Hydrogeol J
DOI 10.1007/s10040-017-1582-4

PAPER

Review: Occurrence of the pathogenic amoeba *Naegleria fowleri* in groundwater

Kelly R. Bright[1] **· Charles P. Gerba**[1]

Received: 1 September 2016 / Accepted: 23 March 2017

Abstract *Naegleria fowleri* is a thermophilic free-living amoeba found worldwide in soils and warm freshwater. It is the causative agent of primary amebic meningoencephalitis, a nearly always fatal disease afflicting mainly children and young adults. Humans are exposed to the organism via swimming, bathing, or other recreational activity during which water is forcefully inhaled into the upper nasal passages. Although many studies have looked at the occurrence of *N. fowleri* in surface waters, limited information is available regarding its occurrence in groundwater and geothermally heated natural waters such as hot springs. This paper reviews the current literature related to the occurrence of *N. fowleri* in these waters and the methods employed for its detection. Case reports of potential groundwater exposures are also included. Despite increased interest in *N. fowleri* in recent years due to well-publicized cases linked to drinking water, many questions still remain unanswered. For instance, why the organism persists in some water sources and not in others is not well understood. The role of biofilms in groundwater wells and plumbing in individual buildings, and the potential for warming due to climate change to expand the occurrence of the organism into new regions, are still unclear. Additional research is needed to address these questions in order to better understand the ecology of *N. fowleri* and the conditions that result in greater risks to bathers.

Published in the special issue "Hydrogeology and Human Health"

✉ Kelly R. Bright
bright@email.arizona.edu

[1] The Water & Energy Sustainable Technology (WEST) Center, Department of Soil, Water & Environmental Science, The University of Arizona, 2959 W. Calle Agua Nueva, Tucson 85745, AZ, USA

Keywords Health · Thermal conditions · Ecology · Groundwater monitoring

Introduction

Naegleria fowleri is a free-living amoeba in the family Vahlkampfidae. It is found worldwide in soils and warm fresh waters. As such, it is found primarily in warmer regions such as the southern tier states in the United States. It has been found isolated from surface waters following rainfall or irrigation and also can be found naturally in warm bodies of water such as river and lake waters, ponds, irrigation canals, cattle tanks, wells (groundwater), hot springs, and other thermally impacted waters such as power plant cooling reservoirs (Bright et al. 2009; Huizinga and McLaughlin 1990; Tyndall et al. 1989). It has also been found in treated drinking water distribution systems (Kelly R. Bright, The University of Arizona, unpublished data, 2016; Cope et al. 2015; Yoder et al. 2012). *N. fowleri* has three morphological life stages including a cyst, flagellate, and trophozoite. The double-walled cyst is environmentally resistant and is often found under adverse environmental conditions (Marshall et al. 1997). When environmental factors improve and food sources are optimal, the cyst can excyst to form the amoeboid or trophozoite state, which can revert back and forth to the flagellate, or swimming stage of the organism, within an hour or less (Visvesvara et al. 2007).

N. fowleri causes a nearly always fatal disease (over a 97% case fatality rate) called primary amebic meningoencephalitis (PAM; John 1982; Martinez and Visvesvara 1997; Schuster and Visvesvara 2004). Over 310 PAM cases have been documented worldwide (Gautam et al. 2012), but the largest numbers of cases have been reported in the United States and, more recently, in Karachi, Pakistan. Humans usually contract

