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

Beni Jequicene Mussengue Chaúque[1], Denise Leal dos Santos[1], Davood Anvari[2], and Marilise Brittes Rott[1]

[1]Universidade Federal do Rio Grande do Sul Instituto de Ciencias Basicas da Saude
[2]Iranshahr University of Medical Sciences

May 16, 2022

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 2021. 71 studies were included and an overall prevalence of FLA in swimming pools and recreational waters of 40.89% (95% CI = 33.97–48.00) was found. Considering the studies published up to 2010 (1977 - 2010) and after 2010 (>2010 - 2021) the prevalence were 51.54% (95% CI = 36.65-66.29) and 37.95% (95% CI = 30.34 – 45.86), respectively. The highest prevalence were found in the American continent (59.52%), in Malaysia (89.33%) and in indoor hot pools 52.27%. In studies that used morphological methods, PCR and both methods simultaneously to identify FLA, the prevalence was 56.41, 22.32 and 39.94%, respectively. Considering only PCR-based studies, the prevalence of *Naegleria* spp., *Acanthamoeba* spp., *Hartmanella* spp. and *Vermamoeba* spp. was 10.01, 15.38, 16.40 and 16.06%, respectively. There is considerable risk of AFL 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.

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

Beni Jequicene Mussengue Chaúque[1,2]*, Denise Leal dos Santos[1], Davood Anvari[3] and Marilise Brittes Rott[1]*

[1] Laboratory of Protozoology, Department of Microbiology, Immunology and Parasitology, Institute of Basic Health Sciences, Universidade Federal do Rio Grande do Sul, Brazil.

[2] Department of Science, Technology, Engineering and Mathematics, Universidade Rovuma, Niassa Branch, Mozambique.

[3] Faculty of Medicine, Iranshahr University of Medical Sciences, Iranshahr, Iran

Beni Jequicene Mussengue Chaúque – benichauq@gmail.com

Denise Leal dos Santos – delealsantos@yahoo.com.br

Davood Anvari – davood_anvari@live.com

Corresponding author*

Corresponding authors: Marilise Brittes Rott and Beni Jequicene Mussengue Chaúque

Mailing address: Laboratory 311, Department of Microbiology, Immunology and Parasitology, Institute of Basic Health Sciences, Department of Parasitology, Universidade Federal do Rio Grande do Sul, Sarmento Leite Street, N 500, Porto Alegre, Rio Grande do Sul 90050-170, Brazil.

Tel.: 3308 4537

Mobile phone: +55 51 999530060

E-mail: marilise.rott@ufrgs.br

Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a review article with no original research data.

Declaration of interests

The authors declare that they have no conflict of interest

SUMMARY

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 2021. 71 studies were included and an overall prevalence of FLA in swimming pools and recreational waters of 40.89% (95% CI = 33.97–48.00) was found. Considering the studies published up to 2010 (1977 - 2010) and after 2010 (>2010 - 2021) the prevalence were 51.54% (95% CI = 36.65-66.29) and 37.95% (95% CI = 30.34 − 45.86), respectively. The highest prevalence were found in the American continent (59.52%), in Malaysia (89.33%) and in indoor hot pools 52.27%. In studies that used morphological methods, PCR and both methods simultaneously to identify FLA, the prevalence was 56.41, 22.32 and 39.94%, respectively. Considering only PCR-based studies, the prevalence of *Naegleria*spp., *Acanthamoeba* spp., *Hartmanella* spp. and *Vermamoeba* spp. was 10.01, 15.38, 16.40 and 16.06%, respectively. There is considerable risk of AFL 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.

1. INTRODUCTION

Free-living amoebae (FLA) are cosmopolitan and ubiquitous microorganisms widely distributed in the environment and can be opportunistic and/or pathogenic (Visvesvara et al., 2007). 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; 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. can cause illnesses in healthy people, such as*Acanthamoeba* keratitis (AK) which primarily affects contact lens (CL) 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. has also been reported to cause skin infections (Murakawa et al., 1995; Paltiel et al. 2004). In addition, 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 & Olender, 2017). The rapid deterioration in the health status of patient 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, contribute to a high prevalence of deaths ($>$ 97%) (Capewell et al., 2010; 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 two forms of life, namely, the trophozoite form (with or without flagellum) which is the active form of the protozoan, in which it feeds, reproduces and expresses 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, 2021; 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 & 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 & Scheid, 2017; Turankar et al., 2019; Nisar et al., 2020; Hubert et al., 2021). 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 (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 & 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 AFL 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.

2. MATERIAL AND METHODS

2.1. Article search strategy

The present study, which aimed to determine the prevalence of free-living amoebae in swimming pools and recreational waters, was planned and carried out based on the PRISMA 2020 guidelines (Page et al., 2021) (Figure 1). The search for scientific articles was performed in different databases, including Web of Science, Scopus, PubMed, ScienceDirect, EMBASE, ProQuest and CAPES periódicos, between December 19 and 27, 2021. 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.

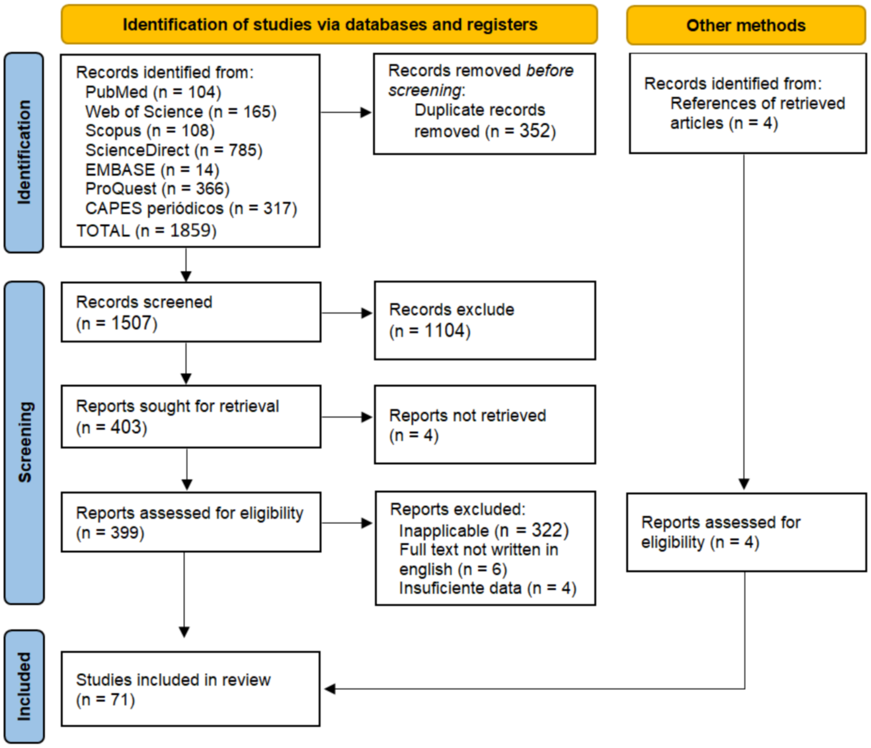


Figure 1: Details of the article retrieval and selection steps based on PRISMA 2020.

2.2. 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 free-living amoebae in swimming pools and human recreation waters were 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.

2.3. 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. Cochrane's Q test (chi-square) and the HigginsI^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.

3. RESULTS

From the total of 1,859 documents returned by the databases accessed, using the search strategy and inclusion criteria described above, 71 articles were selected (Table 1). These studies are distributed in a total of 24 countries, namely Iran (26), Taiwan (10), Egypt (4), Malaysia (4), Brazil (3), Turkey (3), France (2), Italy (2) and USA (2). One study was included from each of the following countries: Belgium, Finland, Germany, Hungary, India, Jamaica, Mexico, Norway, Poland, Portugal, Saudi Arabia, Spain, Sweden, Switzerland and Thailand. Among the studies, 76.06% (54/71) used or included molecular methods to identify FLA, while 23.94% (17/71) used only morphological methods.

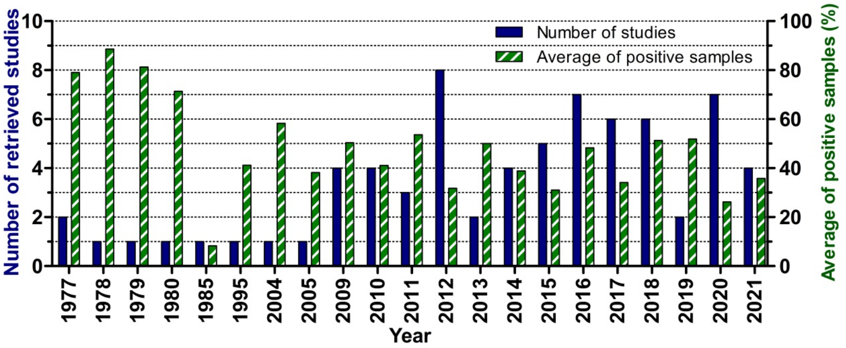


Figure 2. Distribution of selected studies, and mean percentage of positive samples for FLA per year.

The included studies were published between 1977 and 2021, and the distribution of studies by year and the average percentage value of positive samples per year are shown in Figure 2. FLA were detected in at least 1 sample of 98.59% (70/71) of selected studies (Table 1, Table S1).

