water exposure. *N. fowleri* gains access to the CNS by direct invasion through the nasal mucosa and the cribriform plate and causes a rapidly fatal meningoencephalitis. GAE, caused by *Acanthamoeba* spp. and *B. mandrillaris,* is a subacute infection that likely spreads hematogenously from pulmonary or skin lesions to the CNS. The resultant focal neurologic deficits progress over days to months to a diffuse meningoencephalitis and death. Disseminated granulomatous amebic disease involving the skin, lungs, or sinuses (but without CNS infection) with *Acanthamoeba* and *Balamuthia* have also been reported.[5,6] *Acanthamoeba* spp. also cause a subacute to chronic keratitis that is most often associated with contact lens use or corneal trauma, with rare reports of cases occurring after radial keratotomy and laser-assisted in situ keratomileusis (LASIK).[4,7]

ORGANISMS

Naegleria

N. fowleri was named after Malcolm Fowler of Adelaide Children's Hospital of Australia, who (with R.F. Carter) described the initial cases of PAM.[8] Of the approximately 30 species in the genus, *N. fowleri* is the only known pathogen of humans, although other species cause disease in mice (e.g., *Naegleria australiensis* and *Naegleria italica*).[9] Based on sequencing analysis, *N. fowleri* seems to have evolved from the nonpathogenic species *Naegleria lovaniensis* in North America. Globally, eight genotypes of *N. fowleri* have been identified by sequencing with types 1, 2, and 3 endemic to the United States. Type 1 is unique to the United States, and types 2 and 3 appear to be the most common worldwide.[10] Although the pathogenicity among strains appears similar, genotyping isolates can help define connections between cases within an outbreak or cluster.

Naegleria spp. have three life-cycle stages: trophozoites, flagellates, and cysts (Fig. 273.1). The trophozoites are the reproductive stage of the parasite and cause invasive human disease. Trophozoites feed predominantly on bacteria, are 10 to 25 µm in diameter, have pseudopodia, and have a clear nucleus with a prominent dense central nucleolus (Fig. 273.2). The granular cytoplasm can contain ingested red blood cells and leukocytes along with cytoplasmic organelles. On transfer to distilled water or a nonnutrient medium, trophozoites can transform rapidly to a transitory flagellate form, which does not divide or feed. The flagellate form can spontaneously revert to the trophozoite. Among the FLAs, only *Naegleria* has a flagellated life-cycle stage. When trophozoites encyst, the cyst is resistant to environmental stresses and is approximately 9 µm in diameter with a central nucleus and a single-layered wall containing an average of two pores. *N. fowleri* is thermophilic, with trophozoites growing well at temperatures as high as 45°C.[9] Organisms can be cultivated from clinical specimens and grow well in vitro on nonnutrient agar plates coated with bacteria. Like *Acanthamoeba* and *Balamuthia, N. fowleri* can be grown in a cell-free axenic medium, as well as in a chemically defined medium.

Acanthamoeba

Previously, *Acanthamoeba* were grouped by morphology and cyst size into three groups (I, II, and III), but they are now grouped by genetic similarity via newer molecular mechanisms. The genotyping is generally consistent with the morphologic grouping. Seventeen genotypes (T1–17) have been described, and groups T1, T2a, T3–6, T10–12, and T15 have been associated with human disease. Genotype T4 (correlating to the *Acanthamoeba castellanii* complex) is the most commonly identified in the environment and in human disease.[11] The life cycle of *Acanthamoeba* consists of trophozoite and cyst stages (see Fig. 273.1). Trophozoites are 15 to 50 µm in diameter, contain a single nucleus with a prominent central nucleolus, and have distinctive slender, spinelike projections of the plasma membrane (Fig. 273.3). The cysts have a double-layered wall, are less than 18 to 30 µm in diameter, and like *Naegleria* may contain pores in the cyst wall (Fig. 273.4). Trophozoites are the active form of *Acanthamoeba,* feeding on bacteria and environmental debris, whereas the cyst is the inactive but an environmentally resistant stage

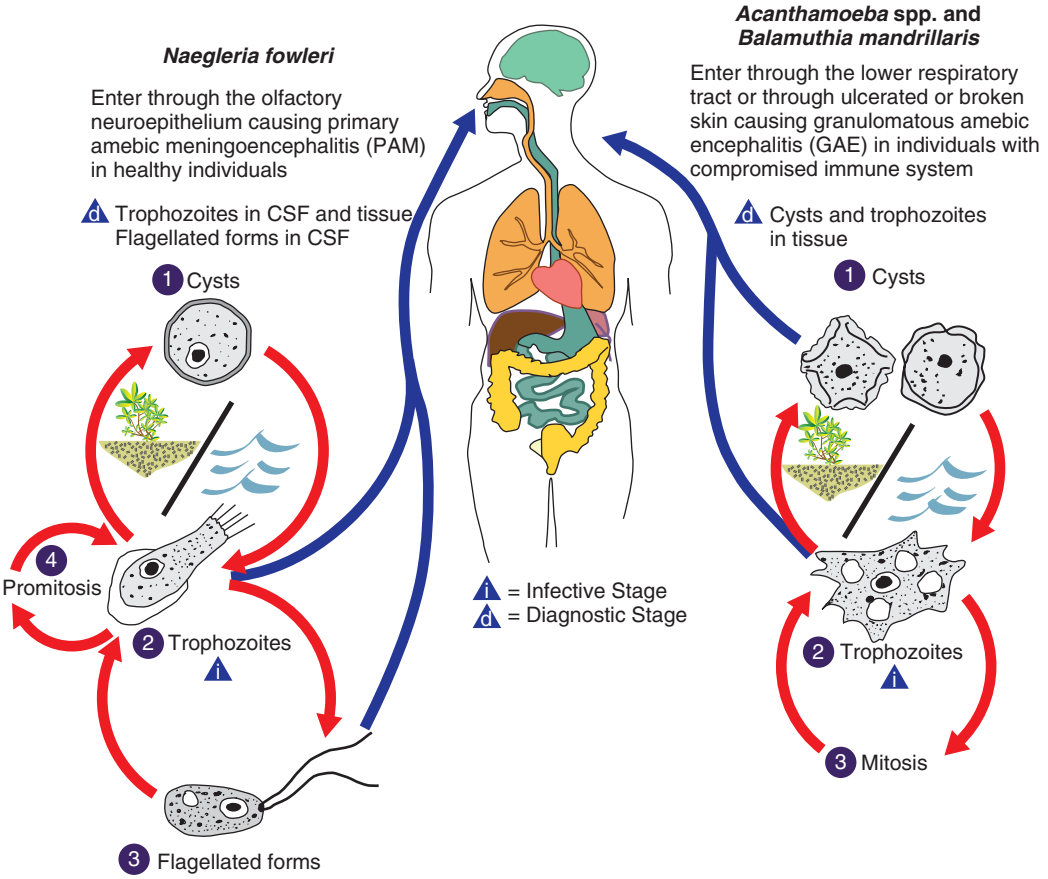


FIG. 273.1 Life cycles of *Naegleria fowleri, Acanthamoeba* spp., and *Balamuthia*, showing stages and proposed portals of entry. *CSF,* Cerebrospinal fluid. *(Courtesy Division of Parasitic Diseases/Centers for Disease Control and Prevention.)*

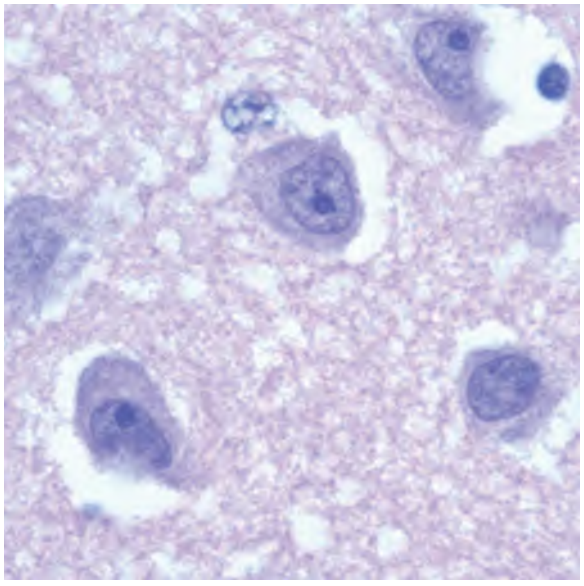


FIG. 273.2 Trophozoites of *Naegleria fowleri* in brain tissue stained with hematoxylin and eosin. Note the clear nucleus with a prominent dense, central nucleolus. *(Courtesy Division of Parasitic Diseases/Centers for Disease Control and Prevention.)*

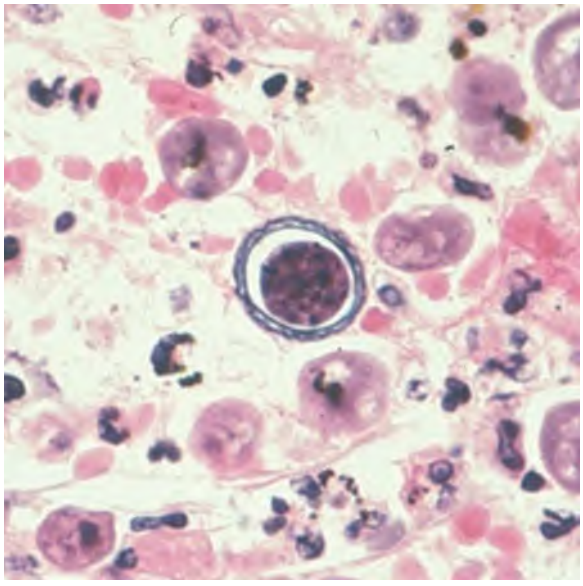


FIG. 273.4 *Acanthamoeba* cyst in brain tissue stained with hematoxylin and eosin. Note the double wall of the cyst. *(Courtesy Division of Parasitic Diseases/Centers for Disease Control and Prevention.)*

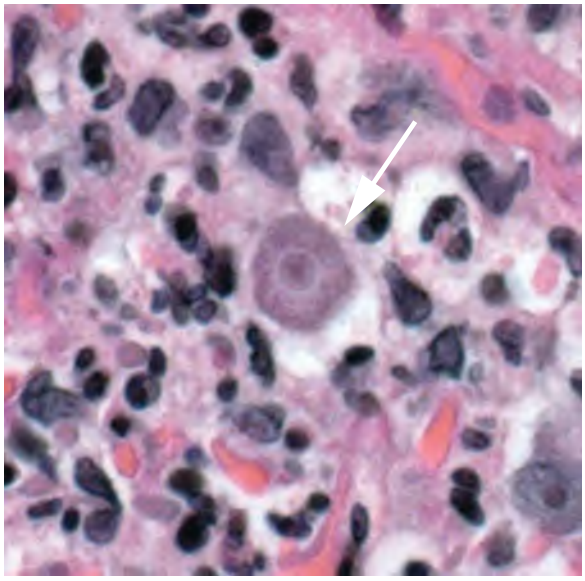


FIG. 273.3 *Acanthamoeba* trophozoite *(arrow)* in tissue stained with hematoxylin and eosin. Note the single nucleus with prominent central nucleolus. *(Courtesy Division of Parasitic Diseases/Centers for Disease Control and Prevention.)*

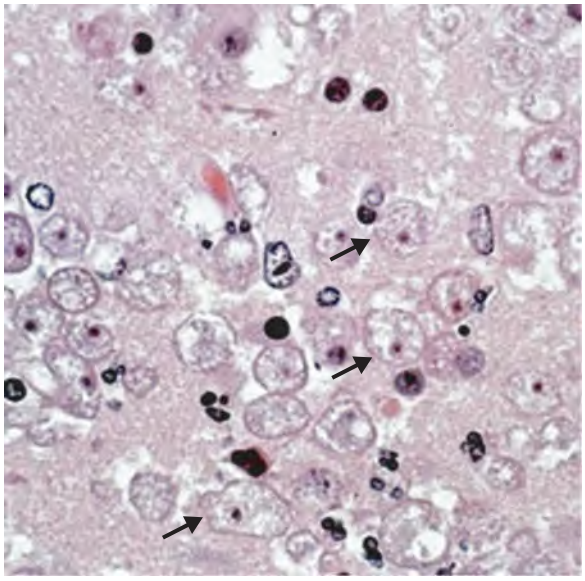


FIG. 273.5 *Balamuthia* trophozoites *(arrows)* in brain tissue stained with hematoxylin and eosin. Trophozoites are uninucleate and cannot be reliably distinguished from *Acanthamoeba* spp. trophozoites without specific immunostaining. *(Courtesy Division of Parasitic Diseases/Centers for Disease Control and Prevention.)*

and may explain the resistance of the organism to antimicrobial therapy, especially in the setting of amebic keratitis.[11] *Acanthamoeba* spp. can be easily cultivated in the laboratory; this is best accomplished by the use of tryptic soy agar with rabbit or horse blood, buffered charcoal yeast extract agar, and nonnutrient agar overlaid with live organisms, such as *Escherichia coli* or *Enterobacter aerogenes*.[1] Like *N. fowleri,* most *Acanthamoeba* spp. can be grown in both cell-free axenic and chemically defined media.[1]

Balamuthia

B. mandrillaris, formerly referred to as a *leptomyxid ameba,* is the only known species of this genus and was originally isolated from the brain of a mandrill baboon in 1986.[12] The life cycle consists of the trophozoite and cyst stages (see Fig. 273.1). *B. mandrillaris* trophozoites have a mean diameter of 30 µm (range, 12 to 60 µm) and are uninucleate (Fig. 273.5). Cysts have a mean diameter of 15 µm (range, 12–30 µm) with a wavy and irregular outer wall that is composed of three layers (Fig. 273.6).[1] On hematoxylin and eosin (H&E)-stained specimens, the organism cannot be reliably differentiated from *Acanthamoeba*. Laboratory growth of *Balamuthia* is more difficult than for *Acanthamoeba* or *Naegleria* because it does not grow on bacteria-coated agar plates and has a long doubling time. Currently, using a cell-free growth medium, axenization, and mammalian cell culture to grow *Balamuthia* in vitro, efforts are being made to better understand the components of the cyst and so offer new drug targets.[11,13] In addition, whole-genome sequencing of several *Balamuthia* strains was recently done. Functional analyses of the genome will offer new insights into *Balamuthia* as an organism and identify potential drug targets.[14]

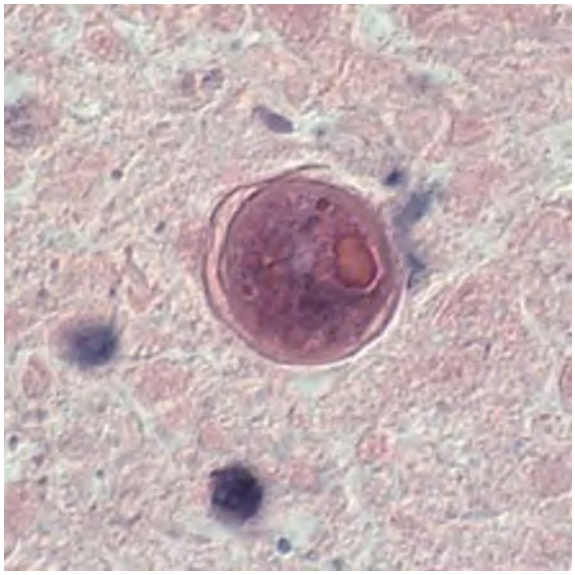


FIG. 273.6 *Balamuthia* cyst in brain tissue stained with hematoxylin and eosin. By light microscopy, only two walls can be distinguished. Specific immunostaining reliably distinguishes *Balamuthia* cysts from those of *Acanthamoeba* spp. *(Courtesy Division of Parasitic Diseases/Centers for Disease Control and Prevention and University of Kentucky Hospital, Lexington, Kentucky.)*

