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REVIEW

Behavioral and Recreational Risk Factors for Free-Living Amebic Infections

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Free-living amebae of the genera *Acanthamoeba*, *Balamuthia*, *Naegleria*, and *Sappinia* are rare causes of infectious diseases in humans with the exception of *Acanthamoeba* keratitis (AK), which is reported in over 10,000 soft contact lens wearers annually worldwide. Unlike several *Acanthamoeba* species, which can cause both AK and granulomatous amebic encephalitis (GAE), only one species of *Naegleria*, *Naegleria fowleri*, is known to infect humans by causing an acute, fulminant, usually lethal, central nervous system (CNS) infection, known as primary amebic meningoencephalitis (PAM).[1–6] Both *Acanthamoeba* species and *N fowleri* are distributed worldwide; found commonly in freshwater; and have even been isolated from tap water, air conditioning systems, and improperly maintained swimming pools.[1–5]

Balamuthia mandrillaris, formerly known as leptomyxid ameba, is another opportunistic, free-living ameba. Like *Acanthamoeba* spp, *B mandrillaris* is capable of causing skin lesions and GAE in individuals with compromised or competent immune systems, who inhale infective spores or develop indolent, granulomatous skin lesions in soil-contaminated wounds. Lastly, *Sappinia pedata*, a recently identified free-living ameba that lives in soil and domestic animal feces, has caused a single case of non-GAE in an immunocompetent Texas farmer.

CNS infections caused by these ubiquitous organisms remain rare despite expanding world populations; but are, nevertheless, increasing today due to a combination of factors including increased freshwater recreational activities during heat waves for PAM, more immunocompromised individuals susceptible to GAE, and more soft contact lens wearers at risk of AK.[6,7]

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The purpose of this review will be to describe the epidemiology, pathophysiology, clinical manifestations, diagnosis, and management of free-living amebic infections. In addition, United States Centers for Disease Control and Prevention (CDC) laboratory-confirmed cases of PAM, *B mandrillaris* GAE, and AK will be analyzed statistically to determine significant risk factors for exposure and infection; and to recommend strategies for the management and prevention of these increasingly described free-living amebic CNS infections.

Materials and Methods

Initially, Medline, Pub Med, Google®, and Google Scholar® search engines were queried for references using all of the key words as medical subject headings terms. The only cases of free-living amebic meningoencephalitis included in the case analyses were cases with CDC laboratory-confirmed detection of *N fowleri*, *Acanthamoeba* spp, or *B mandrillaris* life forms or DNA as detected by polymerase chain reaction (PCR) in cerebrospinal fluid (CSF), brain biopsy, or brain necropsy tissue. Sources of US cases of PAM came from the registry of the CDC's *Naegleria* Workgroup, which ultimately confirmed 121 cases of PAM in the United States during the period 1937 to 2007.[2] Similar analyses were conducted for all CDC laboratory-confirmed cases of GAE caused by *B mandrillaris* ($N = 15$) in the United States during the period, 1999 to 2007. Sources of US cases of *Balmuthia* GAE, or balamuthiasis, came from state departments of public health and the California Encephalitis Project, a joint project launched in 1998 by the California Department of Public Health and the CDC. Similar analyses were conducted for CDC laboratory-confirmed cases of AK during the period, 1987 to 2007 ($N = 73$). Significant behavioral, demographic, and recreational risk factors for PAM, *Balamuthia* GAE, and AK were identified over the study period to make recommendations for the early diagnosis, management, and prevention of these infections.

All categorical variables were analyzed for statistically significant differences by Yates-corrected, chi-square analyses that compared patients with potential risk factors for free-living amebic infections to patients with meningoencephalitis or infectious keratitis of undetermined causes or to other cases of free-living amebic meningoencephalitis or infectious keratitis without risk factors reported during the same time periods. Statistical significance was indicated by p-values ≤ 0.05. As this investigation was a comparative statistical analysis of previously reported CDC-confirmed cases, institutional review board approval was not required.

Results

The Human Free-Living Amebic Infections

Table 1 compares and contrasts the prominent epidemiological, pathological, clinical, and diagnostic features of four free-living amebic infections in humans, and outlines some of their successful treatment strategies. Table 2 presents a step-wise approach for selecting and sending appropriate diagnostic laboratory specimens to the CDC Division of Parasitic Diseases for free-living ameba testing.

