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REVIEW

Prevalence of free-living amoebae in swimming pools and recreational waters, a systematic review and meta-analysis

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Abstract

Free-living amoebae (FLA) are cosmopolitan microorganisms known to be pathogenic to humans who often have a history of contact with contaminated water. Swimming pools and recreational waters are among the environments where the greatest human exposure to FLA occurs. This study aimed to determine the prevalence of FLA in swimming pools and recreational waters, through a systematic review and meta-analysis that included studies published between 1977 and 2022. A total of 106 studies were included and an overall prevalence of FLA in swimming pools and recreational waters of 44.34% (95% CI = 38.57–50.18) was found. Considering the studies published up to 2010 (1977–2010), between 2010 and 2015, and those published after 2010 (> 2010–2022), the prevalence was 53.09% (95% CI = 43.33–62.73) and 37.07% (95% CI = 28.87–45.66) and 45.40% (95% CI = 35.48–55.51), respectively. The highest prevalence was found in the American continent (63.99%), in Mexico (98.35%), and in indoor hot swimming pools (52.27%). The prevalence varied with the variation of FLA detection methods, morphology (57.21%), PCR (25.78%), and simultaneously morphology and PCR (43.16%). The global prevalence by genera was *Vahlkampfia* spp. (54.20%), *Acanthamoeba* spp. (33.47%), *Naegleria* spp. (30.95%), *Hartmannella* spp./*Vermamoeba* spp. (20.73%), *Stenamoeba* spp. (12.05%), and *Vannella* spp. (10.75%). There is considerable risk of FLA infection in swimming pools and recreational waters. Recreational water safety needs to be routinely monitored and, in case of risk, locations need to be identified with warning signs and users need to be educated. Swimming pools and artificial recreational water should be properly disinfected. Photolysis of NaOCl or NaCl in water by UV-C radiation is a promising alternative to disinfect swimming pools and artificial recreational waters.

Keywords Free-living amoebae · Risk of infection · Swimming pool · Recreational waters

Introduction

Free-living amoebae (FLA) are cosmopolitan and ubiquitous microorganisms widely distributed in the environment and can be opportunistic and/or pathogenic

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(Visvesvara et al. 2007; Bellini et al. 2022). They have been isolated from many natural and anthropogenic environmental matrices, including plants, soil, air conditioning dust, bottled mineral water, drinking water treatment and distribution system, and cooling towers (Landell et al. 2013; Maschio et al. 2015; Javanmard et al. 2017; Soares et al. 2017; Wopereis et al. 2020; Pazoki et al. 2020). They have also been isolated from contact lenses, swimming pools, and other recreational waters (Fabres et al. 2016; Bunsuwansakul et al. 2019; Santos et al. 2021; Fabros et al. 2021).

Among its representatives with importance for human health, the genera *Acanthamoeba*, *Naegleria*, and *Balamuthia* stand out. *Acanthamoeba* spp. and its abundance in water bodies seem to be favored by higher temperatures (Kang et al 2020). These protozoa can cause illnesses in healthy people, such as *Acanthamoeba* keratitis (AK) which primarily affects contact lens wearers, usually due to lens wear while swimming or improper lens cleaning (Dos Santos et al. 2018). In immunosuppressed individuals, it can cause granulomatous amebic encephalitis (GAE), which can be fatal (Visvesvara et al. 2007; Sarink et al. 2022). *Acanthamoeba* spp. have also been reported to cause skin infections (Murakawa et al. 1995; Paltiel et al. 2004). In addition, it was isolated from 26% (17/63) of critically ill patient urine samples (Santos et al. 2009); similarly, *Acanthamoeba* (T4) was isolated from 22% (11/50) of urine samples collected from patients with recurrent urinary tract infection (Saberi et al. 2021).

Naegleria fowleri is known as a "brain-eating amoeba" and primarily affects healthy young people using recreational waters, causing primary amoebic meningoencephalitis (PAM) (Fowler and Carter, 1965). PAM is a serious and usually fatal disease if adequate treatment is not initiated at the onset of symptoms (Król-Turmińska and Olender 2017). The rapid deterioration in the health status of patients affected by PAM, combined with the ease of being confused with bacterial meningoencephalitis (since the symptoms are similar), as well as erratic or late diagnosis, contributes to a high prevalence of deaths (> 97%) (Capewell et al. 2015; Johnson et al. 2016). *Balamuthia mandrillaris* and *Sappinia pedatta* also cause encephalitis (Gelman et al. 2001; Visvesvara et al. 2007; Cope et al. 2019); however, there are no reports of the isolation of *S. diploidea*/*pedatta* from swimming pools and recreational waters.

The FLA essentially have three forms of life, namely, the trophozoite form (with or without flagellum) and the flagellated form which are the active forms of the protozoan, in which it may feed, reproduce, and express pathogenicity, and the form of cysts (which is the form of environmental resistance). Cysts have a double-layer wall made essentially of cellulose (Garajová et al. 2019) that protects the protozoan against unfavorable conditions (e.g., food shortages, dissection, extreme pH, and temperatures) or antimicrobial agents (e.g., NaCl, chlorine, drugs, UV, heat) (Aksozek et al. 2002; Thomas et al. 2008; Chaúque and Rott 2021a, b; Chaúque et al. 2021). FLA are considered the "Trojan Horse" of the microbial world, as phylogenetically diverse microorganisms including bacteria, fungi, and viruses survive and multiply within them; these microorganisms are called amoeba-resistant microorganisms (ARM) (Greub and Raoult 2004; Scheid 2014; Delafont et al. 2016; Hubert et al. 2021; Rayamajhee et al. 2021). A wide range of pathogens of public health importance have been described as being ARM, including *Legionella pneumophila*, *Mycobacterium leprae*, *Pseudomonas* spp., *Candida auris*, and various viruses (Maschio et al. 2015; Staggemeier et al. 2016; Balczun and Scheid 2017; Turankar et al. 2019; Nisar et al. 2020; Hubert et al. 2021). The participation of FLA in the environmental persistence of severe acute respiratory syndrome 2 (SARS-CoV-2) has also been proposed (Chaúque et al. 2022; Dey et al 2022). All these aspects that characterize the profile of FLA constitute the main attributes that determine the great importance of these protozoa for human health and the environment.

