, significant publication bias among the included studies was not reported. Furthermore, the current review and meta-analysis, like most similar reviews, encountered certain limitations. These include: a restricted number of studies, the lack of diverse geographical representation, small sample sizes, prevalence reports relying on single studies/datasets, etc. Given these constraints, it is advisable to interpret the study results with care and caution.

Conclusion

The current study reported a relatively high prevalence (18.8%) of *Blastocystis* sp. in water sources, indicating that drinking water could be a source of *Blastocystis* sp. infection. Moreover, a variety of *Blastocystis* STs, particularly zoonotic ones (ST1-ST4, ST6, ST8, and ST10), were identified in water sources, highlighting the potential contamination risk for humans and various animals. These findings, based on limited data and research, underscore the necessity for comprehensive and in-depth studies to gain a thorough understanding of this issue. Overall, prioritizing human health necessitates the implementation of effective monitoring and regulation of drinking water treatment and pollution control.

Ethics approval

The present study was approved by the Ethics Committee of Ardabil University of Medical Sciences, Iran (IR.ARUMS.REC.1403.273).

Authors' Contributions

F.M. and A.A. conceived and designed the study. A.A., F.M., F.H., M.M., and M.R.M. extracted the data. A.A. and F.M. performed the analyses. A.A. and F.M. wrote and revised the paper. All authors read and approved the final article.

Availability of Data and Materials

The datasets supporting the conclusions of this article are included in the article and its additional files.

Disclosure Statement

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplementary Material

Supplementary Table S1
Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3
Supplementary Figure S4
Supplementary Figure S5
Supplementary Figure S6
Supplementary Figure S7

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