Primary Amebic Meningoencephalitis

Naegleria fowleri, the single causative agent of PAM, is a free-living amoeboflagellate that thrives in many types of hot freshwater including geothermal springs and warm water discharges from electrical power plants.[1–5] The parasite feeds on bacteria and organic debris in freshwater, and exists in three life forms; two of which are infective—the environmentally stable cyst form and the motile amoeboid-form, or trophozoite.[8–12] Infective forms invade humans via intact or disrupted nasal mucosa; cross the cribriform plate; migrate along the basilar brain from the olfactory bulbs and tracts to the cerebellum; deeply penetrate the cortex to the periventricular system; and incite a purulent meningoencephalitis with rapid cerebral edema, resulting in early fatal uncal and cerebellar herniation.[1,2,8–18] PAM cases usually occur when it is hot and dry for prolonged periods, causing both higher freshwater temperatures and lower water levels.[2] The incubation period from freshwater exposure and infection to meningoencephalitis may range from 1 to 16 days, but is usually 5 to 7 days.[2] Significant risk factors for PAM in the United States included male sex and warm recreational freshwater exposures in a seasonal pattern (July–August) in a southern tier state (Table 3).[2,13] The background frequency of PAM cases in the United States was zero to three cases per year over the entire 70-year study period, 1937 to 2007; three of the six cases (50%) in a 2007 cluster investigated by the CDC were males (ages 10, 11, and 22 y) who had been wakeboarding in freshwater lakes.[2]

The presenting clinical manifestations of PAM mimic acute bacterial meningitis and include presenting symptoms of headache, anorexia, nausea, vomiting, rhinitis, lethargy, fever, and stiff neck. Disorientation, ataxia, cranial nerve dysfunction (anisocoria, altered senses of smell and taste), mental status changes, seizure activity, and loss of consciousness may follow within hours of initial assessment.

Initial screening laboratory studies are nonspecific and often show peripheral leukocytosis, hyperglycemia, and glycosuria. Blood cultures and peripheral blood Gram stains will be negative for bacteria and other microorganisms. The laboratory diagnosis of PAM may be confirmed by one or more of the following laboratory techniques: (1) microscopic visualization of actively moving *N fowleri* trophozoites in wet mount preparations of freshly centrifuged CSF, not previously frozen or refrigerated; (2) microscopic visualization of *N fowleri* trophozoites in stained slide smears of centrifuged CSF sediments, or stained, fixed brain biopsy specimens; (3) microscopic visualization under ultraviolet light of *N fowleri* trophozoites by immunofluorescent techniques using indirect fluorescent antibodies in slide sections of either hematoxylin and eosin (H&E)-stained unfixed/frozen brain tissue or H&E-stained fixed brain tissue; (4) demonstration of *N fowleri* DNA by PCR from either CSF or brain tissue samples; or (5) microbiological culture of *N fowleri* on agar media.[2,12–14]

Neuroimaging studies in PAM are also nonspecific and may be normal on initial cranial computerized tomography (CT) and magnetic resonance imaging (MRI) scans.[14,19] Subsequent neuroimaging findings may include basilar leptomeningeal enhancement, massive cerebral edema, evidence of elevated intracranial pressure (ICP) (midline shift, compressed ventricles, compressed brainstem and basilar cisterns, and absence of subarachnoid spaces), and multifocal parenchymal lesions, often with evidence of hemorrhagic infarction or necrosis.[14,19] In 1998, Kidney and Kim compared the neuroimaging findings by CT and MRI in a case of *N fowleri*-confirmed PAM and a case of *B mandrillaris*-confirmed GAE.[19] As contrasted with nonspecific, diffuse cerebral edema in PAM, neuroimaging findings in GAE were more localized and included multiple, focal, punctuate, ring-enhancing lesions in the posterior fossa.[19] In 2006, Singh and colleagues described their findings by CT and MRI in five cases of PAM and GAE, and described a wide spectrum of imaging findings that included multifocal parenchymal lesions, pseudotumor-like lesions, meningeal exudates, hemorrhagic infarcts, and cerebral necrosis, with more focal findings in GAE than in PAM cases.[14]

Although usually futile, successful treatment strategies for PAM have included combinations of cerebral edema-reducing therapies (corticosteroids, moderate hyperventilation, diuresis, and hypertonic saline) and specific pharmacotherapy with antifungals (amphotericin B, miconazole, and voriconazole) and synergistic antibiotics (rifampin and azithromycin).[15–18]