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

References	**Year**	**Country**	**Sample source (total)**	**Analyzed samples**	**Positive samples**	**Methods**	**Identity**
Brown And Cursons, 1977	1977	Norway	Swimming areas	50	34	Morphology	*Acanthamoeba* spp., *Naegleria fowleri* and *Naegleria gruberi*
Lyons and Kapur, 1977	1977	USA	Swimming Pool	30	27	Morphology	*Acanthamoeba* spp. And /or *Hartmannella* spp.
Pernin and Riany, 1978	1978	France	Swimming Pool (9)	44	39	Morphology	*Acanthamoeba* spp. *Hartmannella* spp. *Naegleria* spp.

References	**Year**	**Country**	**Sample source (total)**	**Analyzed samples**	**Positive samples**	**Methods**	**Identity**
De Jonckheere, 1979.	1979	Belgium	Swimming Pool	16	13	Morphology	*Acanthamoeba* spp. and *Naegleria* spp.
Janitschke et al., 1980	1980	Germany	Swimming Pool	14	10	Morphology	*Acanthamoeba* spp.
Gogate and Deodhar, 1985	1985	India	Public swimming pool	12	1	Morphology	*N. fowleri*
Vesaluoma et al., 1995	1995	Finland	Public swimming pools and whirlpools (21)	34	14	Morphology	*Acanthamoeba* spp., *Vexillifera* spp., *Flabellula* spp., *Hartmannella* spp., and *Rugipes* spp.
Górnik and Kuźna-Grygiel, 2004.	2004	Poland	Public swimming pools (13)	72	42	Morphology	*Acanthamoeba* spp.
Lekkla et al., 2005	2005	Thailand	Hot springs (13)	68	26	Morphology	*Acanthamoeba* spp. and *Naegleria* spp.
Caumo et al., 2009	2009	Brazil	Swimming pools	65	13	Morphology and PCR	*Acanthamoeba* spp.
Gianinazzi et al., 2009	2009	Switzerland	Indoor hot swimming pools	1	1	Morphology and PCR	*Acanthamoeba lenticulata*
Hsu et al., 2009	2009	Taiwan	Recreational hot springs	55	9	PCR	*Acanthamoeba griffini* and *Acanthamoeba jacobsi*
Hsu et al., 2009a	2009	Taiwan	Mud receation area water	34	20	Morphology and PCR	*Acanthamoeba* spp., *Hartmannella spp.*, and *Naegleria* spp.

References	Year	Country	Sample source (total)	Analyzed samples	Positive samples	Methods	Identity
Init et al., 2010	2010	Malaysia	Public swimming pools (14)	14	14	Morphology	*Acanthamoeba* spp. and *Naegleria* spp.
Huang and Hsu, 2010	2010	Taiwan	Hot springs and waste water in recreation areas	52	11	PCR	*Acanthamoeba* T1, *Acanthamoeba* T2, *Acanthamoeba* T3, *Acanthamoeba* T4, *Acanthamoeba* T5, *Acanthamoeba* T6 and *Acanthamoeba* T15
Gianinazzi et al., 2010	2010	Sweden	Hot springs (4)	31	9	Morphology and PCR/DNA sequencing	*Acanthamoeba healyi, Stenoamoeba* sp., *Hartmannella vermiformis* and *Echinamoeba exundans*
Huang and Hsu, 2010a	2010	Taiwan	Hot springs and hot spring facilities	106	15	Morphology and PCR/DNA sequencing	*Naegleria lovaniensis, Naegleria australiensis, Naegleria clarki, Naegleria americana* and *Naegleria pagei*
Huang and Hsu, 2011	2011	Taiwan	Recreational waters	107	19	PCR	*Naegleria* spp.

References	**Year**	**Country**	**Sample source (total)**	**Analyzed samples**	**Positive samples**	**Methods**	**Identity**
Ithoi et al., 2011	2011	Malaysia	Recreational pools, lakes and streams	33	33	Morphology and PCR	*Naegleria* spp.
Badirzadeh et al., 2011	2011	Iran	Recreational hot springs	28	12	Morphology and PCR	Vahlkampfiid and *Acanthamoeba castellanii* T4
Alves et al., 2012	2012	Brazil	Public swimming pools (7)	7	7	Morphology and PCR	*Acanthamoeba* spp.
Kao et al., 2012	2012	Taiwan	Recreation and drinking water sources (2)	211	13	PCR	*Naegleria philippinensis*, *N. clarki*, *Naegleria gálica*, *N. americana*, *N. australiensis*, *Naegleria dobsoni*, *N. gruberi* and *Naegleria schusteri*
Nazaret at al., 2012	2012	Iran	Recreational waters (22)	50	8	Morphology and PCR	*Hartmannella vermiformis* and *Vannella persistens*
Niyyati et al., 2012	2012	Iran	River recreation areas (10)	55	15	Morphology and PCR	*Acanthamoeba* spp. (T4 e T15) and *Naegleria* spp. (*N. pagei*, *N. clarki* and *Naegleria fultoni*)

References	**Year**	**Country**	**Sample source (total)**	**Analyzed samples**	**Positive samples**	**Methods**	**Identity**
Solgi et al., 2012	2012	Iran	Hot springs	30	8	Morphology and PCR	*Hartmannella vermiformis and Naegleria* (*N. carteri* and *Naegleria* spp.)
Solgi et al., 2012a	2012	Iran	Therapeutic hot springs	60	12	Morphology and PCR	*Acanthamoeba* T4 and T3
Kao et al., 2012a	2012	Taiwan	Recreational hot springs (4)	60	9	Morphology and PCR	*Acanthamoeba* T15, *Acanthamoeba* T4, *Acanthamoeba* T2 and *Acanthamoeba* spp.
Kao et al., 2012b	2012	Taiwan	Hot springs	60	26	Morphology and PCR / DNA sequencing	*N. australiensis, N. lovaniensis*, *Naegleria mexicana* and *N. gruberi*
Moussa et al., 2013	2013	France	Recreational geothermal waters (6)	73	35	Morphology and PCR	*N. fowleri* and *N. lovaniensis*
Tung et al., 2013	2013	Taiwan	Hot springs (1)	25	13	Morphology and PCR / DNA sequencing	*Naegleria* spp. (*N. fowleri*) and *Acanthamoeba* spp.
Kiss et al., 2014	2014	Hungary	Swimming pools (20)	164	68	Morphology and PCR	*Acanthamoeba* spp.
Al-Herrawy et al., 2014	2014	Egypt	Swimming pools (10)	120	59	Morphology and PCR	*Acanthamoeba* spp.

References	**Year**	**Country**	**Sample source (total)**	**Analyzed samples**	**Positive samples**	**Methods**	**Identity**
Sifuentes et al., 2014	2014	USA	Recreational water (33)	103	18	PCR	Thermophilic amoebae and *N. fowleri*
Ji et al., 2014	2014	Taiwan	Hot springs	61	29	Morphology and PCR	*Acanthamoeba* spp.
Ji et al., 2014	2014	Taiwan	Hot springs	61	17	Morphology and PCR	*Naegleria* spp.
Ji et al., 2014	2014	Taiwan	Hot springs	61	11	Morphology and PCR	*Vermamoeba vermiformis*
Behniafar et al., 2015	2015	Iran	Recreational water (Cold and hot springs, and river)	40	7	Morphology and PCR	*Acanthamoeba* spp.
Evyapan et al., 2015	2015	Turkey	Swimming pools and hot springs	50	21	Morphology and PCR	*Acanthamoeba* spp., *Acanthamoeba griffini T3*, *Acanthamoeba castellanii* T4, and *A. jacobsi* T15
Niyyati et al., 2015	2015	Iran	Recreational water (lakes, pools and streams)	60	9	Morphology and PCR	*N. australiensis* and *N. pagei*
Todd et al., 2015	2015	Jamaica	Recreational waters	83	42	Morphology and PCR	*Acanthamoeba* T4, *Acanthamoeba* T5, *Acanthamoeba* T10 and *Acanthamoeba* T11
Niyyati et al., 2015a	2015	Iran	Recreational waters	50	15	Morphology and PCR	*A. castellanii* T4

References	**Year**	**Country**	**Sample source (total)**	**Analyzed samples**	**Positive samples**	**Methods**	**Identity**
Fabres et al., 2016	2016	Brazil	Hot tubs and thermal pools	72	20	Morphology and PCR	*Acanthamoeba* T3 *Acanthamoeba* T4 *Acanthamoeba* T5 *Acanthamoeba* T15
Al-Herrawy et al., 2016	2016	Egypt	Swimming pools (1)	48	30	Morfologia e PCR	*Acanthamoeba* spp., *Naegleria* spp. and *Hartmannella*
Mafi et al., 2016	2016	Iran	Recreational water sources (40)	75	18	Morphology	*Acanthamoeba* spp., *Hartmannella* spp. and Vahlkampfiids (*Naegleria* spp.)
Niyyati et al., 2016	2016	Iran	Recreational and therapeutic geothermal water sources	40	20	PCR	*Acanthamoeba* T4 and T2
Niyyati et al., 2016a	2016	Iran	Recreational waters	25	25	Morphology	Vahlkampfidae spp., *Acanthamoeba* spp., Thecamoeba spp. and *Miniamoebae* spp.
Armand et al., 2016	2016	Iran	Swimming pool	17	12	Morphology and PCR	*Vermamoeba* spp. and *Acanthamoeba* spp.
Latifi et al., 2016	2016	Iran	Hot springs	66	2	Morphology and PCR	*Balamuthia mandrillaris*