Published online: 11 April 2017

the flagellate or trophozoite form of the organism by swimming or bathing or undertaking other forms of recreation in warm surface waters (Bright et al. 2009). Visvesvara et al. (2007) reported *N. fowleri* affecting mostly otherwise healthy children and young adults with a history of swimming or recreational activity in freshwater lakes and ponds. In recent years, nasal rinsing using a neti pot or ritual ablution that includes nasal flushing have been linked to numerous PAM cases, particularly in Pakistan. Interestingly, these cases have occurred more commonly in adults (Shakoor et al. 2011; Yoder et al. 2012). The organism must be forcefully inhaled into the upper nasal passages by activities such as diving or jumping into the water. The trophozoite stage is the infectious form of the organism and is able to penetrate the nasopharyngeal mucosa and travel along the olfactory nerves and through the cribriform plate into the brain (Martinez and Visvesvara 1997), where it produces enzymes and a pore-forming protein that liquefy brain tissues (John 1982; Visvesvara et al. 2007). Clinical symptoms of PAM include severe headache, high fever, nausea, vomiting, nuchal rigidity, confusion, hallucinations, lethargy, seizures, and eventually death resulting from swelling of the brain (Martinez and Visvesvara 1997; Visvesvara et al. 2007). The onset of symptoms usually occurs between 5 and 7 days after exposure to the water, with death following an average of 6.4 days later (Marshall et al. 1997). Because PAM is most often confused with other diseases such as viral flu or bacterial meningitis, proper treatment is often delayed. Even with rapid diagnosis, patient prognosis is most often poor. Nevertheless, a newly available drug called miltefosine has been used in recent years for treating PAM patients. The use of miltefosine in conjunction with other antiparasitic drugs and aggressive management of brain swelling appears to have led to the survival of three patients, although one boy experienced permanent brain damage (Cope et al. 2016; Goldschmidt and Scutti 2016; Linam et al. 2015).

Cases of primary amebic meningoencephalitis (PAM) linked to groundwater and geothermally heated waters

Most cases of PAM caused by *N. fowleri* have been linked to swimming or bathing in warm surface waters. Very few documented cases have been directly linked to exposure to groundwater or geothermal waters and the majority of such cases reported in the scientific literature have occurred in the past 15 years.

Examples of groundwater-related cases

Between 1968 and 1978, eight cases of PAM occurred in New Zealand between Lake Taupo and Matamata, resulting from bathing exposures to geothermal hot springs. All of these cases were fatal (Cursons et al. 2003). In April of 2000, a 10-year-old girl became ill with PAM after bathing in two geothermal pools in the same region. She had immersed her head in the water at both locations. One pool was located at a commercial complex and the water had not been treated other than with the use of sand filtration. This pool also had multiple and extensive cracks in the concrete floor believed to be caused by intrusion of groundwater from below. The second pool was a natural hot spring and was also not treated. Amoebae including *Naegleria lovaniensis* were isolated from both pools using filtration (5-μm pore size) followed by culture on *Escherichia coli* lawns (used as a food source); however, no *N. fowleri* was identified by polymerase chain reaction of the ribosomal internal transcribed spacer (ITS) region using species-specific primers (Cursons et al. 2003).

In the United Kingdom, an 11-year-old contracted *N. fowleri* from a swimming pool supplied with water from a naturally heated hot spring. The temperature of the water from the hot spring was 48 °C (Cain et al. 1981). A nine-year-old girl in California (USA) contracted *N. fowleri* after she and her family had gone swimming several times in San Bernardino National Forest hot springs. This was the same location of an earlier fatal PAM case from 1971. The girl was treated with a cocktail of drugs including amphotericin B, miconazole, rifampin, and sulfisoxazole. She was also treated with medications for increased intracranial pressure and seizures. The girl survived with no lasting adverse effects from the infection (Seidel et al. 1982).

A 38-year-old man from Hong Kong acquired PAM from swimming in a hot spring in mainland China. The man survived the infection following a long course of treatment with amphotericin B, rifampicin and chloramphenicol (Wang et al. 1993). A child in Namibia in southern Africa became infected with *N. fowleri* from stagnant pools that had come from hot springs (Schoeman et al. 1993).

In 2008, a nine-year-old child contracted PAM in Guadeloupe, French West Indies, after swimming in a bath fed with geothermal water. *N. fowleri* was detected in the water source by concentrating 500 ml using filtration (1.2 μm pore size) followed by amoebic culture on an *E. coli* lawn with incubation at 44 °C (and confirmation by PCR using primers specific to the *N. fowleri* ITS region). Nevertheless, the concentration of *N. fowleri* was typically low, ranging from 0 to 22 organisms per liter (Moussa et al. 2013). A later study determined that the source of the *N. fowleri* in the water was the soil and the water acquired the organism as it flowed over the soil (Moussa et al. 2015).