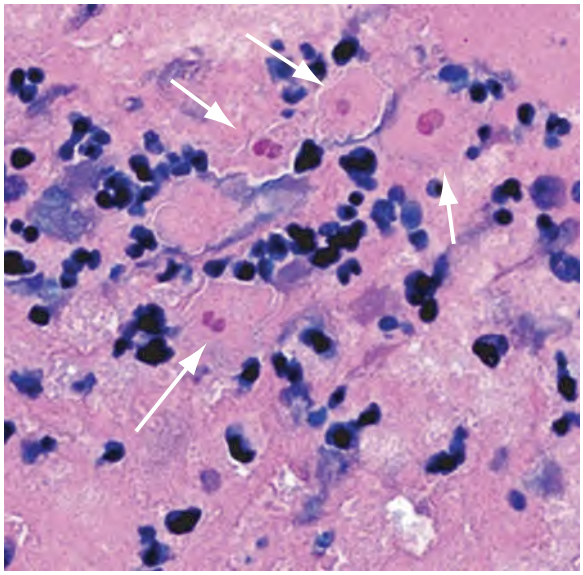


FIG. 273.7 Trophozoites of *Sappinia (arrows)* in brain tissue stained with hematoxylin and eosin. Note the distinctive double nucleus that defines this species. *(Courtesy Division of Parasitic Diseases/Centers for Disease Control and Prevention.)*

Other Free-Living Amebae Reported in Human Disease

Although *Acanthamoeba* spp. are the most common cause of amebic keratitis, *Allovahlkampfia* spp., *Vahlkampfia* spp., *Hartmannella* spp., and *Paravahlkamfia* spp. have each caused at least one human case of subacute keratitis, often in association with contact lens use or corneal trauma.[15–18]

In 2009 a previously healthy man originally diagnosed with viral meningitis was subsequently determined to have PAM caused by *Paravahlkamfia* spp. A newly designed real-time polymerase chain reaction (PCR) assay contributed to this diagnosis and suggests that this is a new species of FLA, termed *Paravahlkamfia francinae*.[19] Of note, he did not receive antimicrobial therapy active against FLAs but spontaneously recovered. This suggests the possibility that some previous survivors of *Naegleria* PAM, which is almost always fatal, may actually have been infected with *Paravahlkamfia* spp. instead. In addition, this case raises the possibility that some previously undiagnosed cases of "aseptic meningitis" may have been unrecognized PAM caused by a mildly virulent FLA.

In 2001 a report described *Sappinia diploidea* as the cause of GAE in a previously healthy 38-year-old man who survived the infection.[20] The identification of this FLA as *Sappinia diploidea* was done by H&E staining and transmission electron microscopy of the patient's excised brain lesion. Two subsequent reports that used molecular diagnostics suggest that this *Sappinia* sp. was most likely either *Sappinia pedata*[21] or a novel species of *Sappinia* that is molecularly closer to *S. pedata* than *S. diploidea*.[22] This is the only case of *Sappinia*-associated human disease reported to date (Fig. 273.7).

Overall, these single-case reports highlight the constant interaction between humans and FLAs and the need for a high index of suspicion for such infections to arrive at an accurate diagnosis.

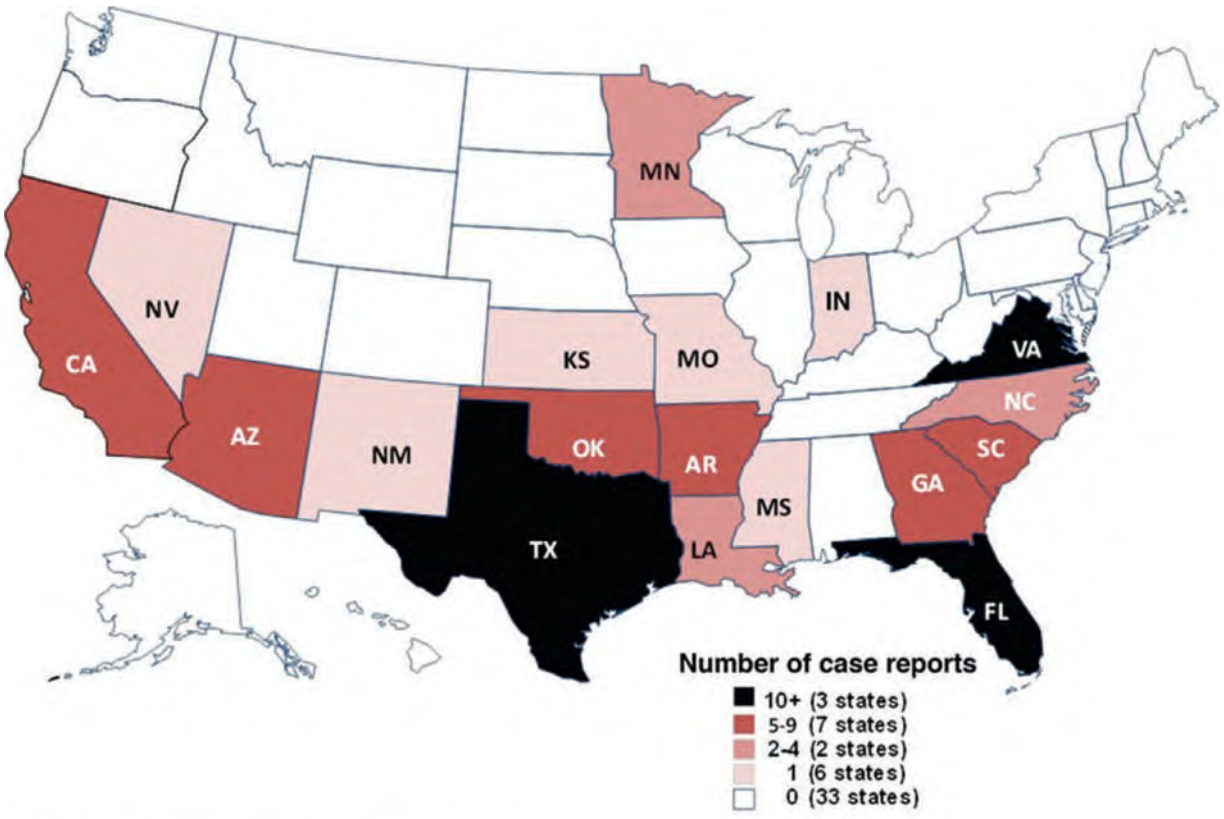
EPIDEMIOLOGY

Naegleria

N. fowleri has been found worldwide in river and lake water samples and in soil.[23] Importantly, *N. fowleri* is not found in seawater. *N. fowleri* has also been found in thermal springs, thermal saline baths, and mud springs. Pathogenic *N. fowleri* are thermophilic and proliferate at temperatures up to 45°C.[9] The presence of *N. fowleri* in fresh water is directly related to water temperature,[24] and *N. fowleri* has been frequently isolated from thermally polluted waters in temperate climates, including as far north in the United States as Minnesota.[25]

In semitropical locations such as Florida, it is not uncommon to isolate at least one *N. fowleri* organism per 25 mL of water.[24] As water temperatures drop in winter, *Naegleria* is typically isolated from lake-bottom sediments; *Naegleria* cysts are stable for up to 8 months at 4°C. Although there have probably been billions of people exposed to *Naegleria*-contaminated fresh water, few develop PAM.[24] The factors that protect most individuals from invasive *Naegleria* infection are not understood. In the southern United States the presence of serum-agglutinating activity against *N. fowleri* in many young adults (but not infants) indicates that subclinical infection or exposure to *Naegleria* is common.[26] PAM has been reported throughout much of the world.[27] Although the true incidence of this entity is unknown, hundreds of cases of PAM have been reported worldwide, with most having a recent history of recreational freshwater exposure. In the United States more than 100 cases of PAM were reported from 1962–2008. Annual reported case numbers ranged from 0 to 8 per year but do not appear to be increasing over time. The median age of patients was 12 years, 79% were male, and only one patient survived. Eighty-five percent of cases occurred in the summer months, and all were in southern-tier states, with more than half in Florida or Texas[28] (Fig. 273.8). Similar findings were noted in a review of 142 US cases from 1937–2013, including three well-documented survivors.[29] In August 2010 a 7-year-old girl died of PAM due to *Naegleria* acquired in Minnesota, expanding the range of this organism over 500 miles north of the previously northernmost known human case in the United States. She had swum in two lakes in the area during the third-hottest summer on record in Minneapolis, possibly an unfortunate consequence of climate change.[25] Several additional cases have been reported recently in non–Southern-tier states, including Kansas, Indiana, and another case in Minnesota.[29]

New reports of PAM continue to appear in the literature, and the current incidence is undoubtedly higher, with some estimates placing the incidence at about 16 deaths annually in the United States.[30] Clusters of PAM cases with common environmental exposures have occurred, including 16 deaths over a 3-year period that were retrospectively traced to a swimming pool in Czechoslovakia with a low chlorine concentration.[31] A young woman died of PAM after swimming in a pool in California that was served by water that was piped in from a mountain hot spring.[32] Recently, a young woman died of PAM after falling out of a raft and being submerged underwater at an artificial whitewater river facility in North Carolina; *N. fowleri* was detected in all of the water



*State of exposure unknown for 4 cases
†11/12 Virginia (VA) cases that occurred during 1937–1957 were identified as part of a retrospective pathology study.

FIG. 273.8 Number of case reports of *Naegleria fowleri* meningitis (*N* = 142) by state of exposure.[29]

samples subsequently tested from the facility.[33] In 2002 two previously healthy children from the same neighborhood in Arizona died of PAM due to *N. fowleri* within 1 day of each other, apparently acquired through exposure to household bathwater.[34] Drinking water for the respective families came from the same untreated community well-water system, and PCR testing revealed *N. fowleri* in the water system.[35] These cases were the first association of a drinking water system with *N. fowleri* infection in the United States.[34] A subsequent study demonstrated *N. fowleri* (detected by PCR) in 16% of 185 wells sampled in Arizona, indicating that transmission in this manner does pose a potential risk.[36] In 2011 two patients in Louisiana died of PAM due to *Naegleria,* both likely secondary to acquisition through sinus irrigation with contaminated tap water. *N. fowleri* was detected in water samples from both households; these were the first US cases reportedly due to exposure to contaminated treated municipal water.[37] PAM deaths related to sinus irrigation or nasal ablution with contaminated tap water have been reported elsewhere, including recently in the US Virgin Islands.[38] In 2013 a young boy died of PAM after likely acquiring the infection through exposure to tap water that was used on a backyard lawn water slide. This occurred at a house that was served by the same water system implicated in the 2011 Louisiana cases previously noted and represented the first time *N. fowleri* was cultured from a US municipal water system (not just the house at which the patients lived); the presence of *N. fowleri* in this water seems to have been related to undetectable chorine levels and high temperatures in the water.[39] Two other recent clusters of PAM due to *N. fowleri* occurred in Pakistan, one a cluster of 13 cases likely due to ablution with contaminated tap water[40] and another cluster of 19 cases exposed only to public water in Karachi.[41] *Naegleria* has recently been found in other drinking water systems worldwide (including in Spain, Australia, Canada, the United Kingdom, and other parts of the United States).[42] No known cases of *N. fowleri* transmission have occurred to date through organ transplantation; although it appears that *N. fowleri* can disseminate outside the CNS and that at least five organ donors had PAM as the cause of their death just before donation, none of the organ recipients in these cases have developed clinically apparent disease due to *N. fowleri*.[43]

Acanthamoeba

Acanthamoeba spp. have been isolated from soil, water, and air in diverse geographic locations.[11,27] In contrast to *Naegleria, Acanthamoeba* growth is inhibited by temperatures above 35°C to 39°C, although recent evidence suggests that *Acanthamoeba* may be able to grow at least inefficiently at higher temperatures.[44] Human exposure to *Acanthamoeba* spp. is likely a common event, as evidenced by the relatively frequent finding of the organisms in pharyngeal swab cultures of healthy individuals.[1] In addition, serologic surveys have demonstrated serum anti-*Acanthamoeba* antibodies in 50% to 100% of some cohorts of healthy people. Persons of Hispanic descent may be less likely to develop antibodies to *Acanthamoeba,* especially *Acanthamoeba polyphaga,* than white persons; the clinical significance of this finding is currently unclear.[45,46] Although exposure of the general population to *Acanthamoeba* appears to be common, GAE caused by *Acanthamoeba* spp. is mostly limited to debilitated or immunosuppressed individuals, although a number of recent cases in immunocompetent individuals have been reported.[1,47–50] Underlying conditions reported in patients with GAE have included acquired immunodeficiency syndrome (AIDS),[47] liver disease, diabetes mellitus, organ transplantation, steroid therapy, chemotherapy, and exposure to rituximab.[1,51,52] Recently, chronic meningitis and meningoencephalitis secondary to *Acanthamoeba* spp. have been reported in several Indian children with no clear immunosuppression, other than malnourishment.[53–55] Whether this represents a syndrome specific to this region or is due to better reporting is unclear at present. Disseminated *Acanthamoeba* infection without overt CNS manifestations has been increasingly recognized, most commonly in AIDS patients but also in transplant patients and those with long-term corticosteroid use.[11,51,56,57] Most commonly, these patients have cutaneous disease, although involvement of the liver, lungs, and bones has been

reported.[6,56] *Acanthamoeba* infection associated with a total artificial heart has also been reported.[58]

Unlike GAE or meningitis, which occurs most commonly in the immunosuppressed, amebic keratitis occurs predominantly in healthy people who wear contact lenses or have had corneal trauma. Worldwide the annual incidence ranges from 0.15 per million to 1.4 per million.[3] In countries with high contact lens usage rates, greater than 80% of cases are likely related to this risk factor. The annual incidence among contact lens wearers is estimated at 1.65 per million to 19.50 per million contact lens wearers with significant associated health care expenditures.[3,59] Amebic keratitis incidence increases during the warmer months, presumptively because of *Acanthamoeba* persistence in warmer temperatures and increased recreational water activity.[3,22] Before 2005 poor contact lens hygiene practices, such as improper lens cleaning, swimming or showering with lenses, or rinsing the lens case with tap water, were associated with this disease, although it had also occurred in persons who reported none of these behaviors.[4] In the United States, from 2005–07 a multistate outbreak involving at least 170 patients was associated with use of Advanced Medical Optics Complete Moisture Plus (AMOCMP), although no specific contamination of the solution was found.[60] After the voluntary removal of AMOCMP the number of cases declined, but not to preoutbreak levels; this new increase in baseline amebic keratitis cases in the outbreak area is currently not understood.[61,62] In addition, an increase of amebic keratitis in other parts of the world, not all of which can be accounted for by AMOCMP use, suggests that amebic keratitis will continue to become more common.[11,63] These examples highlight the fact that commercially available disinfectant solutions are often ineffective against *Acanthamoeba* spp. and that high-risk behavior associated with contact lens use is highly prevalent.[64] The cysts of *Acanthamoeba* spp. in particular may play a critical role in the establishment and persistence of amebic keratitis.[65,66]

Balamuthia

B. mandrillaris infects animals and humans worldwide. Until recently this organism had only rarely been isolated, mostly from soil and water. Now, new PCR methods and cell-free growth media/axenization have made identification and isolation of *Balamuthia* from the environment easier and more common.[17,67] Studies using these methodologies suggest that *Balamuthia* is found worldwide (with regional variability) and that this ameba is more commonly associated with soil than water.[2,67] Like *Naegleria* and *Acanthamoeba,* serologic studies of healthy populations in the United States, Africa, and Australia suggest that many people are exposed to *Balamuthia,* but few develop clinical disease.[2] The first human cases of infection with this organism were reported in 1990.[12] The isolation of *Balamuthia* from potting soil in the home of a fatally infected 3-year-old child that matched the clinical isolate from the patient confirmed the free-living and pathogenic status of *B. mandrillaris*.[68] Worldwide there have been more than 200 cases of human disease reported to date, with a very high mortality rate.[69] Across the world only a small number of patients (12) have survived *Balamuthia* encephalitis in response to a variety of antimicrobial treatments.[69–71] Analysis of US cases suggests that risk factors for balamuthiasis include male sex, Hispanic race, and residence in the most southern states of the United States; infections are more commonly seen in immunocompetent patients than in the immunocompromised. Soil exposure appears to be a common risk factor (85%), though water exposure was also common (66%).[70,72] Whether this epidemiologic pattern is a result of common environmental exposures or a genetic predisposition is currently unknown.