References	**Year**	**Country**	**Sample source (total)**	**Analyzed samples**	**Positive samples**	**Methods**	**Identity**
Mafi et al., 2017	2017	Iran	Swimming pool and Park Ponds (40)	75	18	Morphology	*Acanthamoeba* spp., *Hartmannella* spp. *and Vahlkampfiids* (*Naegleria*)
Al-Herrawy et al., 2017	2017	Egypt	Swimming pool (2)	144	37	Morphology and PCR	*Acanthamoeba* spp. and *Naegleria* spp.
Reyes-Batlle et al., 2017	2017	Spain	Recreational waters (10)	10	1	Morphology and PCR	*Naegleria* spp.
Latifi et al., 2017	2017	Iran	Recreation hot springs	22	12	Morphology and PCR	*Naegleria* spp. (*N. australiensis, N. americana, N. dobsoni, N. pagei, N. polaris,* and *N. fultoni*)
Javanmard et al., 2017	2017	Iran	Public swimming, natural pool and hot springs	33	6	Morphology and PCR	*N. pagei* and *N. gruberi*
Di Filippo et al., 2017	2017	Italy	Geothermal springs	36	26	Morphology and PCR / DNA	*N. australiensis, Naegleria itálica, N. lovaniensis* and *Naegleria* spp.
Vijayakumar, 2018	2018	Saudi Arabia	Pools and recreation waters	27	7	Morphology	*Acanthamoeba* spp.
Hikal et al, 2018	2018	Egypt	Swimming pool (5)	100	79	Morphology and PCR	*Naegleria* spp.
Hikal et al, 2018	2018	Egypt	Swimming pool (5)	100	24	Morphology and PCR	*Naegleria fowleri*

References	**Year**	**Country**	**Sample source (total)**	**Analyzed samples**	**Positive samples**	**Methods**	**Identity**
Poor et al., 2018	2018	Iran	Swimming pools and hot tubs (10)	40	8	Morphology and PCR	*Acanthamoeba* T3 and *Acanthamoeba* T4
Dodangeh et al., 2018	2018	Iran	Recreational hot springs	24	11	Morphology and PCR	*Acanthamoeba castellanii* T4
Latiff et al., 2018	2018	Malaysia	Recreational hot springs (5)	52	38	Morphology	*Acanthamoeba* spp. *Naegleria spp.*
Lares-Jiménez et al., 2018	2018	Mexico	Hot springs (1)	8	8	Morphology and PCR / DNA sequencing	*N. lovaniensis, A. jacobsi, Stenamoeba sp.*, and *Vermamoeba vermiformis*
Haddad et al., 2019	2019	Iran	Hot springs	54	15	Morphology and PCR	*Acanthamoeba castellanii* T4, *Vermamoeba vermiformis*, *N. australiensis, N. pageii* and *N. gruberi*
Hussain et al., 2019	2019	Malaysia	Recreational hot springs (5)	50	38	Morphology and PCR	*Acanthamoeba* T4, T15, T3, T5, T11 and T17
Sarmadian et al., 2020	2020	iran	Swimming pools (6)	6	1	Morphology	*Acanthamoeba* spp.
Değerli et al., 2020	2020	Turkey	Thermal swimming pool	434	148	Morphology and PCR	*Acanthamoeba* spp. and *Naegleria* spp.
Latifi et al., 2020	2020	Iran	Hot springs and beaches	81	54	Morphology and PCR	*Acanthamoeba* (T3, T4 e T5), *V. vermiformis* and *Naegleria* spp.

References	**Year**	**Country**	**Sample source (total)**	**Analyzed samples**	**Positive samples**	**Methods**	**Identity**
Esboei et al., 2020	2020	Iran	Swimming pools	30	12	Morphology and PCR	*Acanthamoeba* T4
Paknejad et al., 2020	2020	Iran	Water from swimming pools and bathtubs	166	31	Morphology and PCR	*Acanthamoeba* T3, *Acanthamoeba* T4, *Acanthamoeba* T11, *Acanthamoeba* spp., *Protacanthamoeba bohemica* and *N. lovaniensis*
Sarmadian et al., 2020	2020	Iran	Swimming pools (6)	576	1	Morphology	*Acanthamoeba* spp.
Attariani et al., 2020	2020	Iran	Swimming pools	42	3	Morphology and PCR	*Acanthamoeba* spp.
Eftekhari-Kenzerki et al., 2021	2021	Iran	Indoor public swimming pools (20)	80	32	Morphology and PCR	*Acanthamoeba* spp.
Aykur and Dagci, 2021	2021	Turkey	Swimming pools	26	3	PCR	*Acanthamoeba* T2, T4 and T5
Reyes-Batlle et al., 2021	2021	Portugal	Swimming pool facilities (20)	20	0	PCR	
Berrilli et al., 2021	2021	Italy	Hot Springs (2)	36	33	Morphology and PCR	*V. vermiformisi*, *N. australiensisi*, *Acanthamoeba* T4 and *Acanthamoeba* T15

Publication bias checked by Egger's regression test, showed that it may have a substantial impact on total prevalence estimate (Egger; bias: 7.0, $P < 0.0001$) (Figure 3).

Hosted file

`image3.wmf` available at https://authorea.com/users/483092/articles/569376-prevalence-of-free-living-amoebae-in-swimming-pools-and-recreational-waters-a-systematic-review-and-meta-analysis

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

Based on the random-effects model meta-analysis, the pooled prevalence of FLA in swimming pools and recreational waters was 40.89% (95% CI = 33.97– 48.00) (Figure 4). The included studies demonstrated a strong heterogeneity (Q = 1900.4, df = 74, $I^2 = 96.1\%$, $P < 0.0001$).

Hosted file

`image4.wmf` available at https://authorea.com/users/483092/articles/569376-prevalence-of-free-living-amoebae-in-swimming-pools-and-recreational-waters-a-systematic-review-and-meta-analysis

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

The global prevalence of FLA in swimming pools and recreational waters considering studies published up to 2010 (1977-2010) was considerably higher 51.54% (95% CI = 36.65 – 66.29) than in studies published after 2010 (>2010-2021) 37.95% (95% CI = 30.34 – 45.86). Considering the continents covered by the selected studies, the highest prevalence 59.52% (95% CI = 31.12 – 84.81) was reported in America and the lowest 34.65% (95% CI = 26.08 – 43.75) in Asia. Among the countries from which more than one study was included, Malaysia had the highest prevalence of FLA in swimming pools and recreational waters 89.33% (95% CI = 70.71 – 99.12), and the lowest prevalence 27.91% (95% CI = 18.73 – 38.13) was recorded in Taiwan. Considering the different sampling sources, the highest prevalence of FLA 52.27% (95% CI = 14.55– 88.50) was obtained in indoor heated pools, and the lowest prevalence 34.75% (95% CI = 20.85– 50.14) was obtained in other recreational waters. The analysis of studies that used only morphological methods to identify free-living amoebae showed the highest prevalence 56.41% (95% CI = 30.09 – 80.92), the lowest prevalence 22.32% (95% CI = 12.28 – 34.33) was obtained from studies based only on molecular methods (PCR), an intermediate prevalence value 39.94% (95% CI = 33.64 – 46.41) 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 ($X^2 = 223.0$, $P < 0.001$), continent ($X^2 = 71.9$, $P = 0.002$), country ($X^2 = 46.4$, $P < 0.001$), sample source ($X^2 = 21.8$, $P = 0.003$) and diagnostic method ($X^2 = 274.0$, $P < 0.001$) (Table 2).

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 > 2010	51.54 (36.65 – 66.29) 37.95 (30.34 – 45.86)	93.6% 96.4%	250.1 1584.3	$P < 0.001$ $P < 0.001$	223.0	$P < 0.001$
Continent Africa America Asia Europe	48.24 (30.49 – 66.22) 59.52 (31.12 – 84.81) 34.65 (26.08 – 43.75) 53.88 (42.63 – 64.93)	95.1% 95.4% 96.2% 91.2%	102.2 108.1 1186.2 169.7	$P < 0.001$ $P < 0.001$ $P < 0.001$ $P < 0.001$	71.9	$P = 0.002$

Subgroup variable	Prevalence (95% CI)	I^2 (%)	Heterogeneity (Q)	P-value	Interaction test (X^2)	P-value
Country Brazil Egypt France Iran Italy Malaysia Taiwan Turkey USA	46.14 (17.16 – 76.64) 47.80 (26.75 – 69.27) 69.62 (27.07 – 97.94) 30.33 (19.52 – 42.37) 82.10 (60.30 – 96.35) 89.33 (70.71 – 99.12) 27.91 (18.73 – 38.13) 30.55 (18.39 – 44.27) 54.31 (0.25 – 99.87)	91.7% 96% - 96.3% - 88.2% 91.2% 76.3% -	24.2 101.1 22.5 675.2 4.6 25.3 136.5 8.4 60.1	$P < 0.001$ $P < 0.001$ $P < 0.001$ $P < 0.001$ $P = 0.031$ $P < 0.001$ $P < 0.001$ $P = 0.014$ $P < 0.001$	46.4	$P < 0.001$
Sample source Hot springs Indoor hot swimming pools Public swimming pool Recreational waters	40.93 (31.53 – 50.68) 52.27 (14.55– 88.50) 45.31 (32.37– 58.58) 34.75 (20.85– 50.14)	92.6% - 97.6% 95.8%	350.5 1.8 1124.2 330.6	$P < 0.001$ $P = 0.169$ $P < 0.001$ $P < 0.001$	21.8	$P = 0.003$
Diagnostic method Morphology PCR Morphology and PCR	56.41 (30.09 – 80.92) 22.32 (12.28 – 34.33) 39.94 (33.64 – 46.41)	98.4% 91.3% 92.6%	961.8 80.8 664.5	$P < 0.001$ $P < 0.001$ $P < 0.001$	274.0	$P < 0.001$

The global prevalence of different genera of FLA in swimming pools and recreational waters (considering only studies that used molecular identification methods) is 10.01%, 15.38%, 16.40% and 16.06% for *Naegleria* spp., *Acanthamoeba* spp., *Hartmanella* spp. and *Vermamoeba* spp. respectively (Table 3). The results of Egger's regression test, as well as the forest plot of the worldwide prevalence of each of these free-living amoeba genera in swimming pools and recreational waters can be seen in figures S1, S2, S3 and S4 of the supplementary material, respectively.