An 18-month-old girl in Australia acquired PAM after exposure to untreated groundwater obtained from a borehole on her family's property (Mackowiak 2010), whereas a 75-year-old man contracted PAM and died after bathing at a hot spring resort in Taiwan. The center for diseases control (CDC) of Taiwan tested the waters and confirmed the presence of *N. fowleri* (Su et al. 2013).

A 47-year-old man in the U.S. Virgin Islands contracted PAM by performing nasal rinsing during ritual ablutions in preparation for Islamic prayers. The water sources supplying the man's household were untreated water from a well and untreated rainwater from a cistern, both of which fed directly into the household plumbing. Three of 17 samples collected from the home were positive for *N. fowleri* (Centers for Disease Control and Prevention 2013). Additionally, a 40-year-old patient in India with a history of using well water exhibited symptoms of PAM and was found to be infected with *N. fowleri* (Gupta et al. 2015).

In 2014, an 11-year-old boy contracted PAM and died after traveling to Costa Rica where he engaged in swimming, zip lining, and using a water slide at a resort hot springs. Subsequently, *N. fowleri* was detected in samples collected from the resort hot springs and river pond using filtration (0.45 μm pore size) and culture on *E. coli* lawns with incubation at 35 °C. Presumptive positive isolates were confirmed by PCR using primers specific to the ITS region as well as sequencing of amplified PCR products of the universal eukaryotic 18S rDNA gene (Booth et al. 2015; Abrahams-Sandi et al. 2015).

In July of 2015, a 21-year-old California woman contracted *N. fowleri* from a private pool that had been filled with water that had been piped in overland from a mountain spring. The temperature of the water entering the pipe a few weeks after her exposure was measured as 10 °C and the temperature of the water entering the pool after traveling overland was 37 °C (Johnson et al. 2016).

In the only documented circumstance in which two cases were identified with exposure to the same water source at approximately the same time, two young children in the Phoenix, Arizona (USA), metropolitan area became ill with PAM after bathing in tap water. The children had no known associations, but one child had recently visited his grandparents' home a few blocks from the other child's residence (Okuda et al. 2004). Neither child had a recent history of swimming or playing in a freshwater lake or pond, but both were known to have a tendency to play in the bath. The tap water was obtained from groundwater from a well that was not routinely chlorinated (Marciano-Cabral et al. 2003). *N. fowleri* was detected in 17 of 19 samples obtained from the two homes. These samples included both sterile cotton gauze Mirasorb sponges that had been passed through the kitchen and bathroom sink traps and residual water (150–350 ml) that was collected from the pipes. In addition, samples of bathwater (60.8 L each) were concentrated using Cuno Micro-Wynd II filters (1 μm pore size). The entire volume was cycled through the filter three times. A volume of 10 ml of each sample was assayed by incubation in tissue culture flasks at either 37 or 44 °C followed by PCR amplification of the *Mp2Cl5* gene which encodes a protein unique to *N. fowleri*. A third volume was concentrated using centrifugation and the pellet was examined by culture on non-nutrient agar plates with heat-killed *E. coli*, with incubation at 44 °C (Marciano-Cabral et al. 2003).