There have now been three documented clusters of transmission of *Balamuthia* through organ transplantation.[73,74] All three donors were young, immunocompetent males. Although one donor was reported to have died of a stroke and the other of acute disseminated encephalomyelitis, the latter donor was subsequently determined to have died of *Balamuthia* GAE. The third donor died from trauma. His postmortem brain and lung examination, done after the liver recipient died of GAE, showed no evidence of *Balamuthia*, although the donor was found to be seropositive for *Balamuthia*. This third case suggests that *Balamuthia* can be transmitted from donors who are asymptomatic. Although 13 patients received organs from these three donors, only 5 (2 liver, 1 kidney-pancreas, and 2 kidney recipients) became symptomatic and were confirmed to have acquired *Balamuthia* from the transplant. In 4 of these patients, illness developed 17 to 24 days posttransplant.[74] Four of these 5 symptomatic patients died despite treatment; the lone survivor had a poor neurologic outcome.[74] Of the remaining 8 patients, 6 were *Balamuthia* seropositive (titer 1:64 or greater). All 8 were empirically treated with a variety of regimens, with the seropositive patients showing declines in antibody titer after treatment. Given the very low rate of GAE among the seropositive patients, it is unclear if any of them would have developed GAE had they not received preemptive therapy.[73,74]

PATHOGENESIS AND PATHOLOGIC FINDINGS

Naegleria

PAM produces a diffuse meningoencephalitis, which affects the cortical gray matter most severely. Animal models[75,76] and autopsy studies[23,27] indicate that CNS invasion by *N. fowleri* occurs after nasal inoculation with amebae by disruption of the olfactory mucosa. The amebae penetrate the respiratory epithelium,[77] as well as the submucosal nervous plexus and the cribriform plate, and gain access to the CNS. In the CNS cortical hemorrhage and edema are seen, and the olfactory bulbs are hemorrhagic and necrotic. *Naegleria* trophozoites are found in the olfactory nerves and the adventitia and perivascular spaces of small to midsize arteries and arterioles.[1] They can be identified in wet mounts of cerebrospinal fluid (CSF) in patients with acute meningoencephalitis.[27,78] No cysts are seen in the brain.[1] A prominent fibrinopurulent leptomeningeal inflammatory infiltrate is usually seen. In addition, in a series of 16 autopsies of patients with PAM, myocarditis was present in 7, although no congestive heart failures or arrhythmias were noted. The inflammatory infiltrate was predominantly neutrophilic, and no amebae were seen in the myocardium.[79] The tissue necrosis elicited by *Naegleria* is likely mediated in part by secreted cysteine proteases and direct phagocytosis by feeding cups found on the trophozoites.[80] The protein Nfa1, which mediates contact between *N. fowleri* pseudopods and target cells, is an important virulence factor for this organism, and mice immunized against this protein exhibit increased survival in experimentally induced PAM.[81–83]

Other potential virulence determinants include nitric oxide production, pore-forming proteins, low-molecular-mass thiol compounds, and calcium-mediated complement resistance. Virulence appears to be mediated by an interaction between the environment and *N. fowleri,* resulting in alterations of the expression of virulence factors by the organism.[84]

Acanthamoeba

Both *Acanthamoeba* spp. and *B. madrillaris* likely gain entry to the body via the skin or the respiratory tract. Although initially contained at the site of entry by the immune system in immunocompromised/debilitated individuals, the amebae can enter the circulation and disseminate to the brain and other organs. The histologic appearance of GAE from *Acanthamoeba* spp. is of parenchymal necrosis and granulomas, although granulomatous tissue reaction may not be present in immunocompromised individuals.[1] When the leptomeninges are involved, two patterns are seen: infiltrates containing equal numbers of polymorphonuclear cells, lymphocytes, and macrophages or infiltrates that are predominantly of lymphocytes and macrophages.[85] Moderate-to-severe cerebral edema occurs, and thus bilateral uncal or cerebellar tonsillar herniations can occur. Necrotizing granulomatous lesions containing perivascular trophozoites and cysts are most frequently located in the cerebellum, midbrain, and brainstem. Multinucleated giant cells are occasionally present within the granulomas.[27] The perivascular location of amebic trophozoites and cysts suggests a hematogenous dissemination of *Acanthamoeba* to the CNS; this is also supported by identification of *Acanthamoeba* in the skin, lung, adrenal glands, and lymph nodes. Amebic skin lesions, sinusitis,[5] and pneumonitis[86] may be sites of primary human infection that lead to hematogenous dissemination.[27]

The histologic appearance of amebic keratitis is similar to that of *Acanthamoeba* infections of other organs. Both amebic cysts and trophozoites are found within the cornea. There is an acute or mixed inflammatory infiltrate that may contain epithelial and giant cells. However, amebae have also been found in tissue in the absence of an inflammatory infiltrate. Corneal neovascularization occurs to a variable extent.[87,88] Involvement

of the posterior chamber of the eye is a rare complication of amebic keratitis. Sterile inflammation of the posterior segment occurs without isolation or visualization of amebic cysts or trophozoites.[87] The corneal ringlike infiltrate caused by *Acanthamoeba* appears to be caused by the neutrophil chemoattractant effect of antigen-antibody complexes in the cornea.[87,88] Amebae adhere to corneal epithelial cells and secrete proteinases that facilitate invasion of the cornea and stroma. Trophozoites and cysts are seen between the lamellae of the cornea, and inflammatory infiltrates in the superficial and middle layers of the corneal stroma are common. Infiltration of nerves causes radial keratoneuritis, and later a characteristic stromal ring infiltrate develops. Anterior uveitis is common. In late stages amebic keratitis is characterized by necrosis, ulceration, descemetocele formation, and perforation of the cornea.[89]

Significant progress has been made toward understanding the molecular basis of pathogenesis in *Acanthamoeba* infections, especially keratitis. The pathology induced by *Acanthamoeba* can be classified into contact-dependent mechanisms, which require the ameba to physically contact the host cell, and contact-independent mechanisms.[11,90] Keratitis begins with the contact-dependent action of trophozoites using a mannose-binding protein to adhere to mannose glycoproteins on the corneal epithelium. The mannose glycoproteins also stimulate the release of cytopathic factors from the parasites, leading to the killing of corneal cells and destruction of the extracellular matrix.[90] In addition, amebic ecto–adenosine triphosphatases (ATPases), neuraminidases, elastases, phospholipases, glycosidases, and proteases, some of which act in a contact-independent manner, potentially play a role in corneal destruction.[11,90] *Acanthamoeba* also evade the immune response by degrading human immunoglobulin A (IgA) antibodies with secreted proteases, switching from trophozoites to cysts, and infecting the cornea, a site that lacks resident antigen-presenting cells, thus avoiding a delayed-type hypersensitivity response or a serum IgG response.[91] Finally, a recent study has shown that *Acanthamoeba* express a pore-forming protein, which can kill both bacteria and mammalian host cells, although the role of this acanthaporin in vivo is unknown.[92]

An ongoing development in the understanding of *Acanthamoeba* pathogenesis at the cellular level is the observation that many bacteria (including *Legionella* spp., some *Mycobacteria, Shigella* spp., *Chlamydia* spp., *Pseudomonas aeruginosa, Vibrio cholerae,* and *Burkholderia* spp.), viruses, and even yeast and protozoa survive within the ameba.[11] This endosymbiosis has several potential sequelae. First, it may render the intracellular microbe more pathogenic for the human host; second, it may facilitate gene transfer between the ameba and bacteria[93]; third, it may allow the microbe to survive an otherwise inhospitable environment; and fourth, in coinfections, *Acanthamoeba* may shield the intracellular microbe from the immune response and antibiotics, thus leading to more fulminant infections with these bacterial species. Although the endosymbiotic relationship of intracellular microbes and ameba continues to be explored, the actual impact of this relationship on human disease remains unclear.[94]

Balamuthia

B. mandrillaris causes a subacute or chronic meningoencephalitis clinically similar to that caused by *Acanthamoeba* spp. However, the histopathologic changes in *Balamuthia* GAE are broader than what is observed in *Acanthamoeba* GAE. In balamuthiasis the histopathology of lesions in the brain parenchyma or the meninges can range from an acute or neutrophilic immune response to a primarily chronic or granulomatous response.[85] Cysts and trophozoites of *B. mandrillaris* are seen in a perivascular pattern and are associated with angiitis and hemorrhagic necrosis of the underlying meninges and brain tissue. The angiotropic location, as well as the fact that organisms have been isolated and transmitted from other tissues (skin,[51] adrenal glands,[2] kidney,[95,96] and liver[96]), suggests that it may be spread hematogenously. An animal model of encephalitis with *B. mandrillaris* has been developed[97] and may be useful in studying the disease pathogenesis. Both immunocompetent and immunocompromised mice are susceptible to intranasal and oral challenge with *B. mandrillaris;* the most likely portal of entry for human disease remains to be determined.[97,98] These mouse models have also shown that CD4 T lymphocytes appear to be more important for resistance to *Balamuthia* than CD8 T lymphocytes.[99] Some progress has been made in understanding how *Balamuthia* crosses the blood-brain barrier (BBB). Several mechanisms likely allow *Balamuthia* to enter the CNS, including amebic surface-expressed galactose-binding proteins and ecto-ATPases, secreted amebic proteases, and phospholipases. In addition, the host response can induce interleukin-6 production and secretion, which by itself can increase BBB permeability by upregulating expression of adhesion molecules on endothelial cells and potentially increasing host immune cell recruitment to the BBB.[100] Despite these advances, much work remains in determining the mechanisms by which *Balamuthia* causes GAE.

CLINICAL MANIFESTATIONS

Table 273.1 lists comparisons of the epidemiologic, clinical, diagnostic, and therapeutic characteristics of *Naegleria, Acanthamoeba,* and *Balamuthia*.

Naegleria

PAM usually occurs in otherwise healthy children and young adults who have had recreational exposure to warm fresh water.[101] The onset of symptoms usually occurs 2 to 5 days after exposure, but apparent incubation periods of up to 2 weeks have been reported. Very early in the illness and consistent with the involvement of the olfactory nerves, the patient may notice changes in taste or smell, followed by an abrupt onset of fever, anorexia, nausea, and vomiting. On initial presentation, headache and meningismus are noted in 86% to 100% of patients, and mental status changes in 66%. Patients rapidly progress to coma and death a median of 5 days after the onset of illness, usually without developing focal neurologic signs.[27] Spinal cord involvement has been reported, and one AIDS patient with *Naegleria* CNS infection has been reported.[98,102]

Acanthamoeba

In contrast to PAM, *Acanthamoeba* GAE is usually an illness of immunocompromised and debilitated individuals, has an insidious onset, and presents with focal neurologic deficits.[1,47] Signs and symptoms in a series of 15 patients with GAE included mental status abnormalities (86%); seizures (66%); fever, headache, and hemiparesis (53%); meningismus (40%); visual disturbances (26%); and ataxia (20%).[103] The duration of illness from presentation until death was 7 to 120 days (mean, 39 days). The incubation period of GAE is difficult to determine, given the ubiquitous environmental presence of these organisms, but *Acanthamoeba* skin ulcers and lesions have been documented for months before the onset of CNS disease.[27,51,104] The skin lesions can be ulcerative, nodular, or subcutaneous abscesses and on biopsy demonstrate amebic granulomas.[51,104,105] Other clinical syndromes that have been described with *Acanthamoeba* infection include pneumonitis,[86] adrenalitis,[86] leukocytoclastic vasculitis,[106] osteomyelitis,[56] sinusitis,[5,104] and infection of a peptic ulcer[107] and an ependymal cyst.[108]

Amebic keratitis is generally a unilateral, sight-threatening infection that leads to corneal ulceration, blindness, and enucleation if not treated promptly.[109] It is frequently misdiagnosed as herpetic, bacterial, or fungal keratitis, resulting in a delay to definitive treatment. The common symptoms are a foreign-body sensation in the affected eye, photophobia, tearing, and pain. Pain out of proportion to the signs of inflammation in early amebic keratitis has been noted, but amebic keratitis can present as a painless keratitis, so the lack of pain should not be used to rule out amebic keratitis.[3] Periods of temporary remission are common, which lead to further delays in diagnosis because they are incorrectly interpreted as responses to antibacterial or antiviral therapy. Signs of amebic keratitis include dendriform epitheliopathy, subepithelial infiltrates and neovascularization, a characteristic corneal ring infiltrate (Fig. 273.9), stromal infiltrates, radial keratoneuritis, hypopyon, and late in the disease, corneal perforation and melt.[3,110] The dendriform epithelial pattern has been described as an early sign of amebic keratitis before stromal involvement. Recognition of this manifestation and high clinical suspicion are important because early treatment increases the chance of a good outcome and more rapid cure.[63] A nonsuppurative keratitis with recurrent ulceration and a waxing and waning clinical course is a typical history, which in part is why amebic keratitis is often misdiagnosed as dendritic keratitis due to herpes simplex virus.[89]

TABLE 273.1 Microbiology, Clinical Characteristics, Diagnosis, and Treatment of Free-Living Amebae Known to Cause Human Disease