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

Genus	Prevalence, % (95% CI)
Naegleria spp. *Acanthamoeba* spp. *Hartmannella* spp. *Vermamoeba* spp.	10.01 (6.59 – 14.05) 15.38 (11.27 – 19.99) 16.40 (

CI: confidence interval; df: degree of freedom.

4. 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, as well as being widely implicated in persistence and / or pseudoresistance of pathogenic bacteria, viruses and fungi in water, including 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 be influenced by the difference in the predominance of risk factors, 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 general prevalence of FLA in swimming pools and recreational waters is 44.785%, however, a higher (51.54%) and lower (37.95%) prevalence value was obtained when considering the data from studies published up to 2010 and studies published after 2010, respectively (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 general prevalence of FLA considering studies that they have simultaneously used morphological and molecular methods coincides with the average prevalence obtained considering 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 & Dkhil, 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 (59.52%), followed by Europe (53.88%). In relation to countries, the highest value of the prevalence of FLA was obtained in Malaysia (89.33%), followed by Italy (82.10%) and France (69.62%), and the lowest values were obtained in Taiwan (27.91), Iran (30.33) and Turkey (30.55). As for the sample source, the indoor hot swimming pools presented a higher value (52.27%) of FLA prevalence, followed by public swimming pools (45.31%) and hot springs (40.93%), recreational waters presented a relatively low value (34.75%). 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).

Our results also show that *Vermamoeba* spp., *Hartmannella*spp. and *Acanthamoeba* spp. are more prevalent, presenting the following prevalence values, 16.06%, 16.40% and 15.38%, respectively (Table 3). The lowest prevalence value was for *Naegleria* spp. (10.01%). These results are in disagreement with the findings of other authors who reported higher prevalence values (Saberi et al., 2020; Fabros et al., 2021). The lower prevalence values found in this study can be explained by the fact that only data from studies that included molecular methods in the identification of amoeba were used to calculate the prevalence of different genera of FLA. As discussed in the previous paragraphs, studies based on molecular methods for identifying FLA report lower prevalence values.

The global prevalence of FLA reported in the present study (44.79%) 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; Rivera et al., 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).

The presence and abundance of FLA in swimming pool water clearly indicates that in addition to these microorganisms being resistant to chlorine in the dosage used in the treatment of drinking water (Thomas et al., 2004; 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).*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 & Rott, 2021). 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, has a residual effect, redosing is not necessary and is simple to apply (Chaúque & Rott, 2021).

The main aspects that constituted limitations for the present study are: 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.

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 free-living amoebae, 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.

ACKNOWLEDGMENT

The authors thank CAPES for the scholarship granted to Beni Chaúque.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest

AUTHOR CONTRIBUTIONS

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, 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.

REFERENCES

Aksozek, A., Mcclellan, K., Howard, K., Niederkorn, J. Y., & Alizadeh, H. (2002). Resistance of *Acanthamoeba castellanii* Cysts to Physical. Chemical, and Radiological Conditions. *Journal of Parasitology* , **88** (3), 621-623. https://doi.org/10.1645/0022-3395(2002)088[0621:ROACCT]2.0.CO;2.

Al-Herrawy, A., Bahgat, M., Mohammed, A. E., Ashour, A. & Hikal, W. (2014). *Acanthamoeba* species in Swimming Pools of Cairo, Egypt. *Iranian journal of parasitology* , **9** (2), 194–201.

Al-Herrawy, A. Z., Gad, M. A., Abd El-Aziz, A., Abou El-Nour, M. F., Shaldoum, F. M., & Salahuldeen, A. (2016). Morphological and molecular detection of potentially pathogenic free-living amoebae in swimming pool samples, *Egyptian Journal of Environmental Research* , **5** , 1-13.

Al-Herrawy, A. Z., Khalil, M. I., El-Sherif, S. S., Omar, F., & Lotfy, W. M. (2017). Surveillance and Molecular Identification of *Acanthamoeba* and *Naegleria* Species in Two Swimming Pools in Alexandria University, Egypt. *Iranian journal of parasitology* , **12** (2), 196–205.

Alves, D. S. M. M., Moraes, A. S., Nitz, N., Oliveira, M. G. C., Hecht, M. M., Gurgel-Gonçalves, R., & Cuba, C. A. C. (2012). Occurrence and characterization of *Acanthamoeba* similar to genotypes T4, T5, and T2/T6 isolated from environmental sources in Brasília, Federal District, Brazil, Experimental Parasitology, **131** (2), 239-244. https://doi.org/10.1016/j.exppara.2012.04.011.

Armand, B., Motazedian, M. H., & Asgari, Q. (2016). Isolation and identification of pathogenic free-living amoeba from surface and tap water of Shiraz City using morphological and molecular methods. Parasitol Res., **115** (1), 63-8. https://doi.org/10.1007/s00436-015-4721-7.

Attariani, H., Turki, H., Shoja, S., Salahi-Moghaddam, A., Ghanbarnejad, A., & Shamseddin, J. (2020). Investigating the frequency of free-living amoeba in water resources with emphasis on *Acanthamoeba* in Bandar Abbas city, Hormozgan province, Iran in 2019-2020. *BMC research notes* , **13** (1), 420. https://doi.org/10.1186/s13104-020-05267-z

Aykur, M., & Dagci, H. (2021). Evaluation of molecular characterization and phylogeny for quantification of *Acanthamoeba* and*Naegleria fowleri* in various water sources, Turkey. *PloS one* , **16** (8), e0256659. https://doi.org/10.1371/journal.pone.0256659.

Badirzadeh, A., Niyyati, M., Babaei, Z., Amini, H., Badirzadeh, H., & Rezaeian, M. (2011). Isolation of free-living amoebae from sarein hot springs in ardebil province, iran. *Iranian journal of parasitology* , **6** (2), 1–8.

Balczun, C., & Scheid, P. L. (2017). Free-Living Amoebae as Hosts for and Vectors of Intracellular Microorganisms with Public Health Significance. *Viruses* , **9** (4), 65. https://doi.org/10.3390/v9040065

Behniafar, H., Niyyati, M., Lasjerdi, Z. (2015). Molecular Characterization of Pathogenic *Acanthamoeba* Isolated from Drinking and Recreational water in East Azerbaijan, Northwest Iran. *Environmental health insights* , **9** , 7–12. https://doi.org/10.4137/EHI.S27811

Berrilli, F., Di Cave, D., Novelletto, A., & Di Filippo, M. M. (2021). PCR-based identification of thermotolerant free-living amoebae in Italian hot springs. Eur J Protistol., **80** , 125812. https://doi.org/10.1016/j.ejop.2021.125812.

Brown, T.J., & Cursons, R.T. (1977). Pathogenic free-living amebae (PFLA) from frozen swimming areas in Oslo, Norway. Scand J Infect Dis.**9** (3), 237-40. https://doi.org/10.3109/inf.1977.9.issue-3.16.

Capewell, L. G., Harris, A. M., Yoder, J. S., Cope, J. R., Eddy, B. A., Roy, S. L., ... & Beach, M. J. (2015). Diagnosis; clinical course; and treatment of primary amoebic meningoencephalitis in the United States; 1937–2013. Journal of the Pediatric Infectious Diseases Society, **4** , 68–75. https://doi.org/10.1093/jpids/piu103.

Caumo, K., Frasson, A. P., Pens, C. J., Panatieri, L. F., Frazzon, A.P., & Rott, M. B. (2009). Potentially pathogenic *Acanthamoeba* in swimming pools: a survey in the southern Brazilian city of Porto Alegre. Ann Trop Med Parasitol., **103** (6), 477-85. https://doi.org/10.1179/136485909X451825.

Chaúque, B. J. M., Benetti, A. D., Corção, G., Silva, C. E., Gonçalves, R. F, & Rott, M. B. (2021). A new continuous-flow solar water disinfection system inactivating cysts of *Acanthamoebacastellanii* , and bacteria. Photochem Photobiol Sci., **20**(1), 123-137. https://doi.org/10.1007/s43630-020-00008-4.

Chaúque, B. J. M., & Rott, M. B. (2021). Photolysis of sodium chloride and sodium hypochlorite by ultraviolet light inactivates the trophozoites and cysts of *Acanthamoeba castellanii* in the water matrix. *J. Water Health* , **19** (1) 190–202. https://doi.org/10.2166/wh.2020.401

De Jonckheere, J. F. (1979). Pathogenic free-living amoebae in swimming pools: survey in Belgium. Ann Microbiol (Paris). **130B** (2), 205-12. PMID: 43689.