Although increasingly prevalent, diseases caused by FLA remain rare; however, the presence of these protozoa, especially in the aquatic environment, is well documented (Milanez et al. 2022; Stapleton 2021; Saburi et al. 2017; Caumo et al. 2009). The presence of FLA in swimming pools and other recreational waters is of concern, as they can be pathogenic or opportunistic and/or lead to the persistence of non-amoebic pathogens in the water, including waters treated with chlorine-based disinfectants (Siddiqui and Khan 2014; Kiss et al. 2014; Dey et al. 2021). It was determined that the prevalence of *Naegleria* spp. in different water sources around the world (considering data from 35 countries) was 26.42%, in recreational water it was 21.27% (10.80–34.11), and in swimming pools was 44.80% (16.19–75.45) (Saberi et al. 2020); however, the global prevalence of FLA in swimming pools and recreational waters remains to be determined. The present systematic review and meta-analysis aimed to determine the prevalence of FLA in swimming pools and recreational waters worldwide.

Methods

Article search strategy

The present study, which aimed to determine the prevalence of FLA in swimming pools and recreational waters, was planned and carried out based on the PRISMA 2020 guidelines (Page et al. 2021) (Fig. 1). The search for scientific articles was performed in different databases, including Web of Science, Scopus, PubMed, ScienceDirect, EMBASE, ProQuest, and CAPES periódicos, between July 4 and 9, 2022. In these databases, articles were retrieved using a combination of the following search terms combined with appropriate Boolean operators: "Free-living amoeba," "swimming pool," "recreational water," "prevalence," "epidemiology," and "hot springs." The references of the selected articles were examined in search of some interesting literature. The search for articles in the database was performed by B.J.M.C, and the accuracy of the searches was verified by D.L.S.

Selection and exclusion criteria

The screening focused essentially on the title and then on the abstract of the articles. All retrieved articles written in English (reporting primary data), with accessible full text, dealing with the presence of FLA in swimming pools and human recreation waters were selected. Studies based on natural surface waters that do not clearly state that the samples were collected in places where human recreational activities certainly take place were not selected. Studies whose data were insufficient, unclear, or duplicated were excluded. Case studies that do not report the prevalence of FLA in swimming pools and human recreation waters were also excluded.

Data analysis procedure

Data were independently extracted and verified by two authors (B.J.M.C and D.L.S); data verification was performed three times. Data extracted from all articles that met the inclusion criteria were included in the calculation of the global prevalence of FLA in swimming pools and recreational waters. To calculate the prevalence of each FLA genera, only data extracted from articles that included molecular methods for the identification of FLA were used. Data analysis was performed by two authors (D.A and B.J.M.C) using Stata software (version 14; Stata Corp, College Station, TX, USA) and GraphPad prism 8.02. A random-effects model meta-analysis was performed to estimate the combined and weighted prevalence of FLA in swimming pools and recreational waters, using a 95% confidence interval, and the results are visualized using a forest plot. Cochran's Q test (chi-square) and the Higgins I^2 statistic were used to calculate the heterogeneity index among the selected studies. I^2 values < 25%, 25%–50%, and > 50% meant low, moderate, and high heterogeneity, respectively. The Egger's test was used to assess the significance of publication bias among the selected studies; $P < 0.001$ was considered significant.

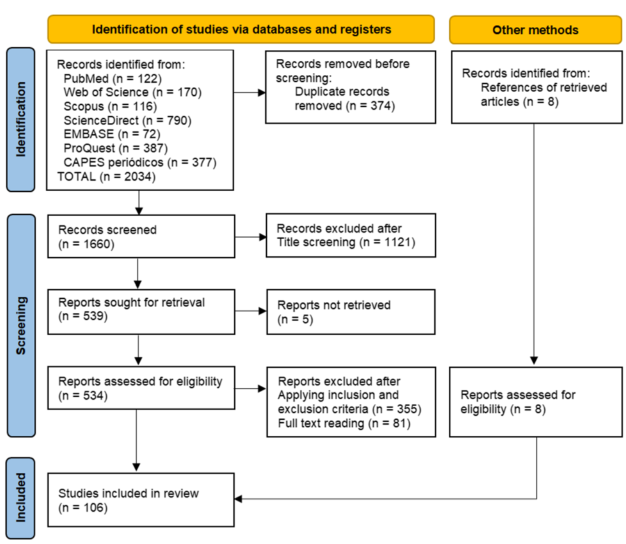

Fig. 1 Details of the article retrieval and selection steps based on PRISMA 2020

Results

From the total of 2034 documents returned by the databases accessed, using the search strategy and inclusion criteria described above, 106 articles were selected (Table 1). These studies are distributed in a total of 30 countries, namely Iran (33), Taiwan (12), Egypt (8), Malaysia (6), Brazil (4), Italy (4), Turkey (4), USA (4), Mexico (3), Saudi Arabia (3), China (2), France (2), Philippines (2), Spain (2), and Thailand (2). One study was included from each of the following countries: Belgium, Bulgaria, Cape Verde, Chile, Finland, Germany, Hungary, India, Jamaica, Japan, Norway, Poland, Portugal, Sweden, and Switzerland. Among the studies, 74.52% (79/106) used or included molecular methods to identify FLA, while 25.47% (27/106) used only morphological methods.

The included studies were published between 1977 and 2022, and the distribution of studies by year and the average percentage value of positive samples per year are shown in Fig. 2. FLA were detected in at least 1 sample of 97.17% (103/106) of selected studies (Table 1).

Publication bias was checked by Egger's regression test, showing that it may have a substantial impact on total prevalence estimate (Egger bias: 6.8, $P < 0.001$) (Fig. 3). This suggests that the reported global prevalence may have been impacted by publication bias.

Based on the random-effects model meta-analysis, the pooled prevalence of FLA in water sources was 44.34% (95% CI = 38.57–50.18). The included studies demonstrated a strong heterogeneity ($Q = 2198.0$, df = 102, $I^2 = 95.4\%$, $P < 0.0001$) (Fig. 4).

The global prevalence of FLA in swimming pools and recreational waters considering studies published up to 2010 (1977–2010) was considerably higher 53.09% (95%

Table 1 Description of included studies reporting the prevalence of live amoebae in swimming pools and recreational waters