	NAEGLERIA FOWLERI	*ACANTHAMOEBA* SPP. (NONKERATITIS DISEASE)	*ACANTHAMOEBA* SPP. (KERATITIS)	*BALAMUTHIA MANDRILLARIS*
Disease	Primary amebic meningoencephalitis (PAM)	Granulomatous amebic encephalitis (GAE); cutaneous lesions; sinus infections	Amebic keratitis	GAE; cutaneous lesions; sinus infections
Epidemiology	Most human cases associated with exposure to recreational warm fresh water	Can acquire from soil, water, air	Corneal trauma; poor contact lens hygiene; association with Advanced Medical Optics Complete Moisture Plus	Can acquire from soil, water, air
Groups at risk	Healthy children and young adults, usually male	Immunocompromised individuals	Contact lens wearers (>80% of cases)	Immunocompromised individuals; healthy children and adults; Hispanic persons
Signs and symptoms at presentation	Headache, neck stiffness; seizures; coma	Headache, neck stiffness; behavioral changes; coma; sinus disease; skin ulcers	Intense pain, photophobia, tearing; dendriform epitheliopathy (early); stromal ring	Headache, fever, cognitive/behavior changes, seizures, hydrocephalus, sinus infection, skin nodules
Clinical course	Prodrome of few days; fulminant disease; without treatment, death usually within 1 week	Prodrome of weeks to months; subacute course; acute stage fatal in weeks	Prodrome of days; subacute to chronic keratitis	Prodrome of weeks to months; subacute course; acute stage fatal in weeks
Laboratory diagnosis	CSF wet mount positive for motile amebae; CSF with polymorphonuclear pleocytosis; no cysts seen in brain tissue; PCR from CSF	Amebae rarely seen in CSF wet mount; cysts seen in brain tissue—test by IFA, IIF, or PCR for definitive identification	Corneal scraping or biopsy to find trophozoites or cysts; confocal microscopy	Amebae rarely isolated from CSF, CSF pleocytosis with mild lymphocytic predominance and protein elevation; cysts seen in brain tissue—test by IFA, IIF, and PCR
Distinct morphologic features	Vesicular nucleus; limacine movement of amebae; flagellate stage; cysts with pores flush at surface	Vesicular nucleus; finger-like pseudopodia projecting from surface; cyst wall with two layers and with pores		Vesicular nucleus with single or multiple nucleoli; ameboid and "spider-like" movements in culture; cyst wall with three layers
In vitro cultivation	Axenic, bacterized, and defined media; tissue culture cells; optimal growth at ≥37°C	Axenic, bacterized, and defined media; tissue culture cells; optimal growth at 37°C (CNS isolates) or optimal growth at 30°C (corneal isolates)		Axenic medium; tissue culture cells; optimal growth at 37°C (bacterized medium not useful)
CT/MRI of head	Nonspecific	Space-occupying or ring-enhancing lesion	Not applicable	Space-occupying or ring-enhancing lesions
Antimicrobial therapy	Intrathecal and IV amphotericin B deoxycholate, fluconazole, rifampin, azithromycin, miltefosine; consider dexamethasone and other adjuncts to manage increased ICP	Pentamidine, azoles, flucytosine, sulfadiazine, miltefosine, amikacin IV and IT, voriconazole	PHMB, chlorhexidine, propamidine, hexamidine, topical and oral voriconazole	Pentamidine, azithromycin or clarithomycin, fluconazole, sulfadiazine, flucytosine, miltefosine

CNS, Central nervous system; *CSF,* cerebrospinal fluid; *CT,* computed tomography; *ICP,* intracranial pressure; *IFA,* immunofluorescent antibody staining; *IIF,* indirect immunofluorescent staining; *IT,* intrathecal; *IV,* intravenous; *MRI,* magnetic resonance imaging; *PCR,* polymerase chain reaction; *PHMB,* polyhexamethylene biguanide.
Modified from Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: Acanthamoeba *spp.*, Balamuthia mandrillaris, Naegleria fowleri, *and* Sappinia diploidea. FEMS Immunol Med Microbiol. *2007;50:1–26.*

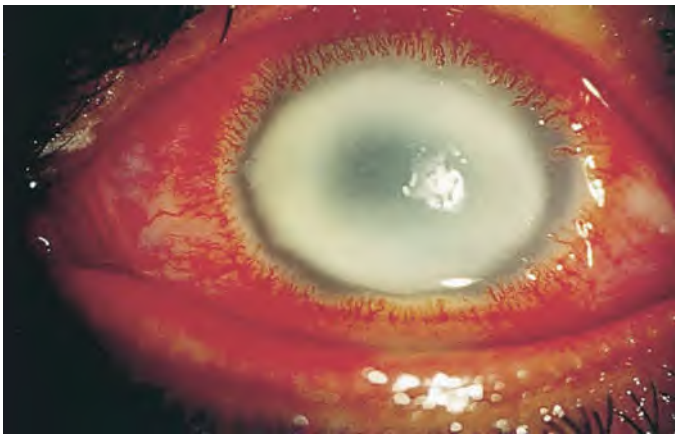


FIG. 273.9 *Acanthamoeba* keratitis with the characteristic ring infiltrate. *(From Garg P, Rao GN. Corneal ulcer: diagnosis and management.* J Comm Eye Health. *1999;12:21.)*

Balamuthia

Unlike *Acanthamoeba, Balamuthia* can cause disease in both immunocompetent (especially in children) and immunocompromised hosts.[70] Subacute or chronic granulomatous meningoencephalitis is the most common clinical presentation, resulting in death 1 week to several months after the onset of neurologic symptoms. Important signs and symptoms include fever, headache, nausea, vomiting, seizure, and focal neurologic signs.[2]

In South America it appears common for patients to present with recognized *Balamuthia* skin lesions preceding GAE, while preceding skin lesions rarely occur in US *Balamuthia* patients.[2,70,111] The skin lesions are usually solitary, poorly defined, and firm or rubbery; have a normal to violaceous coloring; and have preserved sensation.[2] The lesions are usually several centimeters in size, although if untreated they can enlarge and ulcerate; biopsy can show granulomatous inflammation without organisms. The skin lesions can rapidly expand if the patient is treated with systemic corticosteroids.[2,111,112] The lesions most commonly appear on the central face, although other locations such as the lower face, abdomen, and extremities have been reported.[2,111,112] As a rule, it is thought that *Balamuthia* skin lesions herald an eventual progression to CNS involvement in untreated patients, although in a Peruvian cohort, four patients with isolated skin disease were identified, with one patient

spontaneously recovering without treatment and three others improving on systemic antimicrobial therapy directed against *Balamuthia*.[2,113] Recently, a patient with isolated cutaneous *Balamuthia* disease was also identified in the United States.[114]

LABORATORY DIAGNOSIS

Naegleria

PAM should be included in the differential diagnosis for children and young adults with meningoencephalitis or suspected bacterial meningitis, especially if there is a history of recent exposure to fresh water. The peripheral white blood cell (WBC) count is usually elevated, with a predominance of neutrophils. Neuroradiologic imaging ranges from unremarkable to showing diffuse contrast enhancement of the gray matter, cisternal exudates and obliteration, and infarction.[27,115,116] The CSF pressure is often elevated, and the CSF is often hemorrhagic, especially late in the course of the disease. CSF WBC counts may be low early in disease but later range from 400 to 26,000 (median, 2400) WBCs/μL, with neutrophils predominating. The CSF glucose level is low to normal, and the protein level is elevated.

It is important to examine a wet mount of CSF for motile amebic trophozoites. *Naegleria* trophozoites are generally destroyed by the fixation procedure for Gram stain and missed if not looked for on a wet mount. In one series, motile trophozoites in the CSF were seen in 14 of 16 patients with PAM in which a wet mount of CSF was made.[1] However, an antemortem diagnosis was made in only 27% of the 142 patients with PAM in the United States from 1937–2013, highlighting the nonspecific nature of these findings and need for a high index of suspicion for this difficult diagnosis.[29] Wright-Giemsa stain of the CSF can also highlight *N. fowleri* trophozoites.

Serologic diagnosis of PAM has limited utility in the clinical setting because most patients with PAM die soon after infection, which leaves insufficient time to mount a detectable immune response. Nevertheless, a specific antibody response to *N. fowleri* was detected in a California patient who recovered from this disease. In addition, sera collected from several individuals with a history of extensive swimming in freshwater lakes in both the southeastern United States and California had detectable antibodies to *N. fowleri*. Whether these antibodies have protective activity is not clear. More recent diagnostic tools for *Naegleria* infections include molecular methods, such as monoclonal antibodies and PCR for detecting organisms in the CSF; isoenzyme profile analysis from clinically isolated, cultured organisms; and DNA probes.[1] A real-time PCR test that is highly sensitive has recently been developed and is now considered the gold standard for the diagnosis of PAM due to *N. fowleri*.[117,118]

Acanthamoeba

In the past GAE due to *Acanthamoeba* was usually diagnosed only at autopsy. Brain biopsy is the most reliable diagnostic approach because *Acanthamoeba* spp. have only rarely been isolated from the CSF or seen on wet mount; both an immunohistochemical stain and PCR testing are available to aid in the analysis of brain tissue.[53,54,119] Exposure to *Acanthamoeba* results in detectable serum antibodies, but whether this leads to protective immunity is unknown. Serologic studies are generally not useful for diagnosis, although high titers to *Acanthamoeba* were recently used as evidence of *Acanthamoeba* GAE in an immunocompetent patient.[48] Previous studies have shown that antibodies to *Acanthamoeba* exist both in sera of persons without symptoms of disease and in patients who developed GAE or skin infections or both.[45,46]

Neuroimaging generally shows single or multiple space-occupying lesions in the brain, with or without contrast enhancement.[1,27,120] The differential diagnosis in these patients is extensive and includes toxoplasmosis, primary CNS lymphoma, tuberculosis, neurocysticercosis, nocardiosis, aspergillosis, bacterial brain abscess, and GAE secondary to *Balamuthia*. Radiographically, these entities cannot be differentiated reliably, and tissue stained with *Acanthamoeba*-specific antibodies is required for definitive diagnosis. Given the mass lesions, lumbar puncture may be contraindicated because of the risk of herniation. When performed, CSF results have been nondiagnostic, with intermediate elevations in the WBC count, elevated protein, and decreased glucose levels.[1,27] As some GAE cases with wet mounts positive for *Acanthamoeba* have been reported, CSF wet mount is recommended if lumbar puncture is performed.

Acanthamoeba skin infections are often present in patients with GAE. Thus skin nodules or ulcers should be biopsied and examined for *Acanthamoeba* in patients suspected of having GAE. *Acanthamoeba* have been successfully cultured from brain and cutaneous biopsy specimens. PCR of brain tissue has also demonstrated *Acanthamoeba* spp., including on formaldehyde-fixed or paraffin-embedded tissue.[52,120]

The successful treatment of amebic keratitis depends on early diagnosis and treatment. Diagnosis still rests on a high clinical suspicion and demonstrating *Acanthamoeba* in corneal scrapings or biopsy specimens by histopathologic examination, culture, or molecular mechanisms such as PCR or DNA probes, although in vivo confocal microscopy for visualization of cysts is being more commonly used for the diagnosis of amebic keratitis.[3,4,11,63,121] Initial corneal scrapings, Gram stains, and cultures may be misleading because many amebic keratitis cases are associated with coinfection with *Staphylococcus epidermidis, Staphylococcus aureus,* β-hemolytic streptococcus, or *Propionibacterium* spp. Culture of contact lenses and contact lens saline solution has also yielded *Acanthamoeba*.[4,11] Corneal scrapings should be examined under wet mount for motile trophozoites. Spray fixatives may best preserve the morphology of the trophozoites before air drying occurs.[122] The cysts and trophozoites can be visualized with a number of different stains, including H&E, Wright, Giemsa, periodic acid–Schiff, calcofluor white, and acridine orange.[4]

Balamuthia

Like *Acanthamoeba, Balamuthia* GAE is often diagnosed at autopsy, and several of the reported cases of *B. mandrillaris* had initially been ascribed to *Acanthamoeba* because of the difficulty in distinguishing the two morphologically. Given the lack of laboratory or clinical findings specific for *Balamuthia* GAE, tissue stained with *Balamuthia*-specific antibodies or *Balamuthia*-specific PCR from tissue or CSF is required for definitive diagnosis.[2] In brain biopsy specimens of patients with *B. mandrillaris* infection, both the cysts and trophozoites of the organism have been identified, and both an immunohistochemical stain and PCR test are available to aid in the analysis of brain tissue. Isolation of *B. mandrillaris* from CSF has only been reported once; nevertheless, in patients for whom GAE is suspected, wet mount and PCR of the CSF should be done if lumbar puncture is performed.[74,123] The CSF findings usually include a moderate lymphocytic pleocytosis, mild hypoglycorrachia, and an elevated protein level, which occasionally can exceed 1000 mg/dL.[2,70] Neuroimaging results can be quite varied. Many reports describe findings similar to *Acanthamoeba* GAE, such as multifocal lesions ranging from nonenhancing to ring-enhancing or homogeneously enhancing.[2,70] Solitary lesions have also been described,[112] as have mycotic aneurysms.[124,125] Hemorrhage in the ring-enhancing lesion, unusual for most infectious causes of such lesions, has been described in cases of *Balamuthia* and should prompt consideration of *Balamuthia*.[126,127] Although a *Balamuthia* serologic test is available, given the prevalence in apparently healthy seropositive individuals, it is unclear how useful this test is for diagnosis.[2,72]

A multiplex real-time PCR assay, used primarily on CSF or brain tissue, can simultaneously detect *N. fowleri, Acanthamoeba* spp., and *Balamuthia*. This assay targets the 18S ribosomal RNA gene and appears to be both specific and sensitive.[128] Detection of *Sappinia* has now been added to this assay.[54] Another multiplex PCR assay can simultaneously detect *Acanthamoeba, Hartmannella, Vahlkampfia,* and *Naegleria*.[129] An up-and-coming technology is next-generation sequencing in which RNA or DNA is extracted from tissue or CSF, sequenced, and the resulting sequences are queried against databases of known expression or DNA libraries. The advantage of such testing is that it should be highly sensitive and specific and does not require a priori concern for FLAs. The downside of such testing is the cost and relatively slow turnaround.[130,131]

TREATMENT

Naegleria

The optimal treatment for PAM has not been well defined, and few patients have survived this fulminant disease.[1,132]

Clinical Evidence

Amphotericin products form a cornerstone of therapy for PAM due to *Naegleria*. Most survivors of well-documented PAM have received high-dose systemic and intrathecal amphotericin B.[1,132] In vitro data suggest that amphotericin deoxycholate has greater activity against *N. fowleri* than lipid formulations of amphotericin and may therefore be preferred.[29,133] Additional drugs used in patients who have recovered from PAM include fluconazole, rifampin, sulfonamides, and azithromycin.[29,133,134] Increased intracranial pressure has been treated with ventricular drainage, hyperosmolar therapy, dexamethasone, and occasionally hypothermia.[1,133,135] Oral miltefosine, now commercially available in the United States, might be a useful adjunct.[29,133,135] Since 2013 all three surviving US patients with PAM were treated with miltefosine as part of their antimicrobial regimen, whereas among fatal US PAM cases, only about a third received this drug.[133] The three recent US PAM survivors, two children in 2013 and one adolescent in 2016, received a treatment regimen that included intravenous (IV) and intrathecal amphotericin deoxycholate, fluconazole, rifampin, azithromycin, miltefosine, and dexamethasone; two have had good functional outcomes, whereas one was left with profound neurologic deficits.[135] Adjuncts in some of these cases included CSF drainage, hyperosmolar therapy, hyperventilation, and hypothermia.[133] However, several other recent patients treated with similar regimes have not survived. The survival of patients with PAM, especially with a good neurologic outcome, likely depends on several factors, including early diagnosis and treatment, the use of appropriate combination drug therapy, and aggressive management of elevated intracranial pressure.