Occurrence in groundwater and drinking waters with groundwater as a source

Although numerous studies have looked at the occurrence of *N. fowleri* in surface waters (Lee et al. 2002; MacLean et al. 2004; Marciano-Cabral et al. 2003; Sifuentes et al. 2014; Wellings et al. 1977), very few have investigated the amoeba's presence in groundwater. As a direct response to the two PAM cases linked to untreated drinking water wells in the Phoenix (Arizona) area in 2002, the largest study of groundwater to date was conducted (Bright et al. 2009). A total of 113 drinking water supply wells in central and southern Arizona with water temperatures ranging from 18 to 42 °C were surveyed over several months (Bright et al. 2009). Well depths ranged from 100 to >300 m. One-liter samples were collected prior to disinfection and concentrated via centrifugation. The pellet was re-suspended in 5 ml of Page's Amoeba Saline and then the protozoa were further concentrated via filtration (2 μm pore size). This was followed by nested PCR amplification of the *N. fowleri Mp2Cl5* gene. *N. fowleri* was detected in 12 of the 113 wells (10.6%) with 29 of 185 samples testing positive (15.7%) for the amoeba. Ten percent of the first-flush initial samples were positive, whereas 17.2% of the samples were positive for *N. fowleri* when they were collected after the well had been purged three borehole volumes. In addition, 86.2% of all positive samples were from purged samples, suggesting that the organism might be present in the aquifer or within biofilms. A subset of 14 of the wells were sampled multiple times over a period of months. Five of these tested positive every time they were sampled (2–5 times), while others were only transiently positive. It was unclear why some wells were consistently colonized and others were not. No correlations were identified between the presence of *N. fowleri* and other water quality parameters such as temperature, pH, turbidity, and electrical conductivity (Bright et al. 2009).

In a follow up study conducted in Arizona (Laseke et al. 2010), 45 groundwater samples were collected in the greater Phoenix Metropolitan area from six drinking water supply wells with temperatures ranging from 29 to 48.1 °C. Samples were collected in December, August, and September to look at seasonality. From 236.6 to 4402.1 L of each sample were concentrated using Micro-Wynd II cartridge filters. Approximately 27% of all the samples tested positive for the *N. fowleri Mp2Cl5* gene by a nested PCR assay, with five of the six wells testing positive. No correlations could be found between the presence of *N. fowleri* and the temperature of the water.

N. fowleri was isolated from four of 22 (18.2%) one-liter groundwater samples collected from three different regions of Guinea-Bissau, Africa. Other members of the Vahlkampfidae family were also identified in nine additional samples, including one non-pathogenic *Naegleria* species. The samples were allowed to settle for 48 h and then sediments were collected and used to inoculate plates with heat-killed *E. coli*. Presumptive *N. fowleri* isolates were confirmed using conventional PCR utilizing primers specific to the ITS region (Baquero et al. 2014).

In contrast to the results of these studies, no *Naegleria* species were found in groundwaters and well waters in Italy, despite a high prevalence (from 50 to 87%) of other free-living amoebae in the samples. This was also an unexpected result because many of the methods employed were comparable to studies in which *N. fowleri* had been detected. One-liter samples of the water were concentrated via filtration (0.45 μm pore size) and cultured on non-nutrient agar with heat-killed *E. coli*. The PCR assay differed, however, in that its target was the hypervariable diagnostic fragment 3 (DF3) of the 18S rDNA ASA.S1 region (Di Filippo et al. 2015).

In recent years, it has become apparent that Muslim men in Karachi, Pakistan, are being exposed and are becoming infected with *N. fowleri* via ritual ablution that includes nasal rinsing using tap water. Dozens of men have become ill and died each year for the past several years in this city and most of the cases have been identified by one hospital (Shakoor et al. 2011). Due to the lack of reliable public drinking water in Karachi, there is a heavy reliance on storage tanks and wells. Many of these tanks and wells have tested positive for the presence of *N. fowleri*, though it is unclear how great of a role groundwater plays in the exposure to the organism because of the common use of a mixture of water types and the fact that ritual ablutions are often performed both at home and at local mosques (Siddiqui and Khan 2014). It is also unclear why these cases have only been identified in Karachi and not in other cities in the region. It is likely that there is greater physician awareness of the disease in Karachi and that cases are being misdiagnosed in other areas.