References	Country	Sample source (total)	Analyzed samples	Positive samples	Methods	Identity
Brown And Cursons (1977)	Norway	Swimming area	50	34	Morphology	*Acanthamoeba* spp., *Naegleria fowleri*, and *Naegleria gruberi*
Lyons and Kapur (1977)	USA	Swimming pool	30	27	Morphology	*Acanthamoeba* spp. and/or *Hartmannella* spp.
Pernin and Riany (1978)	France	Swimming pool (9)	44	39	Morphology	*Acanthamoeba* spp., *Hartmannella* spp., and *Naegleria* spp.
De Jonckheere (1979)	Belgium	Swimming pool	16	13	Morphology	*Acanthamoeba* spp. and *Naegleria* spp.
Janitschke et al. (1980)	Germany	Swimming pool	14	10	Morphology	*Acanthamoeba* spp.
Scaglia et al. 1983	Italy	Thermal pool and mud basin spa	30	7	Morphology, fluorescent-antibody technique	*N. australiensis*
Gogate and Deodhar (1985)	India	Public swimming pool	12	1	Morphology	*N. fowleri*
Scaglia et al. 1987	Italy	Thermal bath and mud basin (34)	51	34	Morphology, pathogenicity test	*Naegleria* spp., *Acanthamoeba* spp., *Vahlkampfia* spp., and *Hartmannella* spp.
Hamadto et al. 1993	Egypt	Swimming pool (16)	16	12	Morphology, pathogenicity test	*Naegleria* spp. and *Acanthamoeba* spp.
Penas-Ares et al. 1994	Spain	Thermal spa water (12)	12	8	Morphology	*Vahlkampfia longicauda*, *Vahlkampfia salina*, *Vahlkampia baltica*, *Vahlkampfia* sp., *A. polyphaga*, *Acanthamoeba lenticulata*, *Naegleria* sp., *Lingulamoeba* sp., *Paramoeba aesturina*, and *Flabellula* sp.
Vesaluoma et al. (1995)	Finland	Public swimming pool and whirlpool (21)	34	14	Morphology	*Acanthamoeba* spp., *Vexillifera* spp., *Flabellula* spp., *Hartmannella* spp., and *Rugipes* spp.
Munoz et al. 2003	Chile	Swimming pool	8	5	Morphology, PCR	*H. vermiformes*, *Vanella* spp., *Naegleria* spp., and *Acanthamoeba* spp.
Sheehan et al. 2003	USA	Hot spring (22)	22	12	Morphology, PCR	*N. australiensis*, *N. dobsoni*, *N. americana*, *N.pagei*, *N. polaris*, and *N. fultoni*
Izumiyama et al. 2003	Japan	Whirlpool bath and hot spring spa (251)	549	197	Morphology, PCR	*N. fowleri*, *N. lovaniensis*, and *N. australiensis*
Górnik and Kuźna-Grygiel (2004)	Poland	Public swimming pools (13)	72	42	Morphology	*Acanthamoeba* spp.
Tsvetkova et al. 2004	Bulgaria	Swimming pool (6)	31	15	Morphology, PCR	*Acanthamoeba* spp. and *Hartmannella* spp.
Lekkla et al. (2005)	Thailand	Hot spring (13)	68	26	Morphology	*Acanthamoeba* spp. and *Naegleria* spp.
Sukthana et al. 2005	Thailand	Hot spring	57	15	Morphology	*Naegleria* spp. and *Acanthamoeba* spp.
Rezaeian et al 2008	Iran	Swimming pool	2	2	Morphology	*Acanthamoeba* spp.
Caumo et al. (2009)	Brazil	Swimming pool	65	13	Morphology, PCR	*Acanthamoeba* spp.
Gianinazzi et al. (2009)	Switzerland	Indoor hot swimming pool	1	1	Morphology, PCR	*Acanthamoeba lenticulata*

Table 1 (continued)

References	Country	Sample source (total)	Analyzed samples	Positive samples	Methods	Identity
Hsu et al. (2009a, b)	Taiwan	Recreational hot spring	55	9	PCR	*Acanthamoeba griffini* and *Acanthamoeba jacobsi*
Hsu et al. (2009a)	Taiwan	Mud recreation area water	34	20	Morphology, PCR	*Acanthamoeba* spp., *Hartmannella spp.*, and *Naegleria* spp.
Gianinazzi et al. (2010)	Sweden	Hot springs (4)	31	9	Morphology, PCR	*Acanthamoeba healyi*, *Stenoamoeba* sp., *Hartmannella vermiformis*, and *Echinamoeba exundans*
Huang and Hsu (2010a, b)	Taiwan	Hot spring and waste water in recreation area	52	11	PCR	*Acanthamoeba* T1, *Acanthamoeba* T2, *Acanthamoeba* T3, *Acanthamoeba* T4, *Acanthamoeba* T5, *Acanthamoeba* T6, and *Acanthamoeba* T15
Huang and Hsu (2010a)	Taiwan	Hot spring and hot spring facilities	106	15	Morphology, PCR	*Naegleria lovaniensis*, *Naegleria australiensis*, *Naegleria clarki*, *Naegleria americana*, and *Naegleria pagei*
Init et al. (2010)	Malaysia	Public swimming pool (14)	14	14	Morphology	*Acanthamoeba* spp. and *Naegleria* spp.
Lares-Villa et al. 2010	Mexico	Natural recreational water (2)	24	24	PCR	Thermophilic amoebae, thermophilic *Naegleria* spp., and *N. fowleri*
Badirzadeh et al. (2011)	Iran	Recreational hot spring	28	12	Morphology, PCR	Vahlkampfiid and *Acanthamoeba castellanii* T4
Huang and Hsu (2011)	Taiwan	Recreational water	107	19	PCR	*Naegleria* spp.
Ithoi et al. (2011)	Malaysia	Recreational pool, lake, and stream	33	33	Morphology, PCR	*Naegleria* spp.
Nazar et al. 2011	Iran	Water in recreation area	50	16	Morphology, PCR	*Acanthamoeba* spp. T4 and *Acanthamoeba* spp. T5
Alves et al. (2012)	Brazil	Public swimming pool (7)	7	7	Morphology, PCR	*Acanthamoeba* spp.
Kao et al. (2012a, b, c)	Taiwan	Recreation and drinking water source (2)	211	13	PCR	*Naegleria philippinensis*, *N. clarki*, *Naegleria gálica*, *N. americana*, *N. australiensis*, *Naegleria dobsoni*, *N. gruberi*, and *Naegleria schusteri*
Kao et al. (2012a)	Taiwan	Recreational hot spring (4)	60	9	Morphology, PCR	*Acanthamoeba* T15, *Acanthamoeba* T4, *Acanthamoeba* T2, and *Acanthamoeba* spp.
Kao et al. (2012b)	Taiwan	Hot spring	60	26	Morphology, PCR	*N. australiensis, N. lovaniensis*, *Naegleria mexicana*, and *N. gruberi*
Nazar et al. (2012)	Iran	Recreational water (22)	50	8	Morphology, PCR	*Hartmannella vermiformis* and *Vannella persistens*
Niyyati et al. (2012)	Iran	River recreation area (10)	55	15	Morphology, PCR	*Acanthamoeba* spp. (T4 and T15) and *Naegleria* spp. (*N. pagei*, *N. clarki*, and *Naegleria fultoni*)
Rahdar et al. 2012	Iran	Swimming pool (4)	4	2	Morphology, PCR	*Acanthamoeba* T4