Animal Models and In Vitro Assays

Azithromycin was shown to be active against *N. fowleri* in a mouse model of primary amebic encephalitis.[136] The authors report that azithromycin had less activity against *N. fowleri* in vitro but nonetheless protected 100% of mice challenged in a PAM model; other macrolides appear to be less effective. Another report describes the synergistic activity of azithromycin plus amphotericin, both in vitro and in a mouse model.[137] Artemisinin derivatives were investigated in an animal model, but these agents were all inferior to amphotericin B.[138] Corifungin, a polyene macrolide (chemically related to amphotericin) was shown to have better activity against *N. fowleri* than amphotericin, both in vitro and in mouse models. This experimental drug has been granted orphan drug designation for the treatment of PAM by the US Food and Drug Administration (FDA).[139] Passive immunotherapy in animal models has been attempted, and intrathecal administration of anti-*Naegleria* immune serum or monoclonal antibody prolonged the survival of rabbits inoculated intracisternally with *N. fowleri*.[140,141]

In vitro and mouse studies suggest that that both miltefosine and voriconazole have good activity against *N. fowleri*.[142] Phenothiazines (chlorpromazine and trifluoperazine) have also demonstrated inhibitory effects against *N. fowleri* in vitro and in mouse models.[132,141]

Recommendations

Patients with suspected PAM should immediately receive high-dose IV amphotericin (preferably the deoxycholate formulation of amphotericin, although if unavailable, lipid formulations of amphotericin could be given instead) as part of their empirical antiinfective regimen; intrathecal amphotericin may have an additional role in confirmed or highly suspect cases. In the absence of clinical trials to guide therapeutic decisions for this rapidly fatal infection, combination antimicrobial therapy seems warranted and should be administered promptly, given that most survivors received multiple drugs. The addition of azoles, rifampin, azithromycin, miltefosine, or other antimicrobials should be strongly considered. All three recent US survivors of PAM received a treatment regimen that included IV and intrathecal amphotericin deoxycholate, fluconazole, rifampin, azithromycin, miltefosine, and dexamethasone, and some authors now feel this is the preferred first-line treatment regimen for this infection.[135] Aggressive management of increased ICP also seems to be an important therapeutic adjunct. For diagnostic assistance and treatment recommendations, clinicians should contact the Centers for Disease Control and Prevention (CDC) Emergency Operations Center at 770-488-7100.

Acanthamoeba Granulomatous Amebic Encephalitis and Disseminated Disease

Data are limited regarding the treatment of *Acanthamoeba* GAE. Most cases have been diagnosed postmortem, and premortem diagnosis has generally preceded death by only a few days, making evaluation of therapy difficult.

Clinical Evidence

Among the successfully treated patients with GAE, meningitis, and disseminated *Acanthamoeba* disease, all except two were given a combination of antimicrobials. These combination regimens included trimethoprim-sulfamethoxazole (TMP-SMX), flucytosine, and sulfadiazine[132]; penicillin G and chloramphenicol[132]; sulfadiazine, pyrimethamine, and fluconazole[132]; pentamidine, levofloxacin, amphotericin B, flucytosine, rifampin, and itraconazole[132]; pentamidine, flucytosine, itraconazole, topical chlorhexidine, and ketoconazole[132]; IV pentamidine and oral itraconazole[51]; fluconazole, sulfadiazine, and surgical debulking[51]; ketoconazole, rifampin, and TMP-SMX[53,54]; TMP-SMX, rifampin, and surgical debulking[143]; and oral and topical miltefosine with intrathecal and systemic amikacin.[144] The two successfully treated cases in which only a single antimicrobial was used received sulfamethazine or TMP-SMX.[132,145] In one patient with disseminated *Acanthamoeba* and AIDS, the initiation of antiretroviral therapy was felt to be a critical component of the patient's survival, in addition to treatment with itraconazole, flucytosine, rifampin, and topical chlorhexidine.[104] A recent report of an apparently immunocompetent person with *Acanthamoeba* GAE was successfully treated with surgical debulking, miltefosine, and voriconazole for 3 months.[48] Whether this combination would be effective in an immunocompromised patient is unknown. In a recent retrospective review of patients who had nonkeratitis *Acanthamoeba* infections, mortality appeared to be significantly lower among patients who had been treated with miltefosine as a component of their antimicrobial regimens than among patients who did not receive miltefosine, although this study has a number of caveats that make it suggestive but not conclusive.[146]

Animal Models and In Vitro Assays

In mice *Acanthamoeba* infections can be treated with sulfadiazine, rifampin, and flucytosine; however, this requires treatment either before or within 24 hours of infection, an unlikely clinical scenario.[11] In general, the diamidine derivatives (including pentamidine) have the greatest activity against *Acanthamoeba*. Other drugs with in vitro activity include ketoconazole, miconazole, paromomycin, polymyxin, sulfadiazine, TMP-SMX, azithromycin, neomycin, flucytosine, voriconazole, miltefosine, and, to a lesser extent, amphotericin B.[11,87]

Recommendations

The mainstay of successful treatment appears to be early diagnosis and multidrug therapy. On the basis of the experience gained from successfully treated patients, combination regimens might include pentamidine, an azole, a sulfonamide, and possibly flucytosine. Emerging evidence also indicates that miltefosine may be an important component of multidrug therapeutic regimens. However, given the wide range of anecdotally successful regimens previously listed, it is difficult to determine the most efficacious regimen. Because it is now possible to test clinical isolates in vitro for drug susceptibilities, this testing offers one potential way to guide definitive therapy.[144,147]

Acanthamoeba Keratitis

Treatment of amebic keratitis has been notably more successful than that of GAE or PAM, with medical success rates ranging from 75% to 84%.[4,60] Successful treatment requires early diagnosis and aggressive medical management, although surgical débridement and corneal transplantation are sometimes necessary. Recognition of the dendriform pattern on the corneal epithelium, the earliest recognized sign of amebic keratitis should be followed immediately with institution of antiamebic medical therapy.[4] In vitro susceptibility testing for *Acanthamoeba* spp. is available and may prove useful to guide medical therapy; however, there are reports of a poor correlation between in vitro drug susceptibilities and in vivo response of patients with amebic keratitis.[148]

Topical chlorhexidine (0.02%) and polyhexamethylene biguanide (PHMB, 0.02%) are effective against both the trophozoites and the cysts and have become the mainstay of therapy for amebic keratitis.[3,4] Using chlorhexidine or PHMB with a diamidine, propamidine (Brolene) or hexamidine (Desmodine) is common, but neither of the latter medications is available in the United States. Hexamidine monotherapy has been used in France.[4] Other agents that have been used in conjunction with chlorhexidine or PHMB include oral ketoconazole, itraconazole, voriconazole, and topical imidazoles (1%).[4] Miltefosine has excellent activity against trophozoites and is partially active against cysts,[142] but its use in combination with the cationic antiseptics is still investigational.[4] Propamidine isethionate has caused a reversible epithelial keratopathy after prolonged treatment that can be confused with recurrent amebic keratitis.[149] Topical voriconazole by itself and in combination with systemic and intrastromal injections has now been used as an adjunctive therapy to treat several patients who failed chlorhexidine and hexamidine combination therapy.[150] In addition, a recent study suggests that hydroxymethylglutaryl–coenzyme A (HMG-CoA) reductase inhibitors, which can block the *Acanthamoeba* HMG-CoA enzyme, may soon be added to armamentarium to treat amebic keratitis.[13]

The use of corticosteroids in amebic keratitis is controversial. Crystalline keratopathy caused by viridans streptococci has occurred after topical corticosteroid therapy of amebic keratitis in two patients.[14] In animal models and in vitro studies, steroid treatment made the disease worse. However, recent studies revealed no increased risk of treatment failure in patients treated with adjuvant steroids and improved graft survival in patients who did not have inflammation at the time of graft placement.[4]

Recommendations

Topical chlorhexidine or PHMB with or without an adjuvant diamidine or other agent should be applied every hour for the first several days. Treatment is then tapered on the basis of the clinical response. Medical therapy may fail or require adjunctive surgical therapy.[3,4] Neomycin should not be used because it is ineffective against cysts, has limited clinical efficacy data, and can cause hypersensitivity.[4,148] The role of steroids remains unclear.

Balamuthia

Therapy for *B. mandrillaris* infections is not well defined. Systemic infections are associated with a high mortality rate.

Clinical Evidence

Only 12 survivors have been documented among patients in whom brain involvement has occurred. The majority of the survivors were immunocompetent and had early diagnoses of their CNS balamuthiasis, allowing antiamebic treatments to be started relatively early. Survivors were treated with various combinations that included pentamidine, flucytosine, sulfadiazine, and fluconazole or itraconazole, plus one or more of the following drugs: a macrolide, thioridazine/trifluoperazine, liposomal amphotericin, and/or miltefosine.[69,71] In four of eight cases where pentamidine was used and the two cases where thioridazine was used, these drugs were stopped relatively quickly. In Peru three patients who survived CNS balamuthiasis were treated with albendazole, fluconazole, and the addition of TMP-SMX in two cases and miltefosine in one case.[130] Three patients with isolated cutaneous balamuthiasis in Peru were treated with albendazole, itraconazole, and miltefosine; one received this regimen for 6 months and was disease free after 3 years. The US patient with isolated cutaneous disease was treated with azithromycin, fluconazole, sulfadiazine, and briefly miltefosine. Both sulfadiazine and miltefosine were stopped secondary to side effects.[114] There is limited evidence that miltefosine use may be associated with survival, but these data are not conclusive, and fatalities have occurred in patients treated with miltefosine early in their course.[146,151]

Two of these 12 survivors had poor neurologic outcomes, and both cases were associated with an increase in intracranial pressure (ICP) that was only treated after precipitous declines to a comatose state.[125,152] The increased ICP was thought to have been due to cerebral edema (not obstructive hydrocephalus), which may have led to a delay in the recognition of the elevated ICP. Thus, during the management of *Balamuthia* GAE, clinicians should be vigilant about diagnosing and treating elevated ICP.[2,125,152]

One patient with cutaneous disease reportedly noted spontaneous resolution without therapy.[153] Surgical débridement has been used in one patient with isolated cutaneous disease case and one patient who had both cutaneous and CNS disease, although it is unclear if the débridement impacted the outcomes.[2,112]

In Vitro Assays

Prior work suggested that pentamidine, azithromycin, and clarithromycin appear to have some anti-*Balamuthia* activity[154,155]; fluconazole and ketoconazole were poor inhibitors of *Balamuthia* growth; amphotericin B was marginally inhibitory; and TMP-SMX had minimal effect.[154] In vitro data suggest that miltefosine may inhibit *Balamuthia,* but voriconazole had virtually no effect on this organism.[142] A more recent study that interrogated the minimal amebicidal effect of drugs on cysts and trophozoites suggests that amphotericin, miltefosine, and pentamidine have limited efficacy in vitro.[156] A recent in vitro screen using FDA-approved drugs found that amlodipine, apomorphine, demethoxycurcumin, haloperidol, loperamide, prochlorperazine, procyclidine, and resveratrol all had amebicidal activity in two in vitro assays and at concentrations readily achieved in patients.[157]

Recommendations

The mainstay of successful regimens is multidrug therapy, but given the various combinations used, it is difficult to determine the most effective regimen. *Balamuthia* GAE should probably be treated with multidrug antimicrobial regimens that include some combination of pentamidine, flucytosine, a sulfonamide, albendazole, an azole, a macrolide, amphotericin, and miltefosine. Given the poor tolerance of some of these medications (e.g., pentamidine, amphotericin), a reasonable choice is to pursue a regimen that has limited side effects or consider an early change to other antiamebic agents in light of severe side effects. Given the current rarity of survival, it is likely that immune status and early diagnosis and medical therapy are key determinants of prognosis.

PREVENTION

Given the ubiquitous nature of *Acanthamoeba* and *B. mandrillaris,* primary prevention efforts will likely prove difficult. PAM occurs so rarely that active surveillance for *N. fowleri* in public swimming areas is probably not justified as a public health measure. However, a single source of infection, such as a popular swimming area, can cause an outbreak of PAM. Thus, because of the occurrence of clusters of patients with PAM with common environmental exposures,[8,31] health officials should consider closing implicated lakes, rivers, or streams to swimming. Swimmers can decrease their risk by avoiding activities in warm fresh water, especially if the water temperature is particularly high and the water level low, and by keeping their nose shut and avoiding sediment disturbance if they do swim in warm fresh water.[89] Domestic water supplies have recently been implicated as the source of *N. fowleri* in the United States,[28,34] and new water treatment measures are being developed to eliminate FLAs.[158] *N. fowleri* is susceptible to chlorine and can be controlled by adequate chlorination. Given cases recently associated with nasal irrigation with tap water (both for sinus irrigation and ritual ablution), water used for these practices should ideally be sterile. Preliminary experimental work indicates that vaccination using the *nfa1* gene of *Naegleria* may result in an effective immune response, but no clinical data are yet available regarding this approach.[141]

Amebic keratitis associated with contact lens use is generally preventable. Contact lenses should not be worn while swimming or showering, new contact lens cleaning solution should be used nightly, homemade saline solutions should be avoided, contact lens cases should be allowed to air dry each day, and orthokeratology should be avoided. Some authors advocate daily disposable lenses, disposing of the contact lens case after 3 months of use, or microwaving the lens case for 3 minutes on high power. Finally, commercially available contact lens disinfectant solutions that are specifically effective against *Acanthamoeba* are needed.[4,60,159]

RESOURCES

In the United States, the CDC reference laboratory in Atlanta, Georgia currently performs diagnostic testing for all FLAs. Information regarding *N. fowleri* can be found at www.cdc.gov/parasites/naegleria/, for *Acanthamoeba* at www.cdc.gov/parasites/acanthamoeba/, and for *Balamuthia* at www.cdc.gov/parasites/balamuthia/. For round-the-clock diagnostic assistance, specimen collection guidance, shipping instructions, and treatment recommendations regarding all of the FLAs, the CDC Emergency Operations Center can be contacted at 770-488-7100.

Key References

The complete reference list is available online at Expert Consult.

1. Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris, Naegleria fowleri,* and *Sappinia diploidea*. *FEMS Immunol Med Microbiol*. 2007;50:1–26.
2. Bravo FG, Seas C. *Balamuthia mandrillaris* amoebic encephalitis: an emerging parasitic infection. *Curr Infect Dis Rep*. 2012;14:391–396.
3. Dart JK, Saw VP, Kilvington S. *Acanthamoeba* keratitis: diagnosis and treatment update 2009. *Am J Ophthalmol*. 2009;148:487–499, e2.
9. Schuster FL. Cultivation of pathogenic and opportunistic free-living amebas. *Clin Microbiol Rev*. 2002;15:342–354.
10. De Jonckheere JF. Origin and evolution of the worldwide distributed pathogenic amoeboflagellate *Naegleria fowleri*. *Infect Genet Evol*. 2011;11:1520–1528.
11. Siddiqui R, Khan NA. Biology and pathogenesis of *Acanthamoeba*. *Parasit Vectors*. 2012;5:6.
12. Visvesvara GS, Martinez AJ, Schuster FL, et al. Leptomyxid ameba, a new agent of amebic meningoencephalitis in humans and animals. *J Clin Microbiol*. 1990;28:2750–2756.
21. Qvarnstrom Y, da Silva AJ, Schuster FL, et al. Molecular confirmation of *Sappinia pedata* as a causative agent of amoebic encephalitis. *J Infect Dis*. 2009;199:1139–1142.
22. Wylezich C, Walochnik J, Michel R. High genetic diversity of *Sappinia*-like strains (Amoebozoa, Thecamoebidae) revealed by SSU rRNA investigations. *Parasitol Res*. 2009;105:869–873.
25. Kemble SK, Lynfield R, DeVries AS, et al. Fatal *Naegleria fowleri* infection acquired in Minnesota: possible expanded range of a deadly thermophilic organism. *Clin Infect Dis*. 2012;54:805–809.
28. Yoder JS, Eddy BA, Visvesvara GS, et al. The epidemiology of primary amoebic meningoencephalitis in the USA, 1962-2008. *Epidemiol Infect*. 2010;138:968–975.
29. Capewell LG, Harris AM, Yoder JS, et al. Diagnosis, clinical course, and treatment of primary amoebic meningoencephalitis in the United States, 1937-2013. *J Pediatric Infect Dis Soc*. 2015;4:e68–e75.
31. Schuster FL, Visvesvara GS. Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. *Int J Parasitol*. 2004;34:1001–1027.
34. Blackburn BG, Craun GF, Yoder JS, et al. Surveillance for waterborne-disease outbreaks associated with drinking water—United States, 2001-2002. *MMWR Surveill Summ*. 2004;53:23–45.
36. Blair B, Sarkar P, Bright KR, et al. *Naegleria fowleri* in well water. *Emerg Infect Dis*. 2008;14:1499–1501.
37. Yoder JS, Straif-Bourgeois S, Roy SL, et al. Primary amebic meningoencephalitis deaths associated with sinus irrigation using contaminated tap water. *Clin Infect Dis*. 2012;55:e79–e85.
42. Thomas JM, Ashbolt NJ. Do free-living amoebae in treated drinking water systems present an emerging health risk? *Environ Sci Technol*. 2011;45:860–869.
48. Webster D, Umar I, Kolyvas G, et al. Treatment of granulomatous amoebic encephalitis with voriconazole and miltefosine in an immunocompetent soldier. *Am J Trop Med Hyg*. 2012;87:715–718.
51. Walia R, Montoya JG, Visvesvara GS, et al. A case of successful treatment of cutaneous *Acanthamoeba* infection in a lung transplant recipient. *Transpl Infect Dis*. 2007;9:51–54.
53. Singhal T, Bajpai A, Kalra V, et al. Successful treatment of *Acanthamoeba* meningitis with combination oral antimicrobials. *Pediatr Infect Dis J*. 2001;20:623–627.
61. Yoder JS, Verani J, Heidman N, et al. *Acanthamoeba* keratitis: the persistence of cases following a multistate outbreak. *Ophthalmic Epidemiol*. 2012;19:221–225.
63. Patel DV, Rayner S, McGhee CN. Resurgence of *Acanthamoeba* keratitis in Auckland, New Zealand: a 7-year review of presentation and outcomes. *Clin Exp Ophthalmol*. 2010;38:15–20, quiz 87.
70. Cope JR, Landa J, Nethercut H, et al. The epidemiology and clinical features of *Balamuthia mandrillaris* disease in the United States, 1974-2016. *Clin Infect Dis*. 2018 Sep 20. [Epub ahead of print].
74. Farnon EC, Kokko KE, Budge PJ, et al. Transmission of *Balamuthia mandrillaris* by organ transplantation. *Clin Infect Dis*. 2016;63:878–888.
85. Guarner J, Bartlett J, Shieh WJ, et al. Histopathologic spectrum and immunohistochemical diagnosis of amebic meningoencephalitis. *Mod Pathol*. 2007;20:1230–1237.
89. Visvesvara GS, Roy SL, Maguire JH. Pathogenic and opportunistic free-living amebae: *Acanthamoeba* spp., *Balamuthia mandrillaris, Naegleria fowleri,* and *Sappinia pedata*. In: Guerrant RL, Walker DH, Weller PR, eds. *Tropical Infectious Diseases: Principles, Pathogens, and Practice*. Philadelphia: Saunders; 2011:707–713.
94. Paterson GN, Rittig M, Siddiqui R, et al. Is *Acanthamoeba* pathogenicity associated with intracellular bacteria? *Exp Parasitol*. 2011;129:207–210.
101. Centers for Disease Control and Prevention. Primary amebic meningoencephalitis—Arizona, Florida, and Texas, 2007. *MMWR Morb Mortal Wkly Rep*. 2008;57:573–577.
105. Morrison AO, Morris R, Shannon A, et al. Disseminated *Acanthamoeba* infection presenting with cutaneous lesions in an immunocompromised patient: a case report, review of histomorphologic findings, and potential diagnostic pitfalls. *Am J Clin Pathol*. 2016;145:266–270.
110. Patel DV, McGhee CN. *Acanthamoeba* keratitis: a comprehensive photographic reference of common and uncommon signs. *Clin Exp Ophthalmol*. 2009;37:232–238.
116. Singh P, Kochhar R, Vashishta RK, et al. Amebic meningoencephalitis: spectrum of imaging findings. *AJNR Am J Neuroradiol*. 2006;27:1217–1221.
117. Madarova L, Trnkova K, Feikova S, et al. A real-time PCR diagnostic method for detection of *Naegleria fowleri*. *Exp Parasitol*. 2010;126:37–41.
128. Qvarnstrom Y, Visvesvara GS, Sriram R, et al. Multiplex real-time PCR assay for simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris,* and *Naegleria fowleri*. *J Clin Microbiol*. 2006;44:3589–3595.
129. Le Calvez T, Trouilhe MC, Humeau P, et al. Detection of free-living amoebae by using multiplex quantitative PCR. *Mol Cell Probes*. 2012;26:116–120.
132. Schuster FL, Visvesvara GS. Opportunistic amoebae: challenges in prophylaxis and treatment. *Drug Resist Updat*. 2004;7:41–51.
133. Cope JR, Conrad DA, Cohen N, et al. 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*. 2016;62:774–776.
134. Seidel JS, Harmatz P, Visvesvara GS, et al. Successful treatment of primary amebic meningoencephalitis. *N Engl J Med*. 1982;306:346–348.
135. Linam WM, Ahmed M, Cope JR, et al. Successful treatment of an adolescent with *Naegleria fowleri* primary amebic meningoencephalitis. *Pediatrics*. 2015;135:e744.
141. Kim JH, Jung SY, Lee YJ, et al. Effect of therapeutic chemical agents in vitro and on experimental meningoencephalitis due to *Naegleria fowleri*. *Antimicrob Agents Chemother*. 2008;52:4010–4016.
152. Cary LC, Maul E, Potter C, et al. *Balamuthia mandrillaris* meningoencephalitis: survival of a pediatric patient. *Pediatrics*. 2010;125:e699–e703.

References

1. Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris, Naegleria fowleri,* and *Sappinia diploidea*. *FEMS Immunol Med Microbiol*. 2007;50:1–26.
2. Bravo FG, Seas C. *Balamuthia mandrillaris* amoebic encephalitis: an emerging parasitic infection. *Curr Infect Dis Rep*. 2012;14:391–396.
3. Dart JK, Saw VP, Kilvington S. *Acanthamoeba* keratitis: diagnosis and treatment update 2009. *Am J Ophthalmol*. 2009;148:487–499, e2.
4. Chong EM, Dana MR. *Acanthamoeba* keratitis. *Int Ophthalmol Clin*. 2007;47:33–46.
5. Dunand VA, Hammer SM, Rossi R, et al. Parasitic sinusitis and otitis in patients infected with human immunodeficiency virus: report of five cases and review. *Clin Infect Dis*. 1997;25:267–272.
6. Selby DM, Chandra RS, Rakusan TA, et al. Amebic osteomyelitis in a child with acquired immunodeficiency syndrome: a case report. *Pediatr Pathol Lab Med*. 1998;18:89–95.
7. Chang MA, Jain S, Azar DT. Infections following laser in situ keratomileusis: an integration of the published literature. *Surv Ophthalmol*. 2004;49:269–280.
8. Fowler M, Carter RF. Acute pyogenic meningitis probably due to *Acanthamoeba* sp.: a preliminary report. *Br Med J*. 1965;2:740–742.
9. Schuster FL. Cultivation of pathogenic and opportunistic free-living amebas. *Clin Microbiol Rev*. 2002;15:342–354.
10. De Jonckheere JF. Origin and evolution of the worldwide distributed pathogenic amoeboflagellate *Naegleria fowleri*. *Infect Genet Evol*. 2011;11:1520–1528.
11. Siddiqui R, Khan NA. Biology and pathogenesis of *Acanthamoeba*. *Parasit Vectors*. 2012;5:6.
12. Visvesvara GS, Martinez AJ, Schuster FL, et al. Leptomyxid ameba, a new agent of amebic meningoencephalitis in humans and animals. *J Clin Microbiol*. 1990;28:2750–2756.
13. Siddiqui R, Yousuf A, Khan NA. Killing the dead: chemotherapeutic strategies against free living cyst-forming protists (*Acanthamoeba* sp. and *Balamuthia mandrillaris*). *J Eukaryot Microbiol*. 2012;60:291–297.
14. Detering H, Aebischer T, Dabrowski PW, et al. First draft genome sequence of *Balamuthia mandrillaris*, the causative agent of amoebic encephalitis. *Genome Announc*. 2015;3:e01013-15.
15. Aitken D, Hay J, Kinnear FB, et al. Amebic keratitis in a wearer of disposable contact lenses due to a mixed *Vahlkampfia* and *Hartmannella* infection. *Ophthalmology*. 1996;103:485–494.
16. Alexandrakis G, Miller D, Huang AJ. Amebic keratitis due to *Vahlkampfia* infection following corneal trauma. *Arch Ophthalmol*. 1998;116:950–951.
17. Niyyati M, Lorenzo-Morales J, Rezaie S, et al. First report of a mixed infection due to *Acanthamoeba* genotype T3 and *Vahlkampfia* in a cosmetic soft contact lens wearer in Iran. *Exp Parasitol*. 2010;126:89–90.
18. Tolba ME, Huseein EA, Farrag HM, et al. *Allovahlkampfia spelaea* causing keratitis in humans. *PLoS Negl Trop Dis*. 2016;10:e0004841.
19. Visvesvara GS, Sriram R, Qvarnstrom Y, et al. *Paravahlkampfia francinae* n. sp. masquerading as an agent of primary amoebic meningoencephalitis. *J Eukaryot Microbiol*. 2009;56:357–366.
20. Gelman BB, Rauf SJ, Nader R, et al. Amoebic encephalitis due to *Sappinia diploidea*. *JAMA*. 2001;285:2450–2451.
21. Qvarnstrom Y, da Silva AJ, Schuster FL, et al. Molecular confirmation of *Sappinia pedata* as a causative agent of amoebic encephalitis. *J Infect Dis*. 2009;199:1139–1142.
22. Wylezich C, Walochnik J, Michel R. High genetic diversity of *Sappinia*-like strains (Amoebozoa, Thecamoebidae) revealed by SSU rRNA investigations. *Parasitol Res*. 2009;105:869–873.
23. John DT. Primary amebic meningoencephalitis and the biology of *Naegleria fowleri*. *Annu Rev Microbiol*. 1982;36:101–123.
24. Wellings FM, Amuso PT, Chang SL, et al. Isolation and identification of pathogenic *Naegleria* from Florida lakes. *Appl Environ Microbiol*. 1977;34:661–667.
25. Kemble SK, Lynfield R, DeVries AS, et al. Fatal *Naegleria fowleri* infection acquired in Minnesota: possible expanded range of a deadly thermophilic organism. *Clin Infect Dis*. 2012;54:805–809.
26. Marciano-Cabral F, Cline ML, Bradley SG. Specificity of antibodies from human sera for *Naegleria* species. *J Clin Microbiol*. 1987;25:692–697.
27. Martinez AJ. *Free-Living Amebas: Natural History, Prevention, Diagnosis, Pathology and Treatment of Disease*. Boca Raton, FL: CRC Press; 1985.
28. Yoder JS, Eddy BA, Visvesvara GS, et al. The epidemiology of primary amoebic meningoencephalitis in the USA, 1962-2008. *Epidemiol Infect*. 2010;138:968–975.
29. Capewell LG, Harris AM, Yoder JS, et al. Diagnosis, clinical course, and treatment of primary amoebic meningoencephalitis in the United States, 1937-2013. *J Pediatric Infect Dis Soc*. 2015;4:e68–e75.
30. Matanock A, Mehal JM, Liu L, et al. Estimation of undiagnosed *Naegleria fowleri* primary amebic meningoencephalitis, United States. *Emerg Infect Dis*. 2018;24:162–164.
31. Schuster FL, Visvesvara GS. Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. *Int J Parasitol*. 2004;34:1001–1027.
32. Johnson RO, Cope JR, Moskowitz M, et al. Notes from the field: primary amebic meningoencephalitis associated with exposure to swimming pool water supplied by an overland pipe—Inyo County, California, 2015. *MMWR Morb Mortal Wkly Rep*. 2016;65:424.
33. Cope JR, Murphy J, Kahler A, et al. Primary amebic meningoencephalitis associated with rafting on an artificial whitewater river: case report and environmental investigation. *Clin Infect Dis*. 2018;66:548–553.
34. Blackburn BG, Craun GF, Yoder JS, et al. Surveillance for waterborne-disease outbreaks associated with drinking water—United States, 2001-2002. *MMWR Surveill Summ*. 2004;53:23–45.
35. Marciano-Cabral F, MacLean R, Mensah A, et al. Identification of *Naegleria fowleri* in domestic water sources by nested PCR. *Appl Environ Microbiol*. 2003;69:5864–5869.
36. Blair B, Sarkar P, Bright KR, et al. *Naegleria fowleri* in well water. *Emerg Infect Dis*. 2008;14:1499–1501.
37. Yoder JS, Straif-Bourgeois S, Roy SL, et al. Primary amebic meningoencephalitis deaths associated with sinus irrigation using contaminated tap water. *Clin Infect Dis*. 2012;55:e79–e85.
38. Centers for Disease Control and Prevention (CDC). Notes from the field: primary amebic meningoencephalitis associated with ritual nasal rinsing—St. Thomas, U.S. Virgin islands, 2012. *MMWR Morb Mortal Wkly Rep*. 2013;62:903.
39. Cope JR, Ratard RC, Hill VR, et al. The first association of a primary amebic meningoencephalitis death with culturable *Naegleria fowleri* in tap water from a US treated public drinking water system. *Clin Infect Dis*. 2015;60:e36–e42.
40. Shakoor S, Beg MA, Mahmood SF, et al. Primary amebic meningoencephalitis caused by *Naegleria fowleri,* Karachi, Pakistan. *Emerg Infect Dis*. 2011;17:258–261.
41. Ghanchi NK, Khan E, Khan A, et al. *Naegleria fowleri* meningoencephalitis associated with public water supply, Pakistan, 2014. *Emerg Infect Dis*. 2016;22:1835–1837.
42. Thomas JM, Ashbolt NJ. Do free-living amoebae in treated drinking water systems present an emerging health risk? *Environ Sci Technol*. 2011;45:860–869.
43. Roy SL, Metzger R, Chen JG, et al. Risk for transmission of *Naegleria fowleri* from solid organ transplantation. *Am J Transplant*. 2014;14:163–171.
44. Magliano AC, Teixeira MM, Alfieri SC. Revisiting the *Acanthamoeba* species that form star-shaped cysts (genotypes T7, T8, T9, and T17): characterization of seven new Brazilian environmental isolates and phylogenetic inferences. *Parasitology*. 2012;139:45–52.
45. Cerva L. *Acanthamoeba culbertsoni* and *Naegleria fowleri:* occurrence of antibodies in man. *J Hyg Epidemiol Microbiol Immunol*. 1989;33:99–103.
46. Chappell CL, Wright JA, Coletta M, et al. Standardized method of measuring *Acanthamoeba* antibodies in sera from healthy human subjects. *Clin Diagn Lab Immunol*. 2001;8:724–730.
47. Sison JP, Kemper CA, Loveless M, et al. Disseminated *Acanthamoeba* infection in patients with AIDS: case reports and review. *Clin Infect Dis*. 1995;20:1207–1216.
48. Webster D, Umar I, Kolyvas G, et al. Treatment of granulomatous amoebic encephalitis with voriconazole and miltefosine in an immunocompetent soldier. *Am J Trop Med Hyg*. 2012;87:715–718.
49. Rodríguez-Pérez EG, Escandón-Vargas K, Ancer A. Granulomatous amebic encephalitis caused by *Acanthamoeba* sp. in an immunocompetent Mexican adult. *Rev Soc Bras Med Trop*. 2017;50:432.
50. Khanna V, Shastri B, Anusha G, et al. *Acanthamoeba* meningoencephalitis in immunocompetent: a case report and review of literature. *Trop Parasitol*. 2014;4:115–118.
51. Walia R, Montoya JG, Visvesvara GS, et al. A case of successful treatment of cutaneous *Acanthamoeba* infection in a lung transplant recipient. *Transpl Infect Dis*. 2007;9:51–54.
52. Meersseman W, Lagrou K, Sciot R, et al. Rapidly fatal *Acanthamoeba* encephalitis and treatment of cryoglobulinemia. *Emerg Infect Dis*. 2007;13:469–471.
53. Singhal T, Bajpai A, Kalra V, et al. Successful treatment of *Acanthamoeba* meningitis with combination oral antimicrobials. *Pediatr Infect Dis J*. 2001;20:623–627.
54. Saxena A, Mittal S, Burman P, et al. *Acanthamoeba* meningitis with successful outcome. *Indian J Pediatr*. 2009;76:1063–1064.
55. Khurana S, Mewara A, Verma S, et al. Central nervous system infection with *Acanthamoeba* in a malnourished child. *BMJ Case Rep*. 2012;2012:pii:bcr2012007449.
56. Steinberg JP, Galindo RL, Kraus ES, et al. Disseminated acanthamebiasis in a renal transplant recipient with osteomyelitis and cutaneous lesions: case report and literature review. *Clin Infect Dis*. 2002;35:e43–e49.
57. Slater CA, Sickel JZ, Visvesvara GS, et al. Brief report: successful treatment of disseminated *Acanthamoeba* infection in an immunocompromised patient. *N Engl J Med*. 1994;331:85–87.
58. Tan SK, Gajurel K, Tung C, et al. Fatal *Acanthamoeba* encephalitis in a patient with a total artificial heart (syncardia) device. *Open Forum Infect Dis*. 2014;1:ofu057.
59. Collier SA, Gronostaj MP, MacGurn AK, et al; Centers for Disease Control and Prevention (CDC). Estimated burden of keratitis–United States, 2010. *MMWR Morb Mortal Wkly Rep*. 2014;63:1027–1030.
60. Verani JR, Lorick SA, Yoder JS, et al. National outbreak of *Acanthamoeba* keratitis associated with use of a contact lens solution, United States. *Emerg Infect Dis*. 2009;15:1236–1242.
61. Yoder JS, Verani J, Heidman N, et al. *Acanthamoeba* keratitis: the persistence of cases following a multistate outbreak. *Ophthalmic Epidemiol*. 2012;19:221–225.
62. Cheung N, Nagra P, Hammersmith K. Emerging trends in contact lens-related infections. *Curr Opin Ophthalmol*. 2016;27:327–332.
63. Patel DV, Rayner S, McGhee CN. Resurgence of *Acanthamoeba* keratitis in Auckland, New Zealand: a 7-year review of presentation and outcomes. *Clin Exp Ophthalmol*. 2010;38:15–20, quiz 87.
64. Cope JR, Collier SA, Rao MM, et al. Contact lens wearer demographics and risk behaviors for contact lens-related eye infections–United States, 2014. *MMWR Morb Mortal Wkly Rep*. 2015;64:865–870.
65. Siddiqui R, Khan NA, Jarroll EL. The cyst wall carbohydrate composition of *Balamuthia mandrillaris*. *Parasitol Res*. 2009;104:1439–1443.
66. Johnston SP, Sriram R, Qvarnstrom Y, et al. Resistance of *Acanthamoeba* cysts to disinfection in multiple contact lens solutions. *J Clin Microbiol*. 2009;47:2040–2045.
67. Ahmad AF, Andrew PW, Kilvington S. Development of a nested PCR for environmental detection of the pathogenic free-living amoeba *Balamuthia mandrillaris*. *J Eukaryot Microbiol*. 2011;58:269–271.
68. Schuster FL, Dunnebacke TH, Booton GC, et al. Environmental isolation of *Balamuthia mandrillaris* associated with a case of amebic encephalitis. *J Clin Microbiol*. 2003;41:3175–3180.
69. Vollmer ME, Glaser C. A *Balamuthia* survivor. *JMM Case Rep*. 2016;3:e005031.
70. Cope JR, Landa J, Nethercut H, et al. The epidemiology and clinical features of Balamuthia mandrillaris disease in the United States, 1974-2016. *Clin Infect Dis*. 2018 Sep 20. [Epub ahead of print].
71. Moriarty P, Burke C, McCrossin D, et al. *Balamuthia mandrillaris* encephalitis: survival of a child with severe meningoencephalitis and review of the literature. *J Pediatric Infect Dis Soc*. 2014;3:e4–e9.
72. Jackson BR, Kucerova Z, Roy SL, et al. Serologic survey for exposure following fatal *Balamuthia mandrillaris* infection. *Parasitol Res*. 2014;113:1305–1311.
73. Gupte AA, Hocevar SN, Lea AS, et al. Transmission of *Balamuthia mandrillaris* by solid organ transplantation: utility of organ recipient serology to guide clinical management. *Am J Transplant*. 2014;14:1417–1424.
74. Farnon EC, Kokko KE, Budge PJ, et al. Transmission of *Balamuthia mandrillaris* by organ transplantation. *Clin Infect Dis*. 2016;63:878–888.
75. Martinez J, Duma RJ, Nelson EC, et al. Experimental *Naegleria* meningoencephalitis in mice. Penetration of the olfactory mucosal epithelium by *Naegleria* and pathologic changes produced: a light and electron microscope study. *Lab Invest*. 1973;29:121–133.
76. Jaroli KL, McCosh JK, Howard MJ. The role of blood vessels and lungs in the dissemination of *Naegleria fowleri* following intranasal inoculation in mice. *Folia Parasitol (Praha)*. 2002;49:183–188.
77. Rojas-Hernandez S, Jarillo-Luna A, Rodriguez-Monroy M, et al. Immunohistochemical characterization of the initial stages of *Naegleria fowleri* meningoencephalitis in mice. *Parasitol Res*. 2004;94:31–36.
78. Loschiavo F, Ventura-Spagnolo T, Sessa E, et al. Acute primary meningoencephalitis from entamoeba *Naegleria fowleri*. Report of a clinical case with a favourable outcome. *Acta Neurol (Napoli)*. 1993;15:333–340.