Table 1 The human free-living amebic infections

Infections	PAM	GAE		SAE	AK
Pathogens	*Naegleria fowleri*	*Acanthamoeba* spp	*Balamuthia mandrillaris*	*Sappinia pedata*	*Acanthamoeba* spp
Distribution	Worldwide in warm freshwater, bottom sediment, and soil	Worldwide in freshwater and soil	Worldwide in freshwater and soil; more common in southern United States and South America	Demonstrated in soil and tree bark in the United States only	Worldwide in freshwater and soil
Cases reported worldwide (US incidence)	180–200	≤200	Approximately 150	Only one case reported	10,000 cases/y (1–2 cases/1 mo soft contact lens wearers/year-US)
Seasonal occurrence	Summertime or warmest seasons	Year-round	Year-round	Year-round	Year-round
High-risk groups	Immunocompetent children and young adults, especially males with history of freshwater exposures (skiing, wakeboarding) within 2 wk	Immunocompromised children and adults (AIDS, cancer or chemotherapy, organ or bone marrow transplant, liver or renal failure); rarely in the immunocompetent	Immunocompetent children and adults, most often males with soil exposures (dirt-biking, agriculture) and/or of Hispanic origin; less commonly in immunocompromised with AIDS or IV drug use	Immunocompetent farmer with preexisting sinus infection exposed to aerosols of domestic animal feces	Immunocompetent soft contact lens users, more often in females; use of contaminated contact lens cleaning solutions; swimming or showering with soft contact lenses; post corneal trauma
Pathology	Trophozoites penetrate nasal mucosa and cribriform plate and migrate via olfactory nerves to olfactory bulbs and tracts along basilar brain to cerebellum	Hematogenous dissemination from granulomatous skin ulcers or lung granulomas, across blood–brain barrier to CNS	Hematogenous dissemination from granulomatous skin ulcers, often facial, or lung granulomas across blood–brain barrier to CNS	Aerosolized cysts and/or trophozoites enter nasopharynx and directly invade CNS	Soil or stagnant water-dwelling infective cysts and/or trophozoites directly invade corneal epithelium predisposed by prolonged soft contact use, contaminated contact cleaning solutions, or corneal foreign bodies or trauma
Incubation period	Mean 5–7 d (range 1–16 d)	Weeks to months following indolent draining skin ulcers, sinusitis, or pneumonia	Mean 8.5 d (range 1–30 d) following indolent pneumonia or draining granulomas on the face or upper arms	Unknown	Unknown and often misdiagnosed and treated as a bacterial or herpetic keratitis or kerato-conjunctivitis
Clinical features	Fever, headache, stiff neck, nausea, vomiting, specific CN dysfunction (altered senses of smell and taste, anisocoria), seizures, disorientation, coma; more encepahlopathic than meningitic features	Similar to PAM, but more focal neurologic features, early mental status changes, visual loss, photophobia	Same as PAM and GAE with early confusion-disorientation, nonspecific CN dysfunction	Same as PAM, GAE, BAE, with sinusitis, early blurred vision, diplopia, photophobia	Eye pain and foreign body sensation, redness, blurred vision, photophobia, excessive tearing

Table 1 (*Continued*)