Table 1 (continued)

References	Country	Sample source (total)	Analyzed samples	Positive samples	Methods	Identity
Solgi et al. (2012a, b)	Iran	Hot spring	30	8	Morphology, PCR	*Hartmannella vermiformis* and *Naegleria* (*N. carteri* and *Naegleria* spp.)
Solgi et al. (2012a)	Iran	Therapeutic hot spring	60	12	Morphology, PCR	*Acanthamoeba* T4 and T3
Kao et al. 2013a, b	China	Thermal spring water	48	4	PCR	*Naegleria* spp.
Kao et al. 2013a	China	Thermal spring	48	5	PCR	*Acanthamoeba* spp.
Moussa et al. (2013)	France	Recreational geothermal waters (6)	73	35	Morphology, PCR	*N. fowleri* and *N. lovaniensis*
Tung et al. (2013)	Taiwan	Hot spring (1)	25	13	Morphology, PCR	*Naegleria* spp. (*N. fowleri*) and *Acanthamoeba* spp.
Al-Herrawy et al. (2014)	Egypt	Swimming pool (10)	120	59	Morphology, PCR	*Acanthamoeba* spp.
Ji et al. (2014)	Taiwan	Hot spring	61	29	Morphology, PCR	*Acanthamoeba* spp.
Ji et al. (2014)	Taiwan	Hot spring	61	17	Morphology, PCR	*Naegleria* spp.
Ji et al. (2014)	Taiwan	Hot spring	61	11	Morphology, PCR	*Vermamoeba vermiformis*
Kiss et al. (2014)	Hungary	Swimming pool (20)	164	68	Morphology, PCR	*Acanthamoeba* spp.
Onichandran et al. 2014	Philippines	Recreational river (4)	23	12	Morphology, PCR	*Acanthamoeba* spp. and *Naegleria* spp.
Sifuentes et al. (2014)	USA	Recreational water (33)	103	18	PCR	Thermophilic amoebae and *N. fowleri*
Behniafar et al. (2015)	Iran	Recreational water and hot spring	40	7	Morphology, PCR	*Acanthamoeba* spp.
Evyapan et al. (2015)	Turkey	Swimming pool and hot spring	50	21	Morphology, PCR	*Acanthamoeba* spp., *Acanthamoeba griffini T3*, *Acanthamoeba castellanii* T4, and *A. jacobsi* T15
Niyyati et al. (2015a, b)	Iran	Recreational water (lakes, pools, and streams)	60	9	Morphology, PCR	*N. australiensis* and *N. pagei*
Niyyati et al. (2015a)	Iran	Recreational water	50	15	Morphology, PCR	*A. castellanii* T4
Todd et al. (2015)	Jamaica	Recreational water	83	42	Morphology, PCR	*Acanthamoeba* T4, *Acanthamoeba* T5, *Acanthamoeba* T10, and *Acanthamoeba* T11
Al-Herrawy et al. (2016)	Egypt	Swimming pool (1)	48	30	Morphology, PCR	*Acanthamoeba* spp., *Naegleria* spp., and *Hartmannella*
Armand et al. (2016)	Iran	Swimming pool	17	12	Morphology, PCR	*Vermamoeba* spp. and *Acanthamoeba* spp.
Azlan et al. 2016	Malaysia	Recreational lake	7	7	Morphology	*Acanthamoeba* spp.
Fabres et al. (2016)	Brazil	Hot tubs and thermal pool	72	20	Morphology, PCR	*Acanthamoeba* T3, *Acanthamoeba* T4, *Acanthamoeba* T5, and *Acanthamoeba* T15
Latifi et al. (2016)	Iran	Hot spring	66	2	Morphology, PCR	*Balamuthia mandrillaris*
Niyyati et al. (2016a, b)	Iran	Geothermal water source	40	20	PCR	*Acanthamoeba* T4 and T2
Niyyati et al. (2016a)	Iran	Recreational water	25	25	Morphology	Vahlkampfidae spp., *Acanthamoeba* spp., *Thecamoeba* spp., and *Miniamoebae* spp.

Table 1 (continued)

References	Country	Sample source (total)	Analyzed samples	Positive samples	Methods	Identity
Al-Herrawy et al. (2017)	Egypt	Swimming pool (2)	144	37	Morphology, PCR	*Acanthamoeba* spp. and *Naegleria* spp.
Di Filippo et al. (2017)	Italy	Geothermal spring	36	26	Morphology, PCR	*N. australiensis*, *Naegleria itálica*, *N. lovaniensis*, and *Naegleria* spp.
Javanmard et al. (2017)	Iran	Swimming pool and hot spring	33	6	Morphology, PCR	*N. pagei* and *N. gruberi*
Latifi et al. (2017)	Iran	Recreation hot spring	22	12	Morphology, PCR	*Naegleria* spp. (*N. australiensis*, *N. americana*, *N. dobsoni*, *N. pagei*, *N. polaris*, and *N. fultoni*)
Mafi et al. (2017)	Iran	Swimming pool and park pond (40)	75	18	Morphology	*Acanthamoeba* spp., *Hartmannella* spp., and *Vahlkampfiids*
Reyes-Batlle et al. (2017)	Spain	Recreational water (10)	10	1	Morphology, PCR	*Naegleria* spp.
Toula and Elahl 2017	Saudi Arabia	Swimming pool (6)	16	6	Morphology	*Acanthamoeba* spp. and *Naegleria* spp.
Dodangeh et al. (2018)	Iran	Recreational hot spring	24	11	Morphology, PCR	*Acanthamoeba castellanii* T4
Ghaderifar et al. 2018	Iran	Parks pond water (13)	90	31	Morphology, PCR	*Acanthamoeba* T4
Hikal et al (2018)	Egypt	Swimming pool (5)	100	24	Morphology, PCR	*Naegleria fowleri*
Hikal et al. (2018)	Egypt	Swimming pool (5)	100	79	Morphology, PCR	*Naegleria* spp.
Lares-Jiménez et al. (2018)	Mexico	Hot spring (1)	8	8	Morphology, PCR	*N. lovaniensis*, *A. jacobsi*, *Stenamoeba* sp., and *Vermamoeba vermiformis*
Latiff et al. (2018)	Malaysia	Recreational hot spring (5)	52	38	Morphology	*Acanthamoeba* spp. and *Naegleria spp.*
Poor et al. (2018)	Iran	Swimming pool and hot tubs (10)	40	8	Morphology, PCR	*Acanthamoeba* T3 and *Acanthamoeba* T4
Vijayakumar (2018)	Saudi Arabia	Pools and recreation waters	27	7	Morphology	*Acanthamoeba* spp.
Xue et al. 2018	USA	lake recreation areas (10)	160	56	PCR	*N. fowleri*
Gabriel et al. 2019	Malaysia	Recreational place	57	40	Morphology, PCR	*Acanthamoeba* spp. and *Naegleria* spp.
Haddad et al. (2019)	Iran	Hot springs	54	15	Morphology, PCR	*Acanthamoeba castellanii* T4, *Vermamoeba vermiformis*, *N. australiensis*, *N. pageii*, and *N. gruberi*
Hussain et al. (2019)	Malaysia	Recreational hot spring (5)	50	38	Morphology, PCR	*Acanthamoeba* T4, T15, T3, T5, T11, and T17
Maghsoodloorad et al. 2019	Iran	Recreational park water	30	8	Morphology, PCR	*Acanthamoeba* spp. T4 and *Acanthamoeba* spp. T15
Salehi et al. 2019	Iran	Park pool and swimming pool	14	12	Morphology, PCR	*Acanthamoeba* T2, T4, T5, and T11
Attariani et al. (2020)	Iran	Swimming pool	42	3	Morphology, PCR	*Acanthamoeba* spp.
Ballares et al. 2020	Philippines	Recreational water (6)	16	6	Morphology, PCR	*Acanthamoeba* T4, *Acanthamoeba* T5, and *Acanthamoeba* T9
Bonilla-Lemus et al. 2020	Mexico	Recreational water (9)	9	9	Morphology, PCR	*N. australiensis*, *N. gruberi*, *N. fowleri*, *N. clarki*, and *N. pagei*
Değerli et al. (2020)	Turkey	Thermal swimming pool	434	148	Morphology, PCR	*Acanthamoeba* spp. and *Naegleria* spp.