79. Markowitz SM, Martinez AJ, Duma RJ, et al. Myocarditis associated with primary amebic *(Naegleria)* meningoencephalitis. *Am J Clin Pathol*. 1974;62:619–628.
80. Aldape K, Huizinga H, Bouvier J, et al. *Naegleria fowleri:* characterization of a secreted histolytic cysteine protease. *Exp Parasitol*. 1994;78:230–241.
81. Jeong SR, Lee SC, Song KJ, et al. Expression of the *nfa1* gene cloned from pathogenic *Naegleria fowleri* in nonpathogenic *N. gruberi* enhances cytotoxicity against CHO target cells in vitro. *Infect Immun*. 2005;73:4098–4105.
82. Lee YJ, Kim JH, Jeong SR, et al. Production of Nfa1-specific monoclonal antibodies that influences the in vitro cytotoxicity of *Naegleria fowleri* trophozoites on microglial cells. *Parasitol Res*. 2007;101:1191–1196.
83. Lee J, Yoo JK, Sohn HJ, et al. Protective immunity against *Naegleria fowleri* infection on mice immunized with the rNfa1 protein using mucosal adjuvants. *Parasitol Res*. 2015;114:1377–1385.
84. Burri DC, Gottstein B, Zumkehr B, et al. Development of a high- versus low-pathogenicity model of the free-living amoeba *Naegleria fowleri*. *Microbiology*. 2012;158:2652–2660.
85. Guarner J, Bartlett J, Shieh WJ, et al. Histopathologic spectrum and immunohistochemical diagnosis of amebic meningoencephalitis. *Mod Pathol*. 2007;20:1230–1237.
86. Anderlini P, Przepiorka D, Luna M, et al. *Acanthamoeba* meningoencephalitis after bone marrow transplantation. *Bone Marrow Transplant*. 1994;14:459–461.
87. Auran JD, Starr MB, Jakobiec FA. *Acanthamoeba* keratitis. A review of the literature. *Cornea*. 1987;6:2–26.
88. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 10-1985. A 29-year-old native of India with bilateral ulcerative keratitis. *N Engl J Med*. 1985;312:634–641.
89. Visvesvara GS, Roy SL, Maguire JH. Pathogenic and opportunistic free-living amebae: *Acanthamoeba* spp., *Balamuthia mandrillaris, Naegleria fowleri,* and *Sappinia pedata*. In: Guerrant RL, Walker DH, Weller PR, eds. *Tropical Infectious Diseases: Principles, Pathogens, and Practice*. Philadelphia: Saunders; 2011:707–713.
90. Panjwani N. Pathogenesis of *Acanthamoeba* keratitis. *Ocul Surf*. 2010;8:70–79.
91. Clarke DW, Niederkorn JY. The pathophysiology of *Acanthamoeba* keratitis. *Trends Parasitol*. 2006;22:175–180.
92. Michalek M, Sonnichsen FD, Wechselberger R, et al. Structure and function of a unique pore-forming protein from a pathogenic *Acanthamoeba*. *Nat Chem Biol*. 2013;9:37–42.
93. Lamrabet O, Merhej V, Pontarotti P, et al. The genealogic tree of mycobacteria reveals a long-standing sympatric life into free-living protozoa. *PLoS ONE*. 2012;7:e34754.
94. Paterson GN, Rittig M, Siddiqui R, et al. Is *Acanthamoeba* pathogenicity associated with intracellular bacteria? *Exp Parasitol*. 2011;129:207–210.
95. Centers for Disease Control and Prevention. *Balamuthia mandrillaris* transmitted through organ transplantation—Mississippi, 2009. *MMWR Morb Mortal Wkly Rep*. 2010;59:1165–1170.
96. Centers for Disease Control and Prevention. Notes from the field: transplant-transmitted *Balamuthia mandrillaris*—Arizona, 2010. *MMWR Morb Mortal Wkly Rep*. 2010;59:1182.
97. Janitschke K, Martinez AJ, Visvesvara GS, et al. Animal model *Balamuthia mandrillaris* CNS infection: contrast and comparison in immunodeficient and immunocompetent mice: a murine model of "granulomatous" amebic encephalitis. *J Neuropathol Exp Neurol*. 1996;55:815–821.
98. Kiderlen AF, Laube U, Radam E, et al. Oral infection of immunocompetent and immunodeficient mice with *Balamuthia mandrillaris* amebae. *Parasitol Res*. 2007;100:775–782.
99. Kiderlen AF, Radam E, Laube U, et al. Resistance to intranasal infection with *Balamuthia mandrillaris* amebae is T-cell dependent. *J Eukaryot Microbiol*. 2015;62:26–33.
100. Elsheikha HM, Khan NA. Protozoa traversal of the blood-brain barrier to invade the central nervous system. *FEMS Microbiol Rev*. 2010;34:532–553.
101. Centers for Disease Control and Prevention. Primary amebic meningoencephalitis—Arizona, Florida, and Texas, 2007. *MMWR Morb Mortal Wkly Rep*. 2008;57:573–577.
102. Viriyavejakul P, Rochanawutanon M, Sirinavin S. *Naegleria* meningomyeloencephalitis. *Southeast Asian J Trop Med Public Health*. 1997;28:237–240.
103. Martinez AJ. Is *Acanthamoeba* encephalitis an opportunistic infection? *Neurology*. 1980;30:567–574.
104. Carter WW, Gompf SG, Toney JF, et al. Disseminated *Acanthamoeba* sinusitis in a patient with AIDS: a possible role for early antiretroviral therapy. *AIDS Read*. 2004;14:41–49.
105. Morrison AO, Morris R, Shannon A, et al. Disseminated *Acanthamoeba* infection presenting with cutaneous lesions in an immunocompromised patient: a case report, review of histomorphologic findings, and potential diagnostic pitfalls. *Am J Clin Pathol*. 2016;145:266–270.
106. Helton J, Loveless M, White CR Jr. Cutaneous *Acanthamoeba* infection associated with leukocytoclastic vasculitis in an AIDS patient. *Am J Dermatopathol*. 1993;15:146–149.
107. Thamprasert K, Khunamornpong S, Morakote N. *Acanthamoeba* infection of peptic ulcer. *Ann Trop Med Parasitol*. 1993;87:403–405.
108. Ranjan R, Handa A, Choudhary A, et al. *Acanthamoeba* infection in an interhemispheric ependymal cyst: a case report. *Surg Neurol*. 2009;72:185–189.
109. Maycock NJ, Jayaswal R. Update on *Acanthamoeba* keratitis: diagnosis, treatment, and outcomes. *Cornea*. 2016;35:713–720.
110. Patel DV, McGhee CN. *Acanthamoeba* keratitis: a comprehensive photographic reference of common and uncommon signs. *Clin Exp Ophthalmol*. 2009;37:232–238.
111. Galarza M, Cuccia V, Sosa FP, et al. Pediatric granulomatous cerebral amebiasis: a delayed diagnosis. *Pediatr Neurol*. 2002;26:153–156.
112. Botterill E, Yip G. A rare survivor of *Balamuthia* granulomatous encephalitis. *Clin Neurol Neurosurg*. 2011;113:499–502.
113. Bravo F, Sanchez MR. New and re-emerging cutaneous infectious diseases in Latin America and other geographic areas. *Dermatol Clin*. 2003;21:655–668, viii.
114. Chang OH, Liu F, Knopp E, et al. Centrofacial balamuthiasis: case report of a rare cutaneous amebic infection. *J Cutan Pathol*. 2016;43:892–897.
115. Kidney DD, Kim SH. CNS infections with free-living amebas: neuroimaging findings. *AJR Am J Roentgenol*. 1998;171:809–812.
116. Singh P, Kochhar R, Vashishta RK, et al. Amebic meningoencephalitis: spectrum of imaging findings. *AJNR Am J Neuroradiol*. 2006;27:1217–1221.
117. Madarova L, Trnkova K, Feikova S, et al. A real-time PCR diagnostic method for detection of *Naegleria fowleri*. *Exp Parasitol*. 2010;126:37–41.
118. Cope JR, Ali IK. Primary amebic meningoencephalitis: what have we learned in the last 5 years? *Curr Infect Dis Rep*. 2016;18:31.
119. Petry F, Torzewski M, Bohl J, et al. Early diagnosis of *Acanthamoeba* infection during routine cytological examination of cerebrospinal fluid. *J Clin Microbiol*. 2006;44:1903–1904.
120. Shirwadkar CG, Samant R, Sankhe M, et al. *Acanthamoeba* encephalitis in patient with systemic lupus, India. *Emerg Infect Dis*. 2006;12:984–986.
121. Lai S, Asgari M, Henney HR Jr. Non-radioactive DNA probe and polymerase chain reaction procedures for the specific detection of *Acanthamoeba*. *Mol Cell Probes*. 1994;8:81–89.
122. Wright P, Warhurst D, Jones BR. *Acanthamoeba* keratitis successfully treated medically. *Br J Ophthalmol*. 1985;69:778–782.
123. Jayasekera S, Sissons J, Tucker J, et al. Post-mortem culture of *Balamuthia mandrillaris* from the brain and cerebrospinal fluid of a case of granulomatous amoebic meningoencephalitis, using human brain microvascular endothelial cells. *J Med Microbiol*. 2004;53:1007–1012.
124. Hill CP, Damodaran O, Walsh P, et al. *Balamuthia* amebic meningoencephalitis and mycotic aneurysms in an infant. *Pediatr Neurol*. 2011;45:45–48.
125. Orozco L, Hanigan W, Khan M, et al. Neurosurgical intervention in the diagnosis and treatment of *Balamuthia mandrillaris* encephalitis. *J Neurosurg*. 2011;115:636–640.
126. Phillips BC, Gokden M, Petersen E. Granulomatous encephalitis due to *Balamuthia mandrillaris* is not limited to immuno-compromised patients. *Clin Neurol Neurosurg*. 2013;115:1102–1104.
127. Pindyck TN, Dvorscak LE, Hart BL, et al. Fatal granulomatous amebic encephalitis due to *Balamuthia mandrillaris* in New Mexico: a case report. *Open Forum Infect Dis*. 2014;1:ofu062.
128. Qvarnstrom Y, Visvesvara GS, Sriram R, et al. Multiplex real-time PCR assay for simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris,* and *Naegleria fowleri*. *J Clin Microbiol*. 2006;44:3589–3595.
129. Le Calvez T, Trouilhe MC, Humeau P, et al. Detection of free-living amoebae by using multiplex quantitative PCR. *Mol Cell Probes*. 2012;26:116–120.
130. Greninger AL, Messacar K, Dunnebacke T, et al. Clinical metagenomic identification of *Balamuthia mandrillaris* encephalitis and assembly of the draft genome: the continuing case for reference genome sequencing. *Genome Med*. 2015;7:113.
131. Wilson MR, Shanbhag NM, Reid MJ, et al. Diagnosing *Balamuthia mandrillaris* encephalitis with metagenomic deep sequencing. *Ann Neurol*. 2015;78:722–730.
132. Schuster FL, Visvesvara GS. Opportunistic amoebae: challenges in prophylaxis and treatment. *Drug Resist Updat*. 2004;7:41–51.
133. Cope JR, Conrad DA, Cohen N, et al. 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*. 2016;62:774–776.
134. Seidel JS, Harmatz P, Visvesvara GS, et al. Successful treatment of primary amebic meningoencephalitis. *N Engl J Med*. 1982;306:346–348.
135. Linam WM, Ahmed M, Cope JR, et al. Successful treatment of an adolescent with *Naegleria fowleri* primary amebic meningoencephalitis. *Pediatrics*. 2015;135:e744.
136. Goswick SM, Brenner GM. Activities of azithromycin and amphotericin B against *Naegleria fowleri* in vitro and in a mouse model of primary amebic meningoencephalitis. *Antimicrob Agents Chemother*. 2003;47:524–528.
137. Soltow SM, Brenner GM. Synergistic activities of azithromycin and amphotericin B against *Naegleria fowleri* in vitro and in a mouse model of primary amebic meningoencephalitis. *Antimicrob Agents Chemother*. 2007;51:23–27.
138. Gupta S, Ghosh PK, Dutta GP, et al. In vivo study of artemisinin and its derivatives against primary amebic meningoencephalitis caused by *Naegleria fowleri*. *J Parasitol*. 1995;81:1012–1013.
139. Debnath A, Tunac JB, Galindo-Gomez S, et al. Corifungin, a new drug lead against *Naegleria,* identified from a high-throughput screen. *Antimicrob Agents Chemother*. 2012;56:5450–5457.
140. Lallinger GJ, Reiner SL, Cooke DW, et al. Efficacy of immune therapy in early experimental *Naegleria fowleri* meningitis. *Infect Immun*. 1987;55:1289–1293.
141. Kim JH, Jung SY, Lee YJ, et al. Effect of therapeutic chemical agents in vitro and on experimental meningoencephalitis due to *Naegleria fowleri*. *Antimicrob Agents Chemother*. 2008;52:4010–4016.
142. Schuster FL, Guglielmo BJ, Visvesvara GS. In-vitro activity of miltefosine and voriconazole on clinical isolates of free-living amebas: *Balamuthia mandrillaris, Acanthamoeba* spp., and *Naegleria fowleri*. *J Eukaryot Microbiol*. 2006;53:121–126.
143. Fung KT, Dhillon AP, McLaughlin JE, et al. Cure of *Acanthamoeba* cerebral abscess in a liver transplant patient. *Liver Transpl*. 2008;14:308–312.
144. Walochnik J, Aichelburg A, Assadian O, et al. Granulomatous amoebic encephalitis caused by *Acanthamoeba* amoebae of genotype T2 in a human immunodeficiency virus-negative patient. *J Clin Microbiol*. 2008;46:338–340.
145. Sharma PP, Gupta P, Murali MV, et al. Primary amebic meningoencephalitis caused by *Acanthamoeba:* successfully treated with cotrimoxazole. *Indian Pediatr*. 1993;30:1219–1222.
146. Cope JR, Roy SL, Yoder JS, et al Increased Patient Survival: Miltefosine for Treatment Of Free-Living Ameba Infections Caused by *Acanthamoeba* and *Balamuthia*. http://www.cste2.org/confpresentations/uploadedfiles/cste%202013%20miltefosine%20Poster%20final.pdf. Accessed April 17, 2014.
147. McBride J, Ingram PR, Henriquez FL, et al. Development of colorimetric microtiter plate assay for assessment of antimicrobials against *Acanthamoeba*. *J Clin Microbiol*. 2005;43:629–634.
148. Perez-Santonja JJ, Kilvington S, Hughes R, et al. Persistently culture positive *Acanthamoeba* keratitis: in vivo resistance and in vitro sensitivity. *Ophthalmology*. 2003;110:1593–1600.
149. Johns KJ, Head WS, O'Day DM. Corneal toxicity of propamidine. *Arch Ophthalmol*. 1988;106:68–69.
150. Bang S, Edell E, Eghrari AO, et al. Treatment with voriconazole in 3 eyes with resistant *Acanthamoeba* keratitis. *Am J Ophthalmol*. 2010;149:66–69.
151. Shebab KW, Aboul-Nasr K, Elliot SP. *Balamuthia mandrillaris* granulomatous amebic encephalitis with renal dissemination in a previous healthy child: case report and review of the pediatric literature. *J Pediatric Infect Dis Soc*. 2018;7:e163–e168.
152. Cary LC, Maul E, Potter C, et al. *Balamuthia mandrillaris* meningoencephalitis: survival of a pediatric patient. *Pediatrics*. 2010;125:e699–e703.
153. Centers for Disease Control and Prevention. *Balamuthia* amebic encephalitis—California, 1999-2007. *MMWR Morb Mortal Wkly Rep*. 2008;57:768–771.
154. Schuster FL, Visvesvara GS. Axenic growth and drug sensitivity studies of *Balamuthia mandrillaris,* an agent of amebic meningoencephalitis in humans