Occurrence in hot springs

N. fowleri has been known for some time to be present in hot springs. Many public hot springs have signs posted to warn bathers not to immerse their heads in the waters because of the risks of *N. fowleri* infection. According to the Centers for Disease Control and Prevention, five of the 127 cases of PAM which occurred in the United States between 1962 and 2015 with known water exposures were linked to exposure to geothermally heated water such as hot springs (Centers for Disease Control and Prevention 2017). Although numerous PAM cases caused by *N. fowleri* linked to natural geothermal waters have also been identified worldwide, limited studies have been conducted to assess the organism's occurrence in hot springs.

In a study conducted in the French West Indies between 2011 and 2012, 500-ml water samples were collected from four geothermal pools and two warm river bodies (with temperatures ranging from 26.9 and 34.9 °C). Three of the geothermal pools were recreational baths and were regularly washed with detergent and treated with chlorine. Despite this, *N. fowleri* was found in 28 of 73 water samples (38.3%) and 5 of 48 sediment samples (10.4%) by PCR using primers specific to the *N. fowleri* ITS region. A total of 11 of the positive samples were detected in the river water samples. Interestingly, 15 of the 17 geothermal pool samples that were positive for *N. fowleri* were those collected from the baths that were regularly treated with detergent and chlorine. *N. fowleri* was the most common and widely distributed of the free-living amoebae in these waters, though its concentration tended to be low (≤ 22/L). Its prevalence was positively correlated with the turbidity of the waters ($P = 0.02$; Moussa et al. 2013).

A study of hot springs in Thailand identified *N. fowleri* in 35.3% of 68 springs with temperatures ranging from 28 to 65 °C (Lekkla et al. 2005). A survey of sediments, algal mats, and biofilm scrapings of rock surfaces from 23 hot springs in Yellowstone and Grand Teton National Parks found *N. fowleri* by conventional PCR (of the ITS region) in three locations with temperatures ranging from 35 to 40 °C and a pH range of 3.3–7.4 (Sheehan et al. 2003). A study of 22 samples from 11 hot springs in Japan identified *Naegleria* species in 47% of the samples; however, no *N. fowleri* were isolated (Kuroki et al. 1998). Similarly, in a recent study of 22 therapeutic hot springs in northern Iran, *N. fowleri* was not detected in any of the samples despite 54% testing positive for other *Naegleria* species by PCR (Latifi et al. 2017). And finally, in a limited study of three hot springs in Arizona with temperatures ranging from 24 to 42 °C, *N. fowleri* was identified in one hot spring with a temperature of 40.5 °C using centrifugation/filtration to concentrate 1-L grab samples, followed by a nested PCR assay targeting the *Mp2Cl5* gene (Charles P. Gerba, The University of Arizona, unpublished data, 2008).

Discussion

In spite of limited scientific studies investigating the occurrence of *N. fowleri* in groundwater and geothermally heated waters, it is clear that the amoeba is found naturally in these waters, particularly in warmer regions. Also, although *N. fowleri* can be found in the winter months in southern states as well as in northern states with colder waters such as Pennsylvania and Connecticut, the vast majority of recorded cases of PAM in the United States have occurred during the summer (110 of 127 between August and September; no cases between December and March; Centers for Disease Control and Prevention 2017). Recently, PAM cases have been reported in areas farther north than in previous years in states such as Minnesota, Indiana, Missouri, and Kansas. The temperature of shallow groundwaters generally reflects the average air temperatures of the region; therefore, as air temperatures increase due to climate change, water temperatures would also be expected to rise. Nevertheless, more information regarding these habitats and the physical, chemical, and microbial water quality of these waters is necessary to better understand the ecology of the organism and the conditions that lead to its presence and persistence and might lead to greater risks to bathers. For instance, although *N. fowleri* has been strongly linked to warm waters such as hot springs and pools thermally polluted by industry, most studies on groundwater have not been able to correlate the presence of the organism with the temperature of the water, though this is likely due to the typically limited number of samples collected.