Table 1 (continued)

References	Country	Sample source (total)	Analyzed samples	Positive samples	Methods	Identity
El-Badry et al. 2020	Egypt	Swimming pool (7)	28	0	Morphology, PCR	
Esboei et al. (2020)	Iran	Swimming pools	30	12	Morphology, PCR	*Acanthamoeba* T4
Latifi et al. (2020)	Iran	Hot spring and beach	81	54	Morphology, PCR	*Acanthamoeba* (T3, T4 e T5), *V. vermiformis*, and *Naegleria* spp.
Paknejad et al. (2020)	Iran	Swimming pool and bathtub	166	31	Morphology, PCR	*Acanthamoeba* T3, *Acanthamoeba* T4, *Acanthamoeba* T11, *Acanthamoeba* sp., *Protacanthamoeba bohemica*, and *N. lovaniensis*
Sarmadian et al. (2020)	Iran	Swimming pool (6)	6	1	Morphology	*Acanthamoeba* spp.
Sarmadian et al. (2020)	Iran	Swimming pool (6)	576	1	Morphology	*Acanthamoeba* spp.
Zeybek and Türkmen 2020	Turkey	Swimming pool	25	7	Morphology, FISH	
Aykur and Dagci (2021)	Turkey	Swimming pool	26	3	PCR	*Acanthamoeba* T2, T4, and T5
Bakri et al. 2021	Saudi Arabia	Swimming pool	10	4	Morphology, PCR	*Acanthamoeba* spp. and *Naegleria* spp.
Berrilli et al. (2021)	Italy	Hot spring (2)	36	33	Morphology, PCR	*V. vermiformisi*, *N. australiensisi*, *Acanthamoeba* T4, and *Acanthamoeba* T15
Eftekhari-Kenzerki et al. (2021)	Iran	Indoor public swimming pool (20)	80	32	Morphology, PCR	*Acanthamoeba* spp.
Reyes-Batlle et al. (2021)	Portugal	Swimming pool facilities (20)	20	0	PCR	
Nageeb et al. (2022)	Egypt	Swimming pool (2)	8	0	Morphology, PCR	
Rocha et al. 2022	Brazil	Swimming pool (9)	36	15	Morphology	*Acanthamoeba* spp. and *Naegleria* spp.
Salehi et al. 2022	Iran	Swimming pool and park pool	20	17	Morphology, PCR	*Acanthamoeba* (T2, T3, T4, T11, and T15)
Sousa-Ramos et al. 2022	Cape Verde	Recreational fountain and swimming pool	4	2	Morphology, PCR	*Acanthamoeba* sp. T4 and *Vannella* sp.

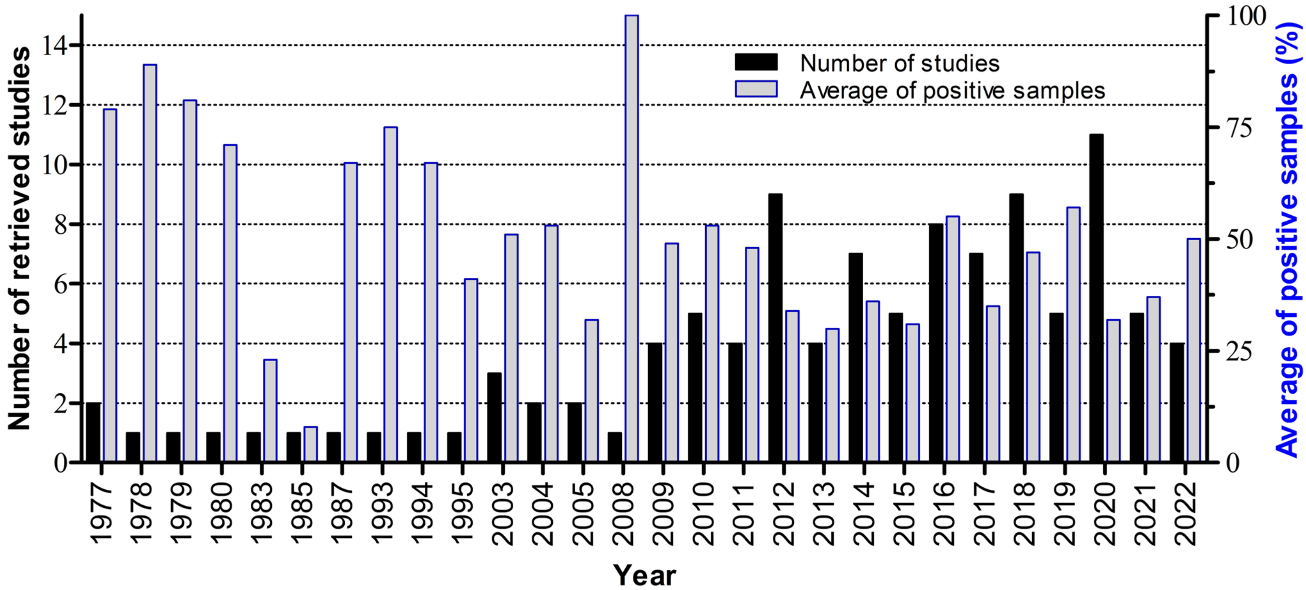

Fig. 2 Distribution of selected studies, and mean percentage of positive samples for FLA per year

