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.
The prevalences of deaths (as well as erratic or late diagnosis, contributes to a high trial meningoencephalitis (since the symptoms are similar), PAM, combined with the ease of being confused with 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. 2022; Dey et al 2022). 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.

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                       | Naegleria spp.                                                         |
| 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, Vahlkampfia baltica, Vahlkampfia sp., A. polyphaga, Acanthamoeba lenticulata, Naegleria spp., 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. lowiensi, 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 jacobi     |
| 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 haileyi, Stenoamoeba sp., Hartmannella vermiformis, and Echinamoeba exudans |
| 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 Naegleria lovaniensis, Naegleria australiensis, Naegleria clarki, Naegleria americana, and Naegleria pagei |
| Huang and Hsu (2010a)               | Taiwan        | Hot spring and hot spring facilities               | 106              | 15               | Morphology, PCR | Naegleria spp.          |
| Init et al. (2010)                  | Malaysia      | Public swimming pool (14)                          | 14               | 14               | Morphology   | Acanthamoeba spp. and Naegleria spp.          |
| Lanes-Villa et al. 2010             | Mexico        | Natural recreational water (2)                     | 24               | 24               | PCR          | Thermophilic amoebae, thermophilic Naegleria spp., and N. fowleri |
| Badizadeh et al. (2011)             | Iran          | Recreational hot spring                            | 28               | 12               | Morphology, PCR | Vahlkampfiid and Acanthamoeba castellanii T4 Naegleria spp. |
| 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 Acanthamoeba spp. |
| Alves et al. (2012)                 | Brazil        | Public swimming pool (7)                           | 7                | 7                | Morphology, PCR | Naegleria philippinensis, N. clarki, Naegleria galica, Na. americana, N. australiensis, Naegleria dobsoni, Na. gruberi, and Naegleria schusteri |
| Kao et al. (2012a, b, c)            | Taiwan        | Recreation and drinking water source (2)           | 211              | 13               | PCR          | Naegleria spp., Hartmannella vermiformis and Vanella persistens |
| Kao et al. (2012a)                  | Taiwan        | Recreational hot spring (4)                        | 60               | 9                | Morphology, PCR | Acanthamoeba T15, Acanthamoeba T4, Acanthamoeba T2, and Acanthamoeba spp. Naegleria mexicana, and N. gruberi |
| 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 Vanella persistens |
| Niyayati 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 |
| 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        | Naegleria spp.                                                          |
| Ji et al. (2014)             | Taiwan      | Hot spring                             | 61               | 17               | Morphology, PCR        | Acanthamoeba 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 castellani T4, A. castellanii T4, and A. jacobi T15 |
| Niyayti et al. (2015a, b)    | Iran        | Recreational water (lakes, pools, and streams) | 60               | 9                | Morphology, PCR        | N. australiensis and N. pagei                                            |
| Niyayti 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                                                 |
| Niyayti et al. (2016a, b)    | Iran        | Geothermal water source                | 40               | 20               | PCR                    | Acanthamoeba T4 and T2                                                  |
| Niyayti et al. (2016a)       | Iran        | Recreational water                     | 25               | 25               | Morphology             | Vahlkampfiae spp., Acanthamoeba spp., Thecamoeba spp., and Miniamoeba 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 italica, 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. fultonii) |
| Mafi et al. (2017) | Iran | Swimming pool and park pond (40) | 75 | 18 | Morphology | Acanthamoeba spp., Hartmannella spp., and Vahlkampfids |
| 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 | N. lovaniensis, A. jacobi, Stenamoeba sp., and Vermamoeba vermiformis |
| Lares-Jiménez et al. (2018) | Mexico | Hot spring (1) | 8 | 8 | Morphology, PCR | Acanthamoeba spp. and Naegleria spp. |
| Latiff et al. (2018) | Malaysia | Recreational hot spring (5) | 52 | 38 | Morphology | Acanthamoeba T3 and Acanthamoeba T4 |
| Poor et al. (2018) | Iran | Swimming pool and hot tubs (10) | 40 | 8 | Morphology, PCR | Acanthamoeba spp. |
| 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 castellanii T4, Vermamoeba vermiformis, N. australiensis, N. pagei, and N. gruberi |
| Haddad et al. (2019) | Iran | Hot springs | 54 | 15 | Morphology, PCR | Acanthamoeba T4, T15, T3, T5, T11, and T17 |
| Hussain et al. (2019) | Malaysia | Recreational hot spring (5) | 50 | 38 | Morphology, PCR | Acanthamoeba spp. |
| Maghsudlooonad et al. 2019 | Iran | Recreational park water | 30 | 8 | Morphology, PCR | Acanthamoeba spp. and Acanthamoeba spp. T4 and Acanthamoeba spp. T15 |
| Salehi et al. 2019 | Iran | Park pool and swimming pool | 14 | 12 | Morphology, PCR | Acanthamoeba spp. and Acanthamoeba spp. 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. |
| References                  | Country       | Sample source (total) | Analyzed samples | Positive samples | Methods          | Identity                                                                 |
|-----------------------------|---------------|-----------------------|-------------------|------------------|------------------|--------------------------------------------------------------------------|
| El-Badry et al. 2020        | Egypt         | Swimming pool (7)     | 28                | 0                | Morphology, PCR   | Acanthamoeba T4                                                          |
| Esboei et al. (2020)        | Iran          | Swimming pools        | 30                | 12               | Morphology, PCR   | Acanthamoeba (T3, T4 e T5), V. vermiciformis, and Naegleria spp.         |
| Latifi et al. (2020)        | Iran          | Hot spring and beach  | 81                | 54               | Morphology, PCR   | Acanthamoeba T3, Acanthamoeba T4, Acanthamoeba T11, Acanthamoeba sp., Protacanthamoeba bohemica, and N. lovaniensis |
| 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  | V. vermiformis, N. australiensis, Acanthamoeba T4, and Acanthamoeba T15 |
| 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. vermiformis, N. australiensis, 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              | Acanthamoeba spp. and Naegleria spp.                                     |
| Nageeb et al. (2022)        | Egypt         | Swimming pool (2)     | 8                 | 0                | Morphology, PCR   | Acanthamoeba T2, T3, T4, T11, and T15                                    |
| Rocha et al. 2022           | Brazil        | Swimming pool (9)     | 36                | 15               | Morphology       | Acanthamoeba (T2, T3, T4, T11, and T15)                                  |
| Salehi et al. 2022          | Iran          | Swimming pool and park pool | 20              | 17               | Morphology, PCR   | Acanthamoeba sp. T4 and Vamella sp.                                      |
| Sousa-Ramos et al. 2022     | Cape Verde    | Recreational fountain and swimming pool | 4               | 2                | Morphology, PCR   | Acanthamoeba sp. T4 and Vamella sp.                                      |
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.
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

| Year       | Prevalence (95% CI) | $I^2$ (%) | Heterogeneity ($Q$) | $P$-value | Interaction test ($\chi^2$) | $P$-value |
|------------|---------------------|----------|---------------------|-----------|-----------------------------|-----------|
| > 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               | $P<0.001$ |                            |           |
| < 2010     | 45.40 (35.48–55.51) | 96.7%    | 1366.2              | $P<0.001$ |                            |           |

Table 2  Subgroup analysis of FLA in water sources

| Subgroup variable | Prevalence (95% CI) | $I^2$ (%) | Heterogeneity ($Q$) | $P$-value | Interaction test ($\chi^2$) | $P$-value |
|-------------------|---------------------|----------|---------------------|-----------|-----------------------------|-----------|
| 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               | $P<0.001$ |                            |           |

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

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

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**Declarations**

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