It is also unclear why certain groundwater wells are persistently colonized by *N. fowleri* while others are not (Bright et al. 2009), despite interventions using high chlorine concentrations in attempts to eradicate the organism from the well casing (Kelly Bright and Charles Gerba, The University of Arizona, unpublished data, 2005). Another mostly unexplored avenue of research is the role of biofilms in the survival and persistence of *N. fowleri* in wells and distribution system pipes/infrastructure (that have groundwater as a source). *N. fowleri* in biofilms are more resistant to disinfectants such as chlorine and chloramines (Goudot et al. 2014; Cope et al. 2015; Miller et al. 2015, 2017). The occurrence of *N. fowleri* in the plumbing of buildings is also a largely unexplored area, though the organism has been found in areas such as sink taps, shower heads, and hot water heaters in individual case investigations (Kelly Bright and Charles Gerba, The University of Arizona, unpublished data, 2009; Marciano-Cabral et al. 2003; Centers for Disease Control and Prevention 2013; Siddiqui and Khan 2014).

Such information is necessary to better educate the public that these waters are not risk free so that they can take the necessary precautions while utilizing these water sources for recreation or other purposes (e.g., ritual ablution) to minimize their exposure. This is especially important due to the perception that such groundwater and natural spring waters are "cleaner" than other recreational water sources.

References

Abrahams-Sandi E, Retana-Moreira L, Castro-Castillo A, Reyes-Batlle M, Lorenzo-Morales J (2015) Fatal meningoencephalitis in child and isolation of *Naegleria fowleri* from hot springs in Costa Rica. Emerg Infect Dis 21(2):382–384

Baquero RA, Reyes-Batlle M, Nicola GG, Navarro CM, Lop'ez-Arencibia A, Esteban JG, Valladares B, Martı'nez-Carretero E, Pinero JE, Lorenzo-Morales J (2014) Presence of potentially pathogenic free-living amoebae strains from well water samples in Guinea-Bissau. Pathogens Glob Health 108(4):206–211

Booth PJ, Bodager D, Slade TA, Jett S (2015) Primary amebic meningoencephalitis associated with hot spring exposure during international travel: Seminole County, Florida, July 2014. MMWR 64(43):1226

Bright KR, Marciano-Cabral F, Gerba CP (2009) Occurrence of *Naegleria fowleri* in Arizona drinking water supply wells. J AWWA 101(11):43–50

Cain ARR, Wiley PF, Brownell B, Warhurst DC (1981) Primary amoebic meningoencephalitis. Arch Dis Child 56:140–143

Centers for Disease Control and Prevention (2013) Primary amebic meningoencephalitis associated with ritual nasal rinsing: St. Thomas, U.S. Virgin Islands, 2012. MMWR 62(45):904

Centers for Disease Control and Prevention (2017) *Naegleria fowleri*: primary amebic meningoencephalitis (PAM)—amebic encephalitis. https://www.cdc.gov/parasites/naegleria/index.html. http://www.cdc.gov/parasites/naegleria/graphs.html#exposure. Accessed 10 February 2017

Cope JR, Ratard RC, Hill VR, Sokol T, Causey JJ, Yoder JS, Mirani G, Mull B, Mukerjee KA, Narayanan J, Doucet M, Qvarnstrom Y, Poole CN, Akingbola OA, Ritter J, Xiong Z, da Silva A, Roellig D, Van Dyke R, Stern H, Xiao L, Beach MJ (2015) The first association of a primary amebic meningoencephalitis death with culturable *Naegleria fowleri* in tap water from a U.S. treated public drinking water system. Clin Infect Dis 60(8):e36–e42

Cope JR, Conrad DA, Cohen N, Cotilla M, DaSilva A, Jackson J, Visvesvara GS (2016) Use of the novel therapeutic agent Miltefosine for the treatment of primary amebic Meningoencephalitis: report of 1 fatal and 1 surviving case. Clin Infect Dis 62(6):774–776

Cursons R, Sleigh J, Hood D, Pullon D (2003) A case of primary amoebic Meningoencephalitis: North Island, New Zealand. N Z Med J 116(1187):U712

Di Filippo MM, Santoro M, Lovreglio P, Monno R, Capolongo C, Calia C, Fumarola L, D'Alfonso R, Berrilli F, Di Cave D (2015) Isolation and molecular characterization of free-living amoebae from different water sources in Italy. Int J Environ Res Public Health 12:3417–3427