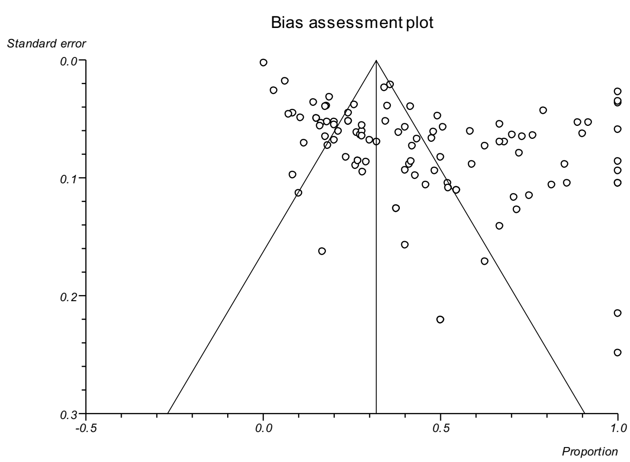

Fig. 3 Result of Egger's bias assessment for the prevalence of free-living amoebae in swimming pools and recreational waters

CI = 43.33–62.73) than in studies published between 2010 and 2015, 37.07% (95% CI = 28.87–45.66), and those published after 2015 (> 2015–2022) 45.40% (95% CI = 35.48–55.51) (Table 2).

Considering the continents covered by the selected studies, the highest prevalence 63.99% (95% CI = 45.03–80.92) was reported in America and the lowest 37.38% (95% CI = 30.12–44.93) in Asia. Among the countries from which more than one study was included, Mexico had the highest prevalence of FLA in swimming pools and recreational waters 98.35% (95% CI = 92.56–99.96), and the lowest prevalence 10.15% (95% CI = 4.99–16.87) was recorded in China (Table 2).

Considering the different sampling sources, the highest prevalence of FLA 52.27% (95% CI = 14.55–88.50) was obtained in indoor hot swimming pools, and the lowest prevalence 39.12% (95% CI = 30.48–48.13) was obtained in hot springs (Table 2).

The analysis of data from studies that used only morphological methods to identify FLA showed the highest prevalence 57.21% (95% CI = 37.99–7535), the lowest prevalence 25.78% (95% CI = 14.18–39.44) was obtained from studies based only on molecular methods (PCR), and an intermediate prevalence value 43.16% (95% CI = 37.73–48.67) was obtained by analyzing studies that simultaneously used morphological and molecular methods (Table 2).

The subgroup analysis revealed that there were statistically significant differences between the overall prevalence of FLA in water sources and year ($X^2 = 449.4$, $P < 0.001$), continent ($X^2 = 156.7$, $P < 0.001$), country ($X^2 = 26.0$, $P < 0.001$), and diagnostic method ($X^2 = 373.5$, $P < 0.001$) (Table 2).

The highest values of the global prevalence of different genera of FLA in swimming pools and recreational waters were from *Vahlkampfia* spp. (54.20%), *Acanthamoeba* spp. (33.47%), and *Naegleria* spp. (30.95%). For other genera, *Hartmannella* spp./*Vermamoeba* spp., *Stenamoeba* spp., and *Vannella* spp., the global prevalence values were 20,73%, 12.05%, and 10.75%, respectively (Table 3). The results of Egger's regression test, as well as the forest plot of the worldwide prevalence of each of these FLA genera

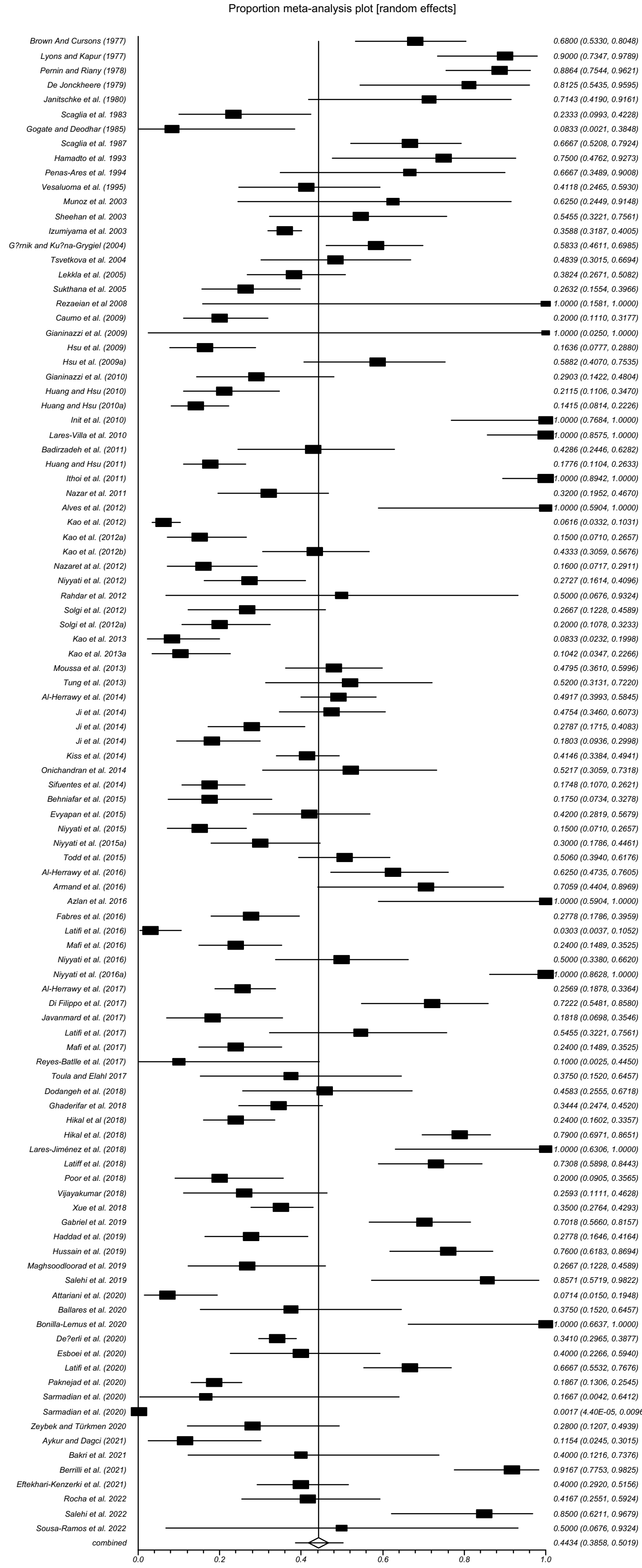

Fig. 4 Forest plot of the worldwide prevalence of free-living amoebae in swimming pools and recreational waters