Değerli, S., Değerli, N., Çamur, D., Doğan, Ö., & İlter, H. (2020). Genotyping by Sequencing of *Acanthamoeba* and *Naegleria* Isolates from the Thermal Pool Distributed Throughout Turkey. Acta Parasit. **65** , 174–186. https://doi.org/10.2478/s11686-019-00148-3.

Delafont, V., Bouchon, D., Héchard, Y., & Moulin, L. (2016). Environmental factors shaping cultured free-living amoebae and their associated bacterial community within drinking water network. Water Res**100** , 382–392. https://doi.org/10.1016/j.watres.2016.05.044.

Dey, R., Folkins, M. A., & Ashbolt, N. J. (2021). Extracellular amoebal-vesicles: potential transmission vehicles for respiratory viruses. NPJ biofilms and microbiomes, 7(1), 25. https://doi.org/10.1038/s41522-021-00201-y

Di Filippo, M. M., Novelletto, A., Di Cave, D., & Berrilli, F. (2017). Identification and phylogenetic position of *Naegleria* spp. from geothermal springs in Italy. Exp Parasitol., **183** , 143-149. https://doi.org/10.1016/j.exppara.2017.08.008.

Diehl, M. L. N., Paes, J., & Rott, M. B. (2021). Genotype distribution of *Acanthamoeba* in keratitis: a systematic review. *Parasitol Res* **120** , 3051–3063. https://doi.org/10.1007/s00436-021-07261-1

Dodangeh, S., Kialashaki, E., Daryani, A., Sharif, M., Sarvi, S., Moghaddam, Y. D., & Hosseini, S. A. (2018). Isolation and molecular identification of *Acanthamoeba* spp. from hot springs in Mazandaran province, northern Iran. J Water Health. **16** (5), 807-813. https://doi.org/10.2166/wh.2018.098.

Dos Santos, D. L,, Kwitko, S., Marinho, D. R., de Araújo, B. S., Locatelli, C. I., & Rott, M. B. (2018). *Acanthamoeba* keratitis in Porto Alegre (southern Brazil): 28 cases and risk factors. Parasitol Res., **117** (3), 747-750. https://doi.org/10.1007/s00436-017-5745-y.

Eftekhari-Kenzerki, R., Solhjoo, K., Babaei, Z., Rezanezhad, H., Abolghazi, A., & Taghipour, A. (2021). High occurrence of *Acanthamoeba* spp. in the water samples of public swimming pools from Kerman Province, Iran. J Water Health*,* **19**(5): 864–871. https://doi.org/10.2166/wh.2021.162.

Esboei, B. R., Fakhar, M., Saberi, R., Barati, M., Moslemi, M., Hassannia, H., Dadimoghadam, Y., & Jalallou, N. (2020). Genotyping and phylogenic study of *Acanthamoeba* isolates from human keratitis and swimming pool water samples in Iran. Parasite epidemiology and control, **11** , e00164. https://doi.org/10.1016/j.parepi.2020.e00164.

Evyapan, G., Koltas, I. S., & Eroglu, F. (2015). Genotyping of *Acanthamoeba* T15: the environmental strain in Turkey, Transactions of The Royal Society of Tropical Medicine and Hygiene, **109** (3), 221–224. https://doi.org/10.1093/trstmh/tru179.

Fabres, L. F., Rosa Dos Santos, S. P., Benitez, L. B., & Rott, M. B. (2016). Isolation and identification of *Acanthamoeba* spp. from thermal swimming pools and spas in Southern Brazil. Acta Parasitol.,**61** (2), 221-7. https://doi.org/10.1515/ap-2016-0031.

Fabros, M. R. L., Diesta, X. R. S., Oronan, J. A., Verdejo, K. S., Garcia, J. S. M., Romey, M. S., & Milanez, G. J. (2021). Current report on the prevalence of free-living amoebae (FLA) in natural hot springs: a systematic review. J Water Health. **19** (4), 563-574. https://doi.org/10.2166/wh.2021.101.

Fowler, M., & Carter, R. F. (1965). Acute pyogenic meningitis probably due to *Acanthamoeba* sp.: a preliminary report. *British medical journal* , **2** (5464), 740–742. https://doi.org/10.1136/bmj.2.5464.734-a.

Garajová, M., Mrva, M., Vaškovicová, N., Martinka, M., Melicherová, J., & Valigurová, A. (2019). Cellulose fibrils formation and organisation of cytoskeleton during encystment are essential for *Acanthamoeba* cyst wall architecture. Sci. Rep., **9** , 4466. https://doi.org/10.1038/s41598-019-41084-6.

Gianinazzi, C., Schild, M., Wüthrich, F., Müller, N., Schürch, N., & Gottstein, B. (2009), Potentially human pathogenic *Acanthamoeba*isolated from a heated indoor swimming pool in Switzerland, Experimental Parasitology, **121** , 2, 180-186. https://doi.org/10.1016/j.exppara.2008.11.001.

Gianinazzi, C., Schild, M., Zumkehr, B., Wüthrich, F., Nüesch, I., Ryter, R., Schürch, N., Gottstein, B., & Müller, N. (2010). Screening of Swiss hot spring resorts for potentially pathogenic free-living amoebae. Exp. Parasitol. **126** (1), 45-53. https://doi.org/10.1016/j.exppara.2009.12.008.

Gogate, A., & Deodhar, L. (1985). Isolation and identification of pathogenic *Naegleria fowleri* (aerobia) from a swimming pool in Bombay. Trans R Soc Trop Med Hyg., **79** (1), 134. https://doi.org/10.1016/0035-9203(85)90258-5.

Gomes, T. S., Vaccaro, L., Magnet, M., Izquierdo, F., Ollero, D., Martínez-Fernández, C., Mayo, L., Moran, M., Pozuelo, M. J., Fenoy, S., Hurtado, C., & del Águila, C. (2020). Presence and interaction of free-living amoebae and amoeba-resisting bacteria in water from drinking water treatment plants. Sci. Total Environ., **719** , 137080. https://doi.org/10.1016/j.scitotenv.2020.137080.

Górnik, K., & Kuźna-Grygiel, W. (2004). Presence of virulent strains of amphizoic amoebae in swimming pools of the city of Szczecin. Ann Agric Environ Med., **11** (2), 233-6. PMID: 15627330.

Greub, G, Raoult, D. (2004). Microorganisms Resistant to Free-Living Amoebae. Clin Microbiol Rev. **17** , 413-33.

Haddad, M. H. F, Khoshnood, S., Mahmoudi, M. R., Habibpour, H., Ali, S. A., Mirzaei, H., Feiz Haddad, R., & Ahmadiangali, K. (2019). Molecular Identification of Free-Living Amoebae (*Naegleria* spp., *Acanthamoeba* spp. and *Vermamoeba* spp.) Isolated from Un-improved Hot Springs, Guilan Province, Northern Iran. Iranian journal of parasitology, **14** (4), 584–591.

Hikal, W.M., Hikal, W., Dkhil, M. A., & Dkhil. M. (2018). Nested PCR assay for the rapid detection of *Naegleria fowleri* from swimming pools in Egypt. Acta Ecologica Sinica, **38** , 102-107. https://doi.org/10.1016/j.chnaes.2017.06.013.

Hsu, B. M., Lin, C. L., & Shih, F. C. (2009a). Survey of pathogenic free-living amoebae and Legionella spp. in mud spring recreation area. Water Res. **43** (11), 2817-28. http://dx.doi.org/10.1016/j.watres.2009.04.002.

Hsu, B-H., Ma, P-H., Liou, T-S., Chen, J-S., & Shih, F-H. (2009). Identification of 18S ribosomal DNA genotype of *Acanthamoeba* from hot spring recreation areas in the central range, Taiwan, Journal of Hydrology, **367** (3–4), 249-254. https://doi.org/10.1016/j.jhydrol.2009.01.018.

Huang, S. W., & Hsu, B. M. (2010). Isolation and identification of*Acanthamoeba* from Taiwan spring recreation areas using culture enrichment combined with PCR. Acta Trop. 2010 Sep;**115** (3), 282-7.

https://doi.org/10.1016/j.actatropica.2010.04.012.

Huang, S. W., & Hsu, B. M. (2010a). Survey of *Naegleria* and its resisting bacteria-*Legionella* in hot spring water of Taiwan using molecular method. Parasitol Res **106** , 1395–1402. https://doi.org/10.1007/s00436-010-1815-0

Huang, S. W., & Hsu, B. M. (2011). Survey of *Naegleria* from Taiwan recreational waters using culture enrichment combined with PCR. Acta Trop., **119** (2-3), 114-8. https://doi.org/10.1016/j.actatropica.2011.04.016.

Hubert, F., Rodier, M.H., Minoza, A., Portet-Sulla, V., Cateau, E., & Brunet, K. (2021). Free-living amoebae promote *Candida auris*survival and proliferation in water. Lett Appl Microbiol., **72**(1), 82-89. https://doi.org/10.1111/lam.13395.

Hussain, R. H. M., Ishak, A. R., Ghani, M. K. A., Khan, N. A., Siddiqui, R., & Anuar, T. S. (2019). Occurrence and molecular characterisation of*Acanthamoeba* isolated from recreational hot springs in Malaysia: evidence of pathogenic potential. Journal of Water and Health**17** (5), 813–825. https://doi.org/10.2166/wh.2019.214.