Infections	PAM	GAE		SAE	AK
Laboratory studies	Trophozoites in CSF wet mounts, stained CSF sediment or brain tissues enhanced by IIF or IFA; *N fowleri* DNA by PCR on CSF or unfixed brain	Both cysts and trophozoites in fixed, stained brain tissue enhanced by IIF or IFA; *Acanthamoeba* DNA by PCR on CSF or unfixed brain	IFA staining of fixed brain tissue; PCR for *Balmuthia* DNA in CSF or brain tissue	Distinctive trophozoites (double nucleus connected by filament, large contractile vacuole) in stained, fixed brain tissue	*Acanthamoeba* cysts and/or trophozoites in corneal smears, fixed, stained corneal scrapings; DNA by PCR; confocal microscopy for dendriform epitheliopathy
Imaging studies by CT and/or MRI	Nonspecific: basilar leptomeningeal enhancement, intraparenchymal lesions and/or hemorrhagic necrosis; evidence of ICP-cerebral edema, midline shift, cisternal and ventricular compression	Nonspecific: multiple space-occupying lesions, with or without ring-enhancing effects	Nonspecific: cerebral edema, hydrocephalus, multiple space-occupying and ring-enhancing in cortex and cerebellum	Single large solitary mass lesion with slight ring-enhancing effect-fronto-parietal or temporo-parietal	Not applicable
Treatment	IV and IT: amphotericin B, azoles-fluconazole, itraconazole, miconazole Synergistic antibiotics: azithromycin PO, rifampin Experimental: chlorpromazine or other phenothiazines, oral miltefosine	IV and IT: azoles IV: azoles, flucytosine, pentamidine, rifampin, trimethoprim/sulfamethoxazole Experimental: phenothiazines; oral miltefosine, topical miltefosine for skin ulcers	IV: azoles-albendazole, fluconazole, itraconazole, pentamidine, flucytosine, sulfadiazine Synergistic macrolides: azithromycin, clarithromycin Experimental: phenothiazines-thioridazine, trifluoperazine; oral miltefosine, topical miltefosine for skin ulcers	IV: pentamidine, flucytosine, itraconazole Synergistic antibiotic: azithromycin po	Topical: 0.02% chlorhexidine, 0.02% polyhexamethylene biguanide, 1% imidazole; PO azoles-itraconazole, ketoconazole, voriconazole
Outcomes (CFRs)	Death within 3–7 d (>95%)	Usually fatal in immunocompromised (90–94%); immunocompetent children most likely to survive	Usually fatal (≥90%)	One survivor in the United States	Treatment successes, 75–85% versus failures, 15–25%: corneal transplant, enucleation

PAM = primary amebic meningoencephalitis; GAE = granulomatous amebic encephalitis; SAE = *Sappinia* amebic encephalitis; AK = *Acanthamoeba* keratitis; BAE = *Balamuthia* amebic encephalitis; IFA = immunofluorescent assays; IIF = indirect immunofluorescence tests; CNS = central nervous system; CN = cranial nerve; CSF = cerebrospinal fluid; PCR = polymerase chain reaction nucleic acid assay; CT = computerized tomography; MRI = magnetic resonance imaging; ICP = intracranial pressure; IV = intravenous; IT: = intrathecal; CFR = case fatality rate.

Several experimental therapies have shown some promise in treating PAM, including chlorpromazine and miltefosine.[20,21] The optimal duration of therapy is unknown, but most survivors have been treated for 10 days.[8]

Today, PAM is best prevented by a combination of educational and behavioral modification strategies including the following.[2,13] (1) Avoid water-related activities, such as swimming, diving, water skiing, and wakeboarding in bodies of warm freshwater, hot springs, and thermally polluted water, such as around coal-burning and nuclear electrical power plants. (2) Avoid similar water-related activities in warm freshwater during prolonged periods of high water temperatures and low water volumes. (3) Hold the nose shut or use nose clips to avoid any traumatic disruptions in the nasal mucosal linings during water-related activities in warm freshwater, such as lakes, rivers, ponds, bayous, and

Table 2 Selecting and sending appropriate diagnostic laboratory specimens to the CDC for free-living ameba testing

Step 1	During the work week, contact the CDC Division of Parasitic Diseases either by email (dpdx@cdc.gov) or by telephone (770-488-4474) to describe your specimens and to request case report and specimen submission forms for free-living amebae testing. Case report forms are also available at www.cdc.gov/ncidod/dpd/parasites/naegleria/free_living_ameba_case_report_doc.
Step 2	On weekends or in emergencies, call the Epidemiology Branch of the CDC Division of Parasitic Diseases at 770-488-7760 for immediate assistance and specific tissue handling instructions.
Step 3	Body fluid and tissue specimens, including blood, biopsy, surgical, or autopsy specimens, may be collected for detection of free-living amebae (*Naegleria*, *Balamuthia*, and *Acanthamoeba* spp). The desired specimens include: • Blood for acute and convalescent serodiagnostic testing. • Tissue slides stained with H&E. • Unstained slides for indirect immunofluorescence studies. • Unfixed brain tissue or CSF for PCR, culture, latex agglutination studies, or wet mounts. • Unfixed corneal scrapings for *Acanthamoeba* spp. • Paraffin-embedded tissue blocks of brain, skin, abscess, sinus, or other tissue biopsies.
Step 4	When shipping specimens to the CDC, follow all shipping guidelines and requirements available at www.dpd.cdc.gov/dpdx/html/diagnosticprocedures.body_dp_otherspec_ship.

CDC = Centers for Disease Control and Prevention; H&E = hematoxylin and eosin; CSF = cerebrospinal fluid; PCR = polymerase chain reaction.