Gautam PL, Sharma S, Puri S, Kumar R, Midha V, Bansal R (2012) A rare case of survival from primary amebic meningoencephalitis. Indian J Crit Care Med 16:34–36

Goldschmidt D, Scutti S (2016) Rare recovery: Florida teen survives brain-eating amoeba. http://www.cnn.com/2016/08/23/health/brain-eating-amoeba-florida-teen-survives/index.html. Accessed 23 August 2016

Goudot S, Herbelin P, Mathieu L, Soreau S, Banas S, Jorand FPA (2014) Biocidal efficacy of monochloramine against planktonic and biofilm-associated *Naegleria fowleri* cells. J Appl Microbiol 116(4):1055–1065

Gupta R, Parashar MK, Kale A (2015) Primary amoebic Meningoencephalitis. J Assoc Physicians India 63(4):69–72

Huizinga HW, McLaughlin GL (1990) Thermal ecology of *Naegleria fowleri* from a power plant cooling reservoir. Appl Environ Microbiol 56(7):2200–2205

John DT (1982) Primary amebic meningoencephalitis and the biology of *Naegleria fowleri*. Annu Rev Microbiol 36:101–123

Johnson RO, Cope JR, Moskowitz M, Kahler A, Hill V, Behrendt K, Molinal L, Fullerton KE, Beach MJ (2016) Primary amebic meningoencephalitis associated with exposure to swimming pool water supplied by an overland pipe: Inyo County, California, 2015. MMWR 65(16):424

Kuroki T, Yagita K, Yabuuchi E, Agata K, Ishima T, Katsube Y, Endo T (1998) Isolation of *Legionella* and free-living amoebae at hot spring spas in Kanagawa, Japan. Kansenshogaku Zasshi 72(10):1050–1055

Laseke I, Korte J, Lamendella R, Kaneshiro ES, Marciano-Cabral F, Oerther DB (2010) Identification of *Naegleria fowleri* in warm ground water aquifers. J Environ Qual 39:147–153

Latifi AR, Niyyati M, Lorenzo-Morales J, Haghighi A, Tabaei SJ, Lasjerdi Z, Azargashb E (2017) Occurrence of *Naegleria* species in therapeutic geothermal water sources, northern Iran. Acta Parasitol 62(1):104–109

Lee SH, Levy DA, Craun GF, Beach MJ, Calderon RL (2002) Surveillance for waterborne-disease outbreaks: United States, 1999–2000. MMWR Surveill Summ 51(8):1–47

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

Linam WM, Ahmed M, Cope JR, Chu C, Visvesvara GS, da Silva AJ, Qvarnstrom Y, Green J (2015) Successful treatment of an adolescent with *Naegleria fowleri* primary amebic Meningoencephalitis. Pediatrics 135(3):e744–e748

Mackowiak PA (2010) Another killer of the Australian bush: a rapidly fatal meningoencephalitis in a child. Clin Infect Dis 50(10):1375–1376

MacLean RC, Richardson DJ, Lepardo R, Marciano-Cabral F (2004) The identification of *Naegleria fowleri* from water and soil samples by nested PCR. Parasitol Res 93(3):211–217

Marciano-Cabral F, MacLean R, Mensah A, LaPat-Polasko L (2003) Identification of *Naegleria fowleri* in domestic water sources by nested PCR. Appl Environ Microbiol 69(10):5864–5869

Marshall M, Naumovitz D, Ortega Y, Sterling CR (1997) Water-borne protozoan pathogens. Clin Microbiol Rev 10(1):67–85

Martinez AJ, Visvesvara GS (1997) Free-living, amphizoic and opportunistic amebas. Brain Pathol 7(1):583–598

Miller HC, Wylie J, Dejean G, Kaksonen A, Sutton D, Braun K, Puzon G (2015) Reduced efficiency of chlorine disinfection of *Naegleria fowleri* in drinking water distribution biofilm. Environ Sci Technol 49(18):11125–11131