Init, I., Lau, Y. L., Fadzlun, A.A., Foead, AI., Neilson, R. S., & Nissapatorn, V. (2010). Detection of free living amoebae,*Acanthamoeba* and *Naegleria* , in swimming pools, Malaysia. Trop Biomed., **27** (3), 566-77. PMID: 21399599.

Ithoi, I., Ahmad, A. F., Nissapatorn, V., Lau, Y. L., Mahmud, R., & Mak, J. W. (2011). Detection of *Naegleria* species in environmental samples from Peninsular Malaysia. PloS one, **6**(9), e24327. https://doi.org/10.1371/journal.pone.0024327.

Jahangeer, M., Mahmood, Z., Munir, N., Waraich, U.E., Tahir, I. M., Akram, M., Ali Shah, S. M., Zulfqar, A., & Zainab, R. (2020).*Naegleria fowleri* : Sources of infection, pathophysiology, diagnosis, and management; a review. Clin Exp Pharmacol Physiol.,**47** (2), 199-212. https://doi.org/10.1111/1440-1681.13192.

Janitschke, K., Werner, H., & Müller, G. (1980). Das Vorkommen von freilebenden Amöben mit möglichen pathogenen Eigenschaften in Schwimmbädern [Examinations on the occurrence of free-living amoebae with possible pathogenic traits in swimming pools (author's transl)]. Zentralbl Bakteriol B. **170** (1-2), 108-22. German. PMID: 7424255.

Javanmard, E., Niyyati, M., Lorenzo-Morales, J., Lasjerdi, Z., Behniafar, H., & Mirjalali, H. (2017). Molecular identification of waterborne free living amoebae (*Acanthamoeba, Naegleria* and*Vermamoeba*) isolated from municipal drinking water and environmental sources, Semnan province, north half of Iran. Exp Parasitol., **183** , 240-244. https://doi.org/10.1016/j.exppara.2017.09.016.

Ji, W. T., Hsu, B. M., Chang, T. Y., Hsu, T.K., Kao, P. M., Huang, K. H., Tsai, S. F., Huang, Y. L., & Fan, C. W. (2014). Surveillance and evaluation of the infection risk of free-living amoebae and Legionella in different aquatic environments. Science of The Total Environment**499** , 212–219. https://doi.org/10.1016/j.scitotenv.2014.07.116.

Johnson, R. O., Cope, J. R., Moskowitz, M., Kahler, A., Hill, V., Behrendt, K., Molina, L., Fullerton, K. E., & Beach, M. J. (2016). Notes from the Field: Primary Amebic Meningoencephalitis Associated with Exposure to Swimming Pool Water Supplied by an Overland Pipe - Inyo County, California, 2015. MMWR Morb Mortal Wkly Rep., **65** (16), 424. https://doi.org/10.15585/mmwr.mm6516a4.

Kao, P. M., Hsu, B. M., Chen, N. H., Huang, K. H., Huang, S. W., King, K. L., & Chiu, Y. C. (2012a). Isolation and identification of*Acanthamoeba* species from thermal spring environments in southern Taiwan. Experimental Parasitology, **130** (4), 354–358. https://doi.org/10.1016/j.exppara.2012.02.008.

Kao, P. M., Tung, M. C., Hsu, B. M., Hsueh, C. J., Chiu, Y. C., Chen, N. H., Shen, S. M., & Huang, Y. L. (2012b). Occurrence and distribution of*Naegleria* species from thermal spring environments in Taiwan. Letters in Applied Microbiology **56** (1), 1–7. https://doi.org/10.1111/lam.12006.

Kao, P-M., Hsu, B-M., Chiu, Y-C., Chen, N-H., Huang, K-H., & Shen, S-M. (2012). Identification of the *Naegleria* Species in Natural Watersheds Used for Drinking and Recreational Purposes in Taiwan, Journal of Environmental Engineering, **138** (8), 893-898. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000549.

Kiss, C., Barna, Z., Vargha, M., & Török, J. K. (2014). Incidence and molecular diversity of *Acanthamoeba* species isolated from public baths in Hungary. Parasitol Res **113** , 2551–2557. https://doi.org/10.1007/s00436-014-3905-x.

Król-Turmińska, K., & Olender, A. (2017). Human infections caused by free-living amoebae. Ann Agric Environ Med., **24** (2), 254-260. https://doi.org/10.5604/12321966.1233568.

Landell, M. F., Salton, J., Caumo, K., Broetto, L., & Rott, M. B. (2013). Isolation and genotyping of free-living environmental isolates of *Acanthamoeba* spp. from bromeliads in Southern Brazil. Exp Parasitol., **134** (3), 290-4. https://doi.org/10.1016/j.exppara.2013.03.028.

Lares-Jiménez, L. F., Borquez-Román, M. A., Lares-García, C., Otero-Ruiz, A., Gonzalez-Galaviz, J. R., Ibarra-Gámez, J. C., & Lares-Villa, F. (2018). Potentially pathogenic genera of free-living amoebae coexisting in a thermal spring. Experimental Parasitology,**195** , 54-58. https://doi.org/10.1016/j.exppara.2018.10.006.

Latiff, N.S. A., Jali, A., Azmi, N. A., Ithoi, I., Sulaiman, W. Y. W., & Yusuf, N. (2018). A ocorrência de *Acanthamoeba* e*Naegleria* em águas recreativas de fontes termais selecionadas em Selangor, Malásia. International Journal of Tropical Medicine**13** (3), 21–24.

Latifi, A., Salami, M., Kazemirad, E., & Soleimani, M. (2020). Isolation and identification of free-living amoeba from the hot springs and beaches of the Caspian Sea. *Parasite epidemiology and control* , **10** , e00151. https://doi.org/10.1016/j.parepi.2020.e00151.

Latifi, A. R., Niyyati, M., Lorenzo-Morales, J., Haghighi, A., Tabaei, S. J., Lasjerdi, Z., & Azargashb, E. (2017). Occurrence of*Naegleria* species in therapeutic geothermal water sources, Northern Iran. Acta Parasitol., **62** (1), 104-109. https://doi.org/10.1515/ap-2017-0012.

Latifi, A. R., Niyyati, M., Lorenzo-Morales, J., Haghighi, A., Tabaei, S. J. S., & Lasjerdi, Z. (2016). Presence of *Balamuthiamandrillaris* in hot springs from Mazandaran province, northern Iran. Epidemiol Infect., **144** (11), 2456-61. https://doi.org/10.1017/S095026881600073X.

Lekkla, A., Sutthikornchai, C., Bovornkitti, S., & Sukthana, Y. (2005). Free-living ameba contamination in natural hot springs in Thailand. Southeast Asian J Trop Med Public Health. 36 Suppl **4** , 5-9. PMID: 16438171.

Lyons, T. B., & Kapur, R. (1977). Limax amoebae in public swimming pools of albany, schenectady, and rensselaer counties, new york: their concentration, correlations, and significance. Applied and Environmental Microbiology, **33** (3), 551–555. https://doi.org/10.1128/aem.33.3.551-555.1977

Mafi, M., Niyyati, M., Haghighi, A., & Lasjerdi, Z. (2017). Contamination of Swimming Pools and Park Ponds with Free Living Amoebae in Tehran. Med J Tabriz Uni Med Sciences Health Services, **38**(6), 2783-2031. https://mj.tbzmed.ac.ir/Article/15215.

Mahmoudi, M. R., Rahmati, B., Seyedpour, S. H., & Karanis, P. (2015). Occurrence and molecular characterization of free-living amoeba species (*Acanthamoeba, Hartmannella,* and *Saccamoeba limax*) in various surface water resources of Iran. Parasitol Res. **114**(12), 4669-74. https://doi.org/10.1007/s00436-015-4712-8.

Maschio, J. V., Corção, G., & Rott, M. B. (2015). Identification of*Pseudomonas* spp. as amoeba-resistant microorganisms in isolates of *Acanthamoeba* . Rev Inst Med Trop Sao Paulo. **57** (1), 81-83. https://doi.org/10.1590/S0036-46652015000100012.

Mavridou, A., Pappa, O., Papatzitze, O., Dioli, C., Kefala, A. M., Drossos, P., & Beloukas, A. (2018). Exotic Tourist Destinations and Transmission of Infections by Swimming Pools and Hot Springs-A Literature Review. *International journal of environmental research and public health* , **15** (12), 2730. https://doi.org/10.3390/ijerph15122730.

Moussa, M., De Jonckheere, J.F., Guerlotté, J., Richard, V., Bastaraud, A., Romana, M., & Talarmin, A. (2013). Survey of Naegleria fowleri in geothermal recreational waters of Guadeloupe (French West Indies). PloS one, **8** (1), e54414. https://doi.org/10.1371/journal.pone.0054414.

Murakawa, G. J., McCalmont, T., Altman, J., Telang, G. H., Hoffman, M. D., Kantor, G. R., & Berger, T. G. (1995). Disseminated acanthamebiasis in patients with AIDS. A report of five cases and a review of the literature. Arch Dermatol., **131** (11), 1291-1296. https://doi.org/10.1001/archderm.1995.01690230069011

Nazar, M., Haghighi, A., Taghipour, N., Ortega-Rivas, A., Tahvildar-Biderouni, F., Mojarad, E.M., & Eftekhar, M., 2012. Molecular identification of *Hartmannella vermiformis* and *Vannella persistens* from man-made recreational water environments, Tehran, Iran. *Parasitol Res* 111, 835–839. https://doi.org/10.1007/s00436-012-2906-x.