Table 2 Subgroup analysis of FLA in water sources

Subgroup variable	Prevalence (95% CI)	I^2 (%)	Heterogeneity (*Q*)	*P*-value	Interaction test (X^2)	*P*-value
Year						
< 2010	53.09 (43.33–62.73)	89.5%	210.4	$P<0.001$	449.4	$P<0.001$
2010–2015	37.07 (28.87–45.66)	93.6%	519.5	$P<0.001$		
> 2015	45.40 (35.48–55.51)	96.7%	1366.2	$P<0.001$		
Continent						
Africa	51.27 (35.08–67.33)	93.5%	107.8	$P<0.001$	156.7	$P<0.001$
America	63.99 (45.03–80.92)	94.5%	201.7	$P<0.001$		
Asia	37.38 (30.12–44.93)	95.7%	1403.3	$P<0.001$		
Europe	51.99 (42.52–61.40)	89.5%	190.6	$P<0.001$		
Country						
Brazil	43.70 (21.99–66.76)	88.7%	26.5	$P<0.001$	26.0	$P<0.001$
China	10.15 (4.99–16.87)	-	0.1	$P=0.737$		
Egypt	51.65 (31.74–71.30)	95.3%	107.0	$P<0.001$		
France	69.62 (27.07–97.94)	-	22.5	$P<0.001$		
Iran	35.11 (24.74–46.26)	95.9%	787.8	$P<0.001$		
Italy	64.76 (37.01–87.95)	92.1%	38.2	$P<0.001$		
Malaysia	87.38 (73.20–96.72)	85%	33.3	$P<0.001$		
Mexico	98.35 (92.56–99.96)	0%	0.1	$P=0.913$		
Philippines	46.29 (31.43–61.48)	-	0.7	$P=0.377$		
Saudi Arabia	32.85 (21.28–45.60)	0%	1.0	$P=0.602$		
Spain	37.68 (1.06–88.08)	-	7.7	$P=0.005$		
Taiwan	26.33 (17.36–36.42)	90.6%	116.4	$P<0.001$		
Thailand	32.68 (21.82–44.57)	-	1.9	$P=0.160$		
Turkey	30.60 (20.92–41.23)	65.6%	8.7	$P=0.033$		
USA	48.70 (22.32–75.47)	95.3%	63.7	$P<0.001$		
Origin						
Hot springs	39.12 (30.48–48.13)	93%	369.0	$P<0.001$	51.6	$P=0.224$
Indoor hot swimming pools	52.27 (14.55–88.50)	-	1.8	$P=0.169$		
Public swimming pools	49.47 (36.87–62.10)	97%	1201.5	$P<0.001$		
Recreational waters	44.44 (33.19–55.99)	95%	538.7	$P<0.001$		
Thermal swimming pools	46.05 (2674–65.99)	88.8%	26.7	$P<0.001$		
Diagnostic method						
Morphology	57.21 (37.99–7535)	97.8%	1083.3	$P<0.001$	373.5	$P<0.001$
PCR	25.78 (14.18–39.44)	94.6%	183.6	$P<0.001$		
Morphology and PCR	43.16 (37.73–48.67)	91.6%	757.6	$P<0.001$		

in swimming pools and recreational waters, can be seen in Fig. S1, S2, S3, S4, S5, and S6 of the supplementary material, respectively.

Discussion

FLA are cosmopolitan microorganisms ubiquitous in all matrices of natural and anthropogenic environments, including water resources. The presence of FLA in pools and recreational waters is worrying, since some of these microorganisms are human pathogens/opportunists, as well as being widely implicated in persistence and/or pseudo-resistance of pathogenic bacteria, viruses, and fungi in water, including in water treated with disinfectants (Thomas et al. 2004; Staggemeier et al. 2016; Mavridou et al. 2018; Gomes et al. 2020; Hubert et al. 2021).

The studies included in present review are distributed by five continents; however, they have a heterogeneous spatial distribution within the territories of the continents; this can suggest differences in the level of FLA importance for health in the contexts of different countries, as well as differences in the frequency of cases diseases associated with the FLA. The frequency of cases of FLA-related diseases can

Table 3 Global prevalence, publication bias, and heterogeneity of FLA in water sources

Genus	Prevalence, % (95% CI)	Cochran *Q*	df	I^2 (%)	*P*-value	Egger bias	*P*-value
Acanthamoeba spp.	33.47 (28.06–39.11)	429.1	55	87.2%	< 0.001	3.4	0.0002
Hartmannella spp./*Vermamoeba* spp.	20.73 (7.73–38.39)	30.55	5	83.8%	0.0008	5.9	0.1363
Naegleria spp.	30.95 (22.85–39.69)	676.0	38	94.4%	< 0.001	4.6	0.0054
Stenamoeba spp.	12.05 (0.08–39.61)	3.2	1	-	0.0732	-	-
Vahlkampfia spp.	54.20 (27.49–79.67)	12.6	2	84.2%	0.0018	-	-
Vannella spp.	10.75 (0.01–37.14)	2.2	1	-	0.133	-	-

CI, confidence interval; *df*, degree of freedom

be influenced by the difference in the predominance of risk factors and the sensitivity of the health surveillance strategy of each country, as well as the heterogeneous distribution of trained professionals carrying out research in this area. In addition, the ease of confusing symptoms of diseases associated with the FLA with those caused by other microorganisms, combined with some cases of rapid deterioration of the patient's health and death (Jahangéer et al. 2020) can contribute to the rarity of reports or even the lack of association of diseases with FLA, especially in contexts where post-mortem study policies are not robust.

Our findings show that the global prevalence of FLA in swimming pools and recreational waters is 44.34%; however, a higher (53.09%) and intermediate (45.40%) prevalence value was obtained when considering the data from studies published up to 2010 and studies published after 2015, respectively. A lower prevalence value (37.07%) was obtained when analyzing data from studies published between 2010 and 2015 (Table 2). A similar result was reported in a study that aimed to determine the prevalence of *Naegleria* spp. in water resources (Saberi et al. 2020). This reduction in the prevalence reported in most recent studies was attributed to the most accurate diagnosis and reduction of false positive results (Jahangeeer et al. 2020; Saberi et al. 2020), as contrary to studies published up to 2010, the vast majority of studies published after 2010 used molecular methods for FLA identification. Curiously, our results show that the overall prevalence of FLA considering studies that used both morphological and molecular methods is close to the mean of the prevalence values obtained from data from studies that used only one of the methods (Table 2). This may suggest that the simultaneous use of these two methods reduces the extreme values obtained separately by each of the methods, and that these methods can be complementary, especially in studies that aim to assess the presence or absence of viable FLA in water samples. The authors agree that the morphological method (generally based on culture) is more laborious and less precise than molecular methods in the identification of FLA (Saberi et al. 2020; Hikal et al. 2018).