Miller HC, Morgan MJ, Wylie JT, Kaksonen AH, Sutton D, Braun K, Puzon GJ (2017) Elimination of *Naegleria fowleri* from bulk water and biofilm in an operational drinking water distribution system. Water Res 110:15–26

Moussa M, De Jonckheere JF, Guerlotte 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):1–11

Moussa M, Tissot O, Guerlotté J, De Jonckheere JF, Talarmin A (2015) Soil is the origin for the presence of *Naegleria fowleri* in the thermal recreational waters. Parasitol Res 114(1):311–315

Okuda DT, Hanna HJ, Coons SW, Bodensteiner JB (2004) *Naegleria fowleri* hemorrhagic Meningoencephalitis: report of two fatalities in children. J Child Neurol 19(3):231–233

Schoeman CJ, van der Vyver AE, Visvesvara GS (1993) Primary amoebic meningo-encephalitis in southern Africa. J Infect 26(2):211–214

Schuster F, Visvesvara GS (2004) Opportunistic amoebae: challenges in prophylaxis and treatment. Drug Resist Updat 7(1):41–51

Seidel JS, Harmatz P, Visvesvara GS, Cohen A, Edwards J, Turner J (1982) Successful treatment of primary amebic Meningoencephalitis. New Engl J Med 306(6):346–348

Shakoor S, Beg MA, Mahmood SF, Bandea R, Sriram R, Noman F, Ali F, Visvesvara GS, Zafar A (2011) Primary amebic meningoencephalitis caused by *Naegleria fowleri*, Karachi, Pakistan. Emerg Infect Dis 17(2):258–261

Sheehan KB, Fagg JA, Ferris MJ, Henson JM (2003) PCR detection and analysis of the free-living amoeba Naegleria in Hot Springs in Yellowstone and Grand Teton National Parks. Appl Environ Microbiol 69(10):5914–5918

Siddiqui R, Khan NA (2014) Primary amoebic meningoencephalitis caused by *Naegleria fowleri*: an old enemy presenting new challenges. PLOS Neglected Trop Dis 8(8):1–8

Sifuentes LY, Choate BL, Gerba CP, Bright KR (2014) The occurrence of *Naegleria fowleri* in recreational waters in Arizona. J Environ Sci Health, Pt A 49:1322–1330

Su MY, Lee MS, Shyu LY, Lin WC, Hsiao PC, Wang CP, Ji DD, Chen KM, Lai SC (2013) A fatal case of *Naegleria fowleri* meningoencephalitis in Taiwan. Korean J Parasitol 51(2):203–206

Tyndall RL, Ironside KS, Metler PL, Tan EL, Hazen TC, Fiermans CB (1989) Effects of thermal additions on the density and distributions of thermophilic amoebae and pathogenic *Naegleria fowleri* in a newly created cooling lake. Appl Environ Microbiol 55(3):722–732

Visvesvara GS, Moura H, Schuster FL (2007) Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. FEMS Immunol Med Microbiol 50(1):1–26

Wang A, Kay R, Poon WS, Ng HK (1993) Successful treatment of amoebic meningoencephalitis in a Chinese living in Hong Kong. Clin Neurol Neurosurg 95(3):249–252

Wellings FM, Amuso PT, Chang SL, Lewis AL (1977) Isolation and Identification of pathogenic *Naegleria* from Florida lakes. Appl Environ Microbiol 34(6):661–667

Yoder JS, Straif-Bourgeois S, Roy SL, Moore TA, Visvesvara GS, Ratard RC, Hill VR, Wilson JD, Linscott AJ, Crager R, Kozak NA, Sriram R, Narayanan J, Mull B, Kahler AM, Schneeberger C, da Silva AJ, Poudel M, Baumgarten KL, Xiao L, Beach MJ (2012) Primary amebic meningoencephalitis deaths associated with sinus irrigation using contaminated tap water. Clin Infect Dis 55(9):e79–e85