Nisar, M. A., Ross, K. E., Brown, M. H., Bentham, R., & Whiley, H. (2020). *Legionella pneumophila* and Protozoan Hosts: Implications for the Control of Hospital and Potable Water Systems. Pathogens.**9** (4), 286. https://doi.org/10.3390/pathogens9040286.

Niyyati, M., Lasjerdi, Z., Nazar, M., Haghighi, A., & Mojarad E. N. (2012). Screening of recreational areas of rivers for potentially pathogenic free-living amoebae in the suburbs of Tehran, Iran. J Water Health, **10** (1), 140–146. https://doi.org/10.2166/wh.2011.068.

Niyyati, M., Lasjerdi, Z., Zarein-Dolab, S., Nazar, M., Behniafar, H., Mahmoudi, M. R., & Mojarad, E. N. (2015). Morphological and Molecular Survey of *Naegleria* spp. in Water Bodies Used for Recreational Purposes in Rasht city, Northern Iran. *Iranian journal of parasitology* , **10** (4), 523–529. PMID: 26811717.

Niyyati, M., Nazar, M., Lasjerdi, Z., Haghighi, A., & Nazemalhosseini-Mojarad, E. (2015a). Reporting of T4 Genotype of*Acanthamoeba* Isolates in Recreational Water Sources of Gilan Province, Northern Iran. Novel Biomed. **3** (1), 20-4. https://doi.org/10.22037/nbm.v3i1.7177.

Niyyati, M., Saberi, R., Latifi, A., & Lasjerdi, Z. (2016). Distribution of *Acanthamoeba* Genotypes Isolated from Recreational and Therapeutic Geothermal Water Sources in Southwestern Iran. *Environmental health insights* , **10** , 69–74. https://doi.org/10.4137/EHI.S38349.

Niyyati, M., Saberi, R., Lorenzo-Morales, J., & Salehi, R. (2016a). High occurrence of potentially-pathogenic free-living amoebae in tap water and recreational water sources in South-West Iran. Trop Biomed.,**33** (1), 95-101. PMID: 33579146.

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., McGuinness, L. A., . . . & Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ (Clinical research ed.), **372** , 71. https://doi.org/10.1136/bmj.n71.

Paknejad, N., Hajialilo, E., Saraei, M., & Javadi, A. (2020). Isolation and identification of *Acanthamoeba* genotypes and *Naegleria*spp. from the water samples of public swimming pools in Qazvin, Iran. J Water Health., **18** (2), 244-251. https://doi.org/10.2166/wh.2019.074.

Paltiel, M., Powell, E., Lynch, J., Baranowski, B., & Martins, C. (2004). Disseminated cutaneous acanthamebiasis: a case report and review of the literature. Cutis., **73** (4), 241-8.

Pazoki, H., Niyyati, M., Javanmard, E., Lasjerdi, Z., Spotin, A., Mirjalali, H., & Behravan, M. R. (2020). Isolation and Phylogenetic Analysis of Free-Living Amoebae (*Acanthamoeba* , *Naegleria* , and *Vermamoeba*) in the Farmland Soils and Recreational Places in Iran. *Acta Parasitol* ., **65** (1), 36-43. https://doi.org/10.2478/s11686-019-00126-9.

Pernin, P., & Riany, A. (1978). Etude sur la présence d'amibes libres' dans les eaux des piscines lyonnaises [Study on the presence of "free-living" amoebae in the swimming-pools of Lyon (author's transl)]. Ann Parasitol Hum Comp., **53** (4), 333-44. French. PMID: 727637.

Poor, B. M., Dalimi, A., Ghafarifar, F., Khoshzaban, F., & Abdolalizadeh, J. (2018). Contamination of swimming pools and hot tubs biofilms with *Acanthamoeba* . Acta Parasit. **63** , 147–153. https://doi.org/10.1515/ap-2018-0016.

Rayamajhee, B., Subedi, D., Peguda, H. K., Willcox, M. D., Henriquez, F. L., & Carnt, N. (2021). A Systematic Review of Intracellular Microorganisms within *Acanthamoeba* to Understand Potential Impact for Infection. Pathogens (Basel, Switzerland), **10** (2), 225. https://doi.org/10.3390/pathogens10020225.

Reyes-Batlle, M., Gabriel, M. F., Rodríguez-Expósito, R., Felgueiras, F., Sifaoui, I., Mourão, Z., de Oliveira Fernandes, E., Piñero, J. E., & Lorenzo-Morales, J. (2021). Evaluation of the occurrence of pathogenic free-living amoeba and bacteria in 20 public indoor swimming pool facilities. MicrobiologyOpen, **10** (1), e1159. https://doi.org/10.1002/mbo3.1159.

Reyes-Batlle, M., Wagner, C., López-Arencibia, A., Sifaoui, I., Martínez-Carretero, E., Valladares, B., Piñero, J. E., & Lorenzo-Morales, J. (2017). Isolation and molecular characterization of a *Naegleria* strain from a recreational water fountain in Tenerife, Canary Islands, Spain. Acta Parasitol., **62** (2), 265-268. https://doi.org/10.1515/ap-2017-0033.

Rivera, F., Ramírez, P., Vilaclara, G., Robles, E., & Medina, F. (1983). A survey of pathogenic and free-living amoebae inhabiting swimming pool water in Mexico City. Environ Res., **32** (1), 205-11. https://doi.org/10.1016/0013-9351(83)90207-4.

Rivera, F., Ramírez, E., Bonilla, P., Calderón, A., Gallegos, E., Rodríguez, S., Ortiz, R., Zaldívar, B., Ramírez, P., & Durán, A. (1993). Pathogenic and free-living amoebae isolated from swimming pools and physiotherapy tubs in Mexico. Environ Res., **62** (1), 43-52. https://doi.org/10.1006/enrs.1993.1087.

Saberi, R., Seifi, Z., Dodangeh, S., Najafi, A., Hosseini, S. A, Anvari, D., Taghipour, A., Norouzi, M., & Niyyati, M. (2020). A systematic literature review and meta-analysis on the global prevalence of*Naegleria* spp. in water sources. Transbound Emerg Dis.,**67** (6), 2389-2402. https://doi.org/10.1111/tbed.13635.

Saburi, E., Rajaii, T., Behdari, A., Kohansal, M. H., & Vazini, H. (2017). Free-living amoebae in the water resources of Iran: a systematic review. J Parasit Dis., **41** (4), 919-928. https://doi.org/10.1007/s12639-017-0950-2.

Santos, D. L., Virginio, V. G., Kwitko, S., Marinho, D. R., Araujo, B. S., Locatelli, C. I., & Rott, M. B. (2021). Profile of contact lens wearers and associated risk factors for *Acanthamoeba* spp. Editora Atena, cap. 14. https://doi.org/10.22533/at.ed.543210120.

Santos, L. C., Oliveira, M. S., Lobo, R. D., Higashino, H. R., Costa, S. F., van der Heijden, I. M., Giudice, M. C., Silva, A. R., & Levin, A. S. (2009). *Acanthamoeba* spp. in urine of critically ill patients. Emerging infectious diseases, **15** (7), 1144–1146. https://doi.org/10.3201/eid1507.081415.

Sarink, M. J., van der Meijs, N. L., Denzer, K., Koenderman, L., Tielens, A. G. M., van Hellemond, J. J. (2022). Three encephalitis-causing amoebae and their distinct interactions with the host. Trends Parasitol. **38** (3), 230-245. https://doi.org/10.1016/j.pt.2021.10.004.

Sarmadian, H., Hazbavi, Y., Didehdar, M., Ghannadzadeh, M. J., Hajihossein, R., Khosravi, M., & Ghasemikhah, R. (2020). Fungal and parasitic contamination of indoor public swimming pools in Arak, Iran. The Journal of the Egyptian Public Health Association, **95**(1), 8. https://doi.org/10.1186/s42506-020-0036-3.

Scheid, P. (2014). Relevance of free-living amoebae as hosts for phylogenetically diverse microorganisms. Parasitol Res., **113**(7), 2407-14. https://doi.org/10.1007/s00436-014-3932-7.

Siddiqui, R., & Khan, N. A. (2014). Primary Amoebic Meningoencephalitis Caused by *Naegleria fowleri* : An Old Enemy Presenting New Challenges. PLoS Negl. Trop. Dis. **8** , e3017. doi:10.1371/journal.pntd.0003017

Sifuentes, L. Y., Choate, B. L., Gerba, C. P., & Bright, K. R. (2014). The occurrence of *Naegleria fowleri* in recreational waters in Arizona. J Environ Sci Health A Tox Hazard Subst Environ Eng.,**49** (11), 1322-30. https://doi.org/10.1080/10934529.2014.910342.

Soares, S. S., Souza, T. K., Berté, F. K., Cantarelli, V. V., & Rott, M. B. (2017). Occurrence of Infected Free-Living Amoebae in Cooling Towers of Southern Brazil. Curr Microbiol., **74** (12), 1461-1468. https://doi.org/10.1007/s00284-017-1341-8.

Solgi, R., Niyyati, M., Haghighi, A., & Mojarad, E. N. (2012). Occurrence of Thermotolerant Hartmannella vermiformis and Naegleria Spp. in Hot Springs of Ardebil Province, Northwest Iran. Iranian journal of parasitology, **7** (2), 47–52.

Solgi, R., Niyyati, M., Haghighi, A., Taghipour, N., Tabaei, S .J., Eftekhar, M., & Mojarad, E. M. (2012a). Thermotolerant*Acanthamoeba* spp. isolated from therapeutic hot springs in Northwestern Iran. J Water Health. **10** (4), 650-6. https://doi.org/10.2166/wh.2012.032.