Table 3 Analysis of significant risk factors for primary amebic meningoencephalitis (PAM)—United States, 1937–2007, $N = 121$

Risk factors	X^2	*p*-Value
Male sex	73.210	0.0001*
Recreational freshwater exposure	47.515	0.001*
Seasonal (summer) exposure, July–September	105.875	0.00001*
Exposure in a southern tier state†	12.042	0.001*

X^2: Yates-corrected, two-tailed chi-square test values.
*Statistically significant, $p \leq 0.05$.
†Fifteen southern tier states: AR, AZ, CA, FL, GA, LA, MO, MS, NC, NM, NV, OK, SC, TX, and VA.

hot springs. (4) Avoid similar water-related activities in drainage ditches, retention or oxidation ponds, and irrigation canals. (5) Avoid digging in or stirring up the sediment during all water-related activities in shallow, warm freshwater areas.[2,13]

Granulomatous Amebic Encephalitis

GAE is a chronic infection of the brain that may disseminate to other organs hematogenously and usually occurs in immunosuppressed patients with AIDS or organ transplants, or in patients receiving chemotherapy for cancer or tuberculosis.[6,22–25] GAE may be caused by several species of *Acanthamoeba* or by another, phylogenetically related, free-living ameba, *B mandrillaris*. *Acanthamoeba* species and *B mandrillaris* are distributed worldwide in freshwater and soil, and can cause GAE year-round.[25] The portal of entry for these opportunistic pathogens is through the respiratory tract or ulcerating skin wounds with hematogenous spread to the CNS and, less commonly, with dissemination to other organs in the severely immunocompromised.[26]

To date, at least 250 cases of *Acanthamoeba* GAE and 150 cases of *Balamuthia* GAE have been reported, with acanthamoebiasis still confined mostly to the immunocompromised and balamuthiasis affecting both immunocompromised and immunocompetent individuals.[27–30] Besides immunocompromise, other potential risk factors for balamuthiasis may include contact with stagnant freshwater or with contaminated soil, often through agricultural work, desert motorcycling, dirt-biking, or even gardening.[30] The risk factors for balamuthiasis are analyzed in Table 4.

The incubation period for *Acanthamoeba* GAE could extend for weeks or months after primary inoculation in the skin, sinuses, or lungs, with subsequent draining ulcers, chronic sinusitis, or pneumonia.[30] Although primary inoculation with *B mandrillaris* is also via the skin or lungs, the incubation period is shorter than in *Acanthamoeba* GAE with a mean of 8.5 days and a range of 1 to 30 days.[26] The clinical presentation of GAE from either causative pathogen is the same with early behavioral and personality changes, fever, depressed mental status, seizures, photophobia, visual loss, and nonspecific cranial nerve dysfunction, followed by signs of increased ICP, including headache, nausea, vomiting, and loss of consciousness.[31,32]

The laboratory diagnosis of GAE from either causative pathogen is also similar with cysts and

Table 4 Analysis of significant risk factors for *Balamuthia mandrillaris* granulomatous amebic encephalitis (GAE)—United States, 1999–2007, $N = 15$

Risk factors	X^2	*p*-Value
Male sex	5.538	0.019*
Exposure in a southern tier state†	4.800	0.028*
Exposure in southern California	19.200	0.0001*
Hispanic ethnicity	2.462	0.117
Hispanic ethnicity in California	4.167	0.041*
Contact with soil	0.167	0.683
Initial skin wound contaminated with soil or stagnant freshwater	5.000	0.025*

X^2: Yates-corrected, two-tailed chi-square test values.
*Four southern tier states: CA, FL, GA, and TX.
†Statistically significant, $p \leq 0.05$.

trophozoites rarely identified in the CSF, but more often identified in fixed and stained skin ulcer biopsies, brain biopsies, and post-mortem brain tissue. Recently, immunodiagnostic tests, such as indirect immunofluorescent ultraviolet microscopy and indirect immunofluorescent antibody ultraviolet microscopy with specific antipathogen antibodies, and new PCR assays for identification of pathogen DNA have been developed for diagnostic specimens.[33] In 2006, Qvarnstrom and colleagues at the CDC described a new multiplex real-time PCR assay for the simultaneous detection of *Acanthamoeba* spp, *B mandrillaris*, and *N fowleri*, which will permit rapid and specific detection of a single free-living ameba in clinical specimens within 5 hours.[33]