and other animals. *J Clin Microbiol*. 1996;34:385–388.
155. Schuster FL, Visvesvara GS. Efficacy of novel antimicrobials against clinical isolates of opportunistic amebas. *J Eukaryot Microbiol*. 1998;45:612–618.
156. Ahmad AF, Heaselgrave W, Andrew PW, et al. The in vitro efficacy of antimicrobial agents against the pathogenic free-living amoeba *Balamuthia mandrillaris*. *J Eukaryot Microbiol*. 2013;60:539–543.
157. Kalsoom H, Baig AM, Khan NA, et al. Laboratory testing of clinically approved drugs against *Balamuthia mandrillaris*. *World J Microbiol Biotechnol*. 2014;30:2337–2342.
158. Vernhes MC, Benichou A, Pernin P, et al. Elimination of free-living amoebae in fresh water with pulsed electric fields. *Water Res*. 2002;36:3429–3438.
159. Patel A, Hammersmith K. Contact lens-related microbial keratitis: recent outbreaks. *Curr Opin Ophthalmol*. 2008;19:302–306.
160. Martin-Navarro CM, Lorenzo-Morales J, Machin RP, et al. Inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase and application of statins as a novel effective therapeutic approach against *Acanthamoeba* infections. *Antimicrob Agents Chemother*. 2013;57:375–381.
161. Davis RM, Schroeder RP, Rowsey JJ, et al. *Acanthamoeba* keratitis and infectious crystalline keratopathy. *Arch Ophthalmol*. 1987;105:1524–1527.
162. Martinez DY, Seas C, Bravo F, et al. Successful treatment of *Balamuthia mandrillaris* amoebic infection with extensive neurological and cutaneous involvement. *Clin Infect Dis*. 2010;51:e7–e11.

274 Malaria (*Plasmodium* Species)

Rick M. Fairhurst[a] *and Thomas E. Wellems*

SHORT VIEW SUMMARY

Definition
- A history of travel and exposure in a malaria-endemic area is typical.
- Uncomplicated malaria can easily be confused with the "flu": fever, accompanied by headaches, body aches, and malaise.
- Severe malaria can present with any one of the following: diminished consciousness, convulsions, respiratory distress, prostration, hyperparasitemia, severe anemia, hypoglycemia, jaundice, renal insufficiency, hemoglobinuria, shock, cessation of eating and drinking, repetitive vomiting, or hyperpyrexia.

Epidemiology
- Malaria is endemic in tropical and subtropical areas of Africa, South America, Asia, and Oceania.
- Malaria is transmitted by the bite of a female *Anopheles* mosquito. Falciparum malaria usually occurs within 6 months of exposure, but other malarias can occur more than a year after exposure.
- Transmission can also occur congenitally and by blood transfusion, imported mosquitoes, or autochthonous mosquitoes infected by immigrants.

Microbiology
- Plasmodia are parasitic protozoa of the Apicomplexa phylum.
- *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae,* and *Plasmodium knowlesi* are the major human malaria parasites. Mixed infections can occur.

Laboratory Diagnosis
- Identification of parasites is by expert examination of Giemsa-stained thick and thin blood smears.
- Rapid diagnostic tests are available (e.g., BinaxNOW in the United States [US]) and are accurate for *P. falciparum* and *P. vivax* but less so for other species. Polymerase chain reaction is the most sensitive test but is not rapidly available.

Therapy (See Tables 274.3 and 274.4 for Dosages)
- Treatment depends primarily on *Plasmodium* species, disease severity, drug-resistance epidemiology, patient age, and pregnancy status.
- Uncomplicated malaria
  - Chloroquine-sensitive *P. falciparum* (Mexico, Central America west of the Panama Canal, Haiti, the Dominican Republic, and most areas of the Middle East), *P. vivax, P. ovale, P. malariae,* and *P. knowlesi* can be treated with oral chloroquine phosphate.
  - Chloroquine-resistant *P. falciparum* (most areas of the world) or chloroquine-resistant *P. vivax* (Papua New Guinea and Indonesia) can be treated with oral artemether-lumefantrine (Coartem, the preferred treatment), or with atovaquone-proguanil (Malarone), quinine sulfate *plus* doxycycline, or mefloquine (dihydroartemisinin-piperaquine is registered and available in some countries outside the US).
  - Relapses of *P. vivax* and *P. ovale* malaria can be prevented with oral primaquine phosphate (contraindicated in pregnant or breastfeeding women and glucose-6-phosphate dehydrogenase [G6PD]–deficient persons).
- Severe malaria
  - Intravenous artesunate (preferred but available only from the Centers for Disease Control and Prevention in the US), quinidine gluconate (not available in the US), or quinine dihydrochloride (not available in the US).

Prevention
- Chemoprophylaxis decisions depend primarily on drug-resistance epidemiology, patient age, and pregnancy status.
  - Areas with chloroquine-sensitive malaria: chloroquine phosphate
  - Areas with mefloquine-sensitive malaria: mefloquine
  - All areas: atovaquone-proguanil or doxycycline
  - Areas with mostly *P. vivax:* primaquine (contraindicated in pregnant or breastfeeding women and G6PD-deficient persons)
- Mosquito repellents and avoidance measures

THE MALARIA PROBLEM

Malaria remains an overwhelming problem in tropical developing countries, accounting for an estimated 216 million cases and 445,000 deaths in 2016.[1–3] Nearly 40% of the world's population is at risk for acquiring malaria.[4–7] In sub-Saharan Africa, most severe cases and deaths occur in children younger than 5 years and in pregnant women.

The introduction of chloroquine and dichlorodiphenyltrichloroethane (DDT) at the end of World War II brought dramatic new power to malaria control efforts worldwide. With postwar economic recovery and a renewed spirit of international cooperation, optimism ran high that the widespread use of these new compounds would eliminate malaria, and in 1955, the World Health Organization (WHO) launched its campaign to eradicate the disease. This goal proved overly optimistic, and the centrally organized DDT spraying programs at the core of the campaign were discontinued in 1967. The campaign, nevertheless, brought regional successes that coincided with other factors to reduce malaria incidence in many areas of the world (e.g., in Asia) (Fig. 274.1A).[8]

A stark exception to this general progress has been sub-Saharan Africa, where malaria remains deeply entrenched. Even the most committed spraying and eradication programs in endemic areas of this region could not defeat malaria's efficient transmission by the *Anopheles gambiae* mosquito.[9] The wide availability and use of chloroquine did, however, boost the health of young African children who suffer most from *Plasmodium falciparum,* the species responsible for the deadliest forms of malaria. As chloroquine became increasingly available in the 1950s to 1970s, death rates from malaria in Africa began to drop, approaching half the level of the prechloroquine years.[10]

Unfortunately, the massive use of chloroquine (hundreds of tons sufficient for hundreds of millions of treatments annually) in the 1980s[11] selected for chloroquine-resistant *P. falciparum* strains in Southeast Asia that entered and spread across Africa. In the 1980s and 1990s,

[a]All material in this chapter is in the public domain, except any borrowed figures or tables.