Staggemeier, R., Arantes, T., Caumo, K. S., Rott, M. B., & Spilki, F. R. (2016). Detection and quantification of human adenovirus genomes in*Acanthamoeba* isolated from swimming pools. An Acad Bras Cienc.,**88** Suppl 1:635-41. https://doi.org/10.1590/0001-3765201620150151.

Stapleton, F. (2021). The epidemiology of infectious keratitis. Ocul Surf., S1542-0124(21)00089-6. https://doi.org/10.1016/j.jtos.2021.08.007.

Thomas, V., Loret, J. F., Jousset, M., & Greub, G. (2008). Biodiversity of amoebae and amoebae-resisting bacteria in a drinking water treatment plant. Environ Microbiol., **10** , 2728–2745. https://doi.org/10.1111/j.1462-2920.2008.01693.x.

Thomas, V., Bouchez, T., Nicolas, V., Robert, S., Loret, J. F., & Lévi, Y. (2004). Amoebae in domestic water systems: resistance to disinfection treatments and implication in *Legionella* persistence. J. Appl. Microbiol., **97** , 950–963. https://doi.org/10.1111/j.1365-2672.2004.02391.x

Todd, C. D., Reyes-Batlle, M., Piñero, J. E., Martínez-Carretero, E., Valladares, B., Streete, D., Lorenzo-Morales, J., & Lindo, J. F. (2015). Isolation and molecular characterization of *Acanthamoeba*genotypes in recreational and domestic water sources from Jamaica, West Indies. J Water Health. **13** (3), 909-19. https://doi.org/10.2166/wh.2015.232.

Tung, M. C., Hsu, B. M., Tao, C.W., Lin, W. C., Tsai, H. F., Ji, D., Shen, S. M., Chen, J. S., Shih, F. C., & Huang, Y. L. (2013). Identification and significance of *Naegleria fowleri* isolated from the hot spring which related to the first primary amebic meningoencephalitis (PAM) patient in Taiwan. International Journal for Parasitology, **43** (9), 691–696. https://doi.org/10.1016/j.ijpara.2013.01.012.

Turankar, R. P., Lavania, M., Darlong, J., Siva Sai, K. S. R., Sengupta, U., & Jadhav, R. S. (2019). Survival of *Mycobacterium leprae* and association with *Acanthamoeba* from environmental samples in the inhabitant areas of active leprosy cases: A cross sectional study from endemic pockets of Purulia, West Bengal. Infect Genet Evol.,**72** , 199-204. https://doi.org/10.1016/j.meegid.2019.01.014.

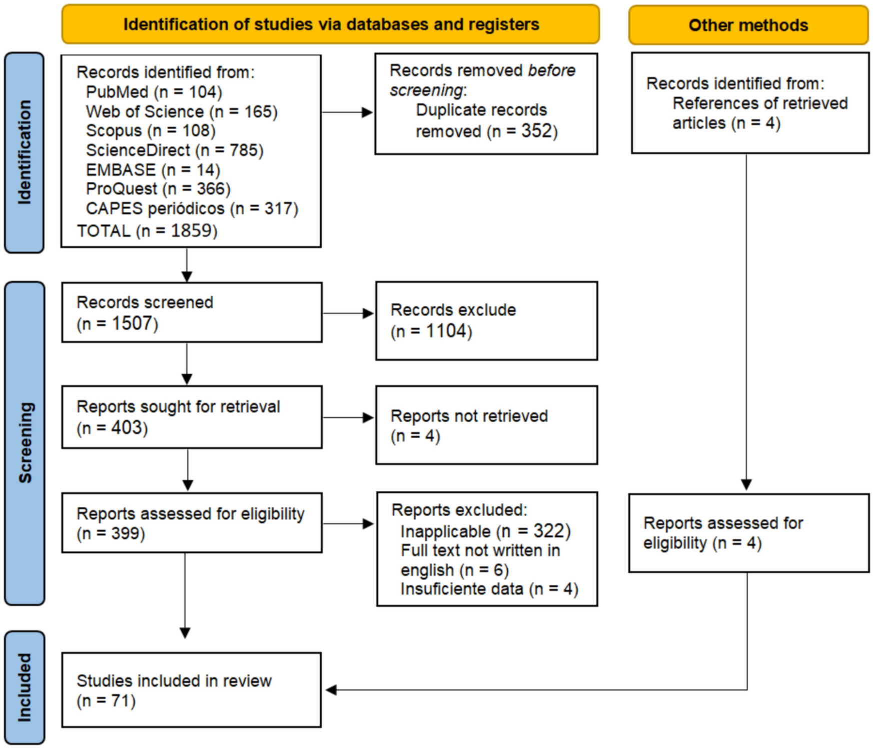
Vesaluoma, M., Kalso, S., Jokipii, L., Warhurst, D., Pönkä, A., & Tervo, T. (1995). Microbiological quality in Finnish public swimming pools and whirlpools with special reference to free living amoebae: a risk factor for contact lens wearers?. *The British journal of ophthalmology* , **79** (2), 178–181. https://doi.org/10.1136/bjo.79.2.178.

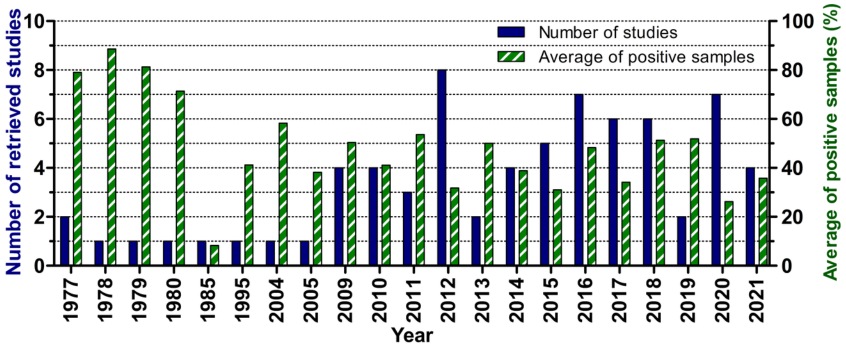
Vijayakumar, R. (2018). Isolation, identification of pathogenic *Acanthamoeba* from drinking and recreational water sources in Saudi Arabia. Journal of advanced veterinary and animal research, **5** (4), 439–444. https://doi.org/10.5455/javar.2018.e296.

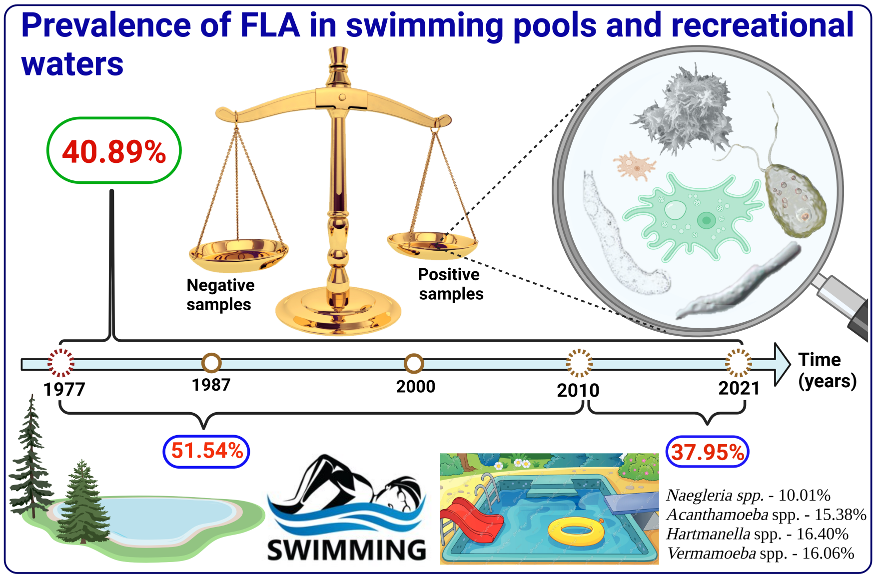
Visvesvara, G. S. (2013). Infections with free-living amebae. Handb Clin Neurol., **114** , 153-68. https://doi.org/10.1016/B978-0-444-53490-3.00010-8.

Visvesvara, G. S., Moura, H., & Schuster, F. L. (2007). Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp.,*Balamuthia mandrillaris, Naegleria fowleri,* and *Sappinia diploidea* . FEMS Immunol Med Microbiol., **50** (1), 1-26. https://doi.org/10.1111/j.1574-695X.2007.00232.x.

Wopereis, D. B., Bazzo, M. L., de Macedo, J. P., Casara, F., Golfeto, L., Venancio, E., de Oliveira, J. G., Rott, M. B., & Caumo, K. S. (2020). Free-living amoebae and their relationship to air quality in hospital environments: characterization of *Acanthamoeba* spp. obtained from air-conditioning systems. Parasitology., **147** (7), 782-790. https://doi.org/10.1017/S0031182020000487.






Prevalence of FLA in swimming pools and recreational waters
40.89%
Negative samples
Positive samples
1977
1987
2000
2010
2021
Time (years)
51.54%
37.95%
SWIMMING
Naegleria spp. - 10.01%
Acanthamoeba spp. - 15.38%
Hartmanella spp. - 16.40%
Vermamoeba spp. - 16.06%