Neuroimaging studies by axial CT and/or MRI in GAE are nonspecific and often include single to multiple space-occupying lesions in the brain from the frontal cortex to the cerebellum with ring enhancing and other focal effects slightly more common in balamuthiasis than in acanthamoebiasis.[14,19] Evidence of cerebral edema with increased ICP will often be present and may include midline shifts, cisternal and ventricular compression, and hydrocephalus.[14,19]

Treatment strategies for GAE will include combinations of critical care techniques to reduce increased ICP, craniotomy for biopsy or excision of mass lesions, and combination pharmacotherapy with antifungals, antiprotozoal agents, synergistic antibiotics, and several experimental therapies that have shown promise in vitro, such as phenothiazines. Although case fatality rates in GAE are very high (90%–94% in acanthamoebiasis and ≥90% in balamuthiasis), successful drug treatment combinations in acanthamoebiasis have included intravenous pentamidine isethionate, flucytosine (5-flurocytosine), amphotericin B, the benzimidazole antifungals (albendazole), the triazole antifungals (itraconazole and fluconazole), the synergistic antibiotics, rifampin and trimethroprim/sulfamethoxazole (TMP/SMX) (or amikacin or oral sulfadiazine), and topical ketoconazole or miltefosine for skin ulcers.[26,34–37] In 2008, Aichelburg and colleagues in Vienna reported treating a patient successfully with disseminated tuberculosis and acanthamoebiasis with topical and oral miltefosine, a phosphocholine analog used to treat visceral leishmaniasis, and a combination of intravenous fluconazole, TMP/SMX, synergistic antibiotics (amikacin), and four tuberculostatic drugs.[22] Successful intravenous drug treatment combinations in balamuthiasis have included azoles (albendazole, fluconazole, or itraconazole), flucytosine, pentamidine, sulfadiazine, and synergistic macrolide antibiotics (azithromycin or clarithromycin) and phenothiazines (thioridazine or trifluoperazine).[29,31] In 2004, Schuster and Visvesvara demonstrated that the phenothiazines demonstrated in vitro efficacy against *B mandrillaris* in clinical specimens.[34] The optimum duration of drug therapy for GAE is unknown, but most survivors have been treated for many weeks to months.[29–31,35–37] In 2010, Martinez and coworkers reported the successful treatment of *B mandrillaris*-confirmed GAE in a patient with extensive cutaneous and neurological involvement with prolonged therapy with albendazole, fluconazole, and miltefosine.[38]

A genetic predisposition to *B mandrillaris* GAE has now been identified in American Hispanics, who appear less able to produce effective antibodies against the free-living amebae, and may be predisposed by more frequent contact with *Balamuthia*-contaminated soils and aerosols in agricultural occupations.[39,40]

Prevention and control strategies for GAE should include (1) consideration of GAE in organ transplant and immunocompromised patients with encephalitis and skin ulcers not improving with standard therapies; (2) recognition of genetic risk factors for acanthamoebiasis and balamuthiasis in Hispanics less able to produce antibodies against causative free-living amebae; and (3) recognition of other soil or stagnant freshwater risk factors in both immunocompetent and immunosuppressed patients with skin ulcers and unexplained meningoencephalitis.[39,40]

Acanthamoeba Keratitis

Acanthamoeba spp also cause a subacute to chronic keratitis that has occurred following prolonged wearing of, showering, swimming, or sleeping with soft contact lens.[41–44] AK has also been reported after using contaminated contact lens cleansing solutions, following corneal trauma, and, rarely, after radial keratotomy.[41–44] The incidence rate of AK has been increasing worldwide and is now reported to be 10,000 cases per year or 1 to 2 cases per 1 million soft contact lens wearers in the United States, or approximately 10,000 cases per year among contact lens users worldwide.[42,43]

A significant outbreak of AK in US contact lens wearers was first confirmed by the CDC in January 2007 after an increasing number of cases were reported in Chicago, Illinois, in late 2006.[42,43] In March 2007, the CDC completed a retrospective survey analysis of AK cases from 22 national ophthalmology centers and documented an increase in US culture-confirmed cases of AK beginning in 2004, a widespread geographic distribution.[42,43] By June 2007, the CDC had received reports from state public health departments and ophthalmologists from 37 US states and Puerto Rico identifying 221 patients with AK, 158 of whom had culture-positive AK.[42,43] A risk factor analysis of culture-confirmed cases demonstrated a significant association between AK in soft contact lens wearers and the use of a specific brand of multi-purpose contact lens cleanser solution, Complete® MoisturePlus™ (Advanced Medical Optics, Santa Ana, CA, USA).[42–44] This product was recalled immediately and removed from the US market. Contact lens wearers were advised to: (1) stop using the product immediately and discard remaining solutions; (2) choose an alternative contact lens solution; (3) discard current contact lens storage containers; and (4) see an eye-care provider if

experiencing any signs of eye infection, including eye pain, redness, blurred vision, photophobia, excessive tearing, or foreign body sensation.[43,44] An analysis of significant risk factors for AK is presented in Table 5.