The subgroup analysis considering the distribution of the studies by the continents showed that FLA are more prevalent in the swimming pools and recreational water from America (63.99%), followed by Europe (51.99%) and Africa (51.27%). In relation to countries, the highest value of the prevalence of FLA was obtained in Mexico (98.35%), followed by Malaysia (87.38%), France (69.62%), and Italy (64.76%), and the lowest values were obtained in China (10.15%), Taiwan (26.33%), Turkey (30.60), and Thailand (32.68%). As for the sample source, the indoor hot swimming pools presented a higher value (52.27%) of FLA prevalence, followed by public swimming pools (49.47%) and thermal swimming pools (46.05%). The genera *Vahlkampfia* spp., *Acanthamoeba* spp., and *Naegleria* spp. were more prevalent, presenting the following prevalence values, 54.20%, 33.47%, and 30.95%, respectively (Table 3). The lowest prevalence value was for *Vannella* spp. (10.75%). These results are in accordance with other authors whose studies reported high prevalence of FLA (*Acanthamoeba* spp. 48.5%, *Naegleria* spp. 46.0%, *Vermamoeba* spp. 4.7%, and *Balamuthia* spp. 0.7%) in hot springs (Fabros et al. 2021). Saberi et al. (2020) reported the following prevalence values for *Naegleria* spp. 44.80%, 32.88%, and 21.27%, in swimming pools, hot springs, and recreational waters, respectively. The subgroup analysis showed that prevalence values are statistically different ($P < 0.001$) for all variables studied (Table 2). These findings are in accordance with other studies that reported a variable distribution in abundance and diversity of FLA species around the world (Jahangéer et al. 2020; Saberi et al. 2020; Fabros et al. 2021).

The global prevalence of FLA reported in the present study (44.34%) is worrying, since direct contact between humans and these waters is often established. In addition, several studies have reported the isolation of several potentially pathogenic FLA (Caumo et al. 2009; Alves et al. 2012; Behniafar et al. 2015;) and others with proven pathogenicity in ex vivo and in vivo trials (Brown and Cursons 1977; Janitschke et al. 1980; Rivera et al. 1983, 1993; Gianinazzi et al. 2009). Most of these FLA are identified as *N. fowleri*, *Acanthamoeba* spp., and *Balamuthia mandrillaris*. Most isolates of *Acanthamoeba* spp. reported as pathogens are distributed among the T5, T11, T15, T3, and T4 genotypes, and among them, the T4 genotype is more prevalent in hot springs (Mahmoudi et al. 2015; Fabros et al. 2021) and is associated with most cases of *Acanthamoeba* keratitis (Diehl et al. 2021; Bellini et al. 2022). The presence and abundance of FLA in swimming pool water clearly indicate that in addition to these microorganisms being resistant to chlorine in the dosage used in the treatment of drinking water (Thomas et al. 2004; Majid et al. 2017; Gomes et al. 2020), they are also resistant to chlorine and other disinfectants in the dosage used for swimming pools and artificial recreational waters (Rivera et al. 1983; Kiss et al. 2014; Zeybek et al. 2017). *Acanthamoeba castellanii* trophozoites and cysts have been reported to be resistant to exposure for more than 2 h to NaOCl and NaCl at concentrations up to 8 mg/L and 40 g/L, respectively. On the other hand, exposure to the combined effect of NaOCl or NaCl with ultraviolet C (UV-C) radiation resulted in rapid inactivation of trophozoites even when lower concentrations of NaOCl and NaCl were used (Chaúque and Rott 2021a, b). Cyst inactivation was achieved by twice as long exposure (300 min) to the combined effect of NaOCl or NaCl and UV-C, with redosing of NaOCl. Despite having demonstrated that both methods are effective, and that they have a strong potential to be used in the effective disinfection of swimming pool water, it was found that the use of NaCl is more cost-effective, as it is cheaper and has a residual effect; redosing is not necessary and is simple to apply (Chaúque and Rott 2021a, b). On the other hand, the use of solar UV radiation (UV-A and B) in place of UV-C (which depends on electricity) can further reduce the cost of the disinfection process. The effectiveness of using solar UV to photolyse NaOCl to inactivate chlorine-resistant microorganisms has been previously documented (Zhou et al. 2014). Readers interested in solar water disinfection technology applicable to recreational water treatment are directed to the appropriate literature (Chaúque and Rott 2021a; Chaúque et al. 2022).

The main aspects that constituted limitations for the present study are the following: the lack of studies carried out in most countries of the world; the heterogeneous distribution of the number of studies among the included countries; difference in FLA identification methods among many studies and discrepancy in the number of samples considered positive by the morphological and molecular method in the same study. The loss of isolates from positive samples in some studies, due to fungal contamination of non-nutrient agar plates prior to molecular identification of the amoebae, was also a limitation.

Conclusion

It is concluded that the prevalence of FLA in swimming pools and recreational waters is high and, therefore, of concern, since there is a risk of contracting infection by pathogenic amoebae or other pathogens (such as fungi, bacteria, and viruses) that may be harbored and dispersed by FLA in water (Mavridou et al. 2018). Thus, it is necessary to implement disinfection techniques that are effective in eliminating microorganisms, including FLA, in swimming pools and artificial recreational waters. The use of the combined effect of NaCl and UV-C has great potential to be used to eliminate or minimize the risk of infection by FLA in swimming pools and other artificial recreational waters. The potential risk of infection by FLA in natural recreational waters needs to be routinely quantified by health surveillance. Warning signs need to be placed where there is minimal risk of infection by FLA, and people using these water bodies need to be educated about the potential risk and possible safety measures. These measures include not diving in recreational waters wearing contact lenses, preventing water from entering the airways and eyes, and avoiding jumping into the water. Health care workers (especially those working near recreational water use sites with risk of infection by FLA) need to be trained to be on the lookout for symptoms suggestive of infection by FLA, especially in summer.

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Author contribution B.J.M.C. conceived the idea, wrote the project, collected and analyzed the data, and wrote the manuscript. D.S. participated in the conception of the idea, performed the data verification, and wrote and revised the manuscript. D.A. performed data analysis and manuscript review. M.B.R. managed the project and reviewed the manuscript. All authors approved the publication of this version of the manuscript.

Data availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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