The presenting clinical manifestations of AK include a prodrome of days of unilateral ocular redness, foreign body sensation, and excessive tearing, followed by intense ocular pain. Confocal microscopy will confirm dendriform epitheliopathy; and corneal smears or fixed, stained corneal scrapings often demonstrate *Acanthamoeba* spp cysts and/or trophozoites.[41–43] PCR assays for the detection of *Acanthamoeba* nucleic acids will also confirm diagnosis.[42,43] Early treatment with topical 0.02% chlorhexadine, 0.02% polyhexamethylene biguanide, or 1% imidazole, often combined with an oral azole (itraconazole, ketoconazole, or voriconazole), is successful in over 75% of cases; with corneal transplant or enucleation reserved for treatment failures.[42,43]

Prevention and control strategies for AK include (1) avoiding showering and swimming while wearing contact lenses; (2) using only sterile commercial contact lens cleansings solutions, rather than homemade solutions; (3) disinfecting contact lenses every night in sterile cleansing solutions; (4) allowing contact lens cases to air dry during the day; and (5) switching to daily disposable contact lenses, and disposing of their cases every 3 months, or microwaving their cases for 3 min on high power every 3 months.[41–43]

Conclusions

Once considered non-pathogenic, free-living amebae have emerged over recent decades as significant pathogenic threats to human health for several reasons including the following. (1) Free-living amebae are widely distributed in soil and freshwater throughout the temperate and tropical world, have environmentally stable cyst forms for over-wintering, and have taken advantage of longer warm seasons to parasitize humans in their outdoor pursuits.[7] (2) Some free-living amebae are frequently opportunistic, but can also evade host responses in immunocompetent individuals, such as *Acanthamoeba* spp, *B mandrillaris*, and *S pedata*.[44] (3) Free-living amebae are resistant to antimicrobial monotherapy and require combined therapy with a variety of antimicrobials.[44] (4) Free-living amebic infections are often difficult to diagnose unless suspected; the laboratory is alerted to the possibility of amebic forms in diagnostic specimens; and confirmatory immunological and molecular tests are available, usually at distant reference labs (see Table 2). (5) Lastly, some ethnic groups, such as American Hispanics, may be genetically predisposed to GAE because they cannot muster protective antibody responses to phylogenetically related *Acanthamoeba* spp and *B mandrillaris*.[39,40]

Travel medicine clinicians should suspect free-living amebic infections of the CNS in refractory cases of meningoencephalitis initially managed as aseptic or bacterial infections, especially in patients predisposed to such infections by regions visited, behavioral practices, ethnicity, or immunosuppression. In addition, travel medicine clinicians should advise patients not to shower or swim with contact lenses on, should suspect AK in soft contact lens wearers with refractory keratitis, and refer probable AK cases to ophthalmologists for further evaluation and treatment. Future investigations will be required to determine the significance of freshwater wakeboarding, popular among adolescents, as a significant recreational risk factor for PAM and to determine any dose-response effects of global warming on rising freshwater temperatures and the multiplication and infectivity of aquatic free-living amebae.

Table 5 Analysis of significant risk factors for *Acanthamoeba* keratitis (AK)—United States, 1987–2007, $N = 73$

Risk factors	X^2	*p*-Value
Male sex	0.986	0.321
Female sex	0.986	0.321
Wearing soft contact lenses	53.041	0.0001*
Disinfecting contact lenses less than recommended by manufacturer	8.000	0.005*
Using homemade disinfectant solutions	14.519	0.0001*
Swimming with contact lenses in place	0.116	0.733
Showering with contact lenses in place	34.717	0.0001*

X^2: Yates-corrected, two-tailed chi-square test values.
*Statistically significant, $p \leq 0.05$.

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Declaration of Interests

The author states he has no conflicts of interest to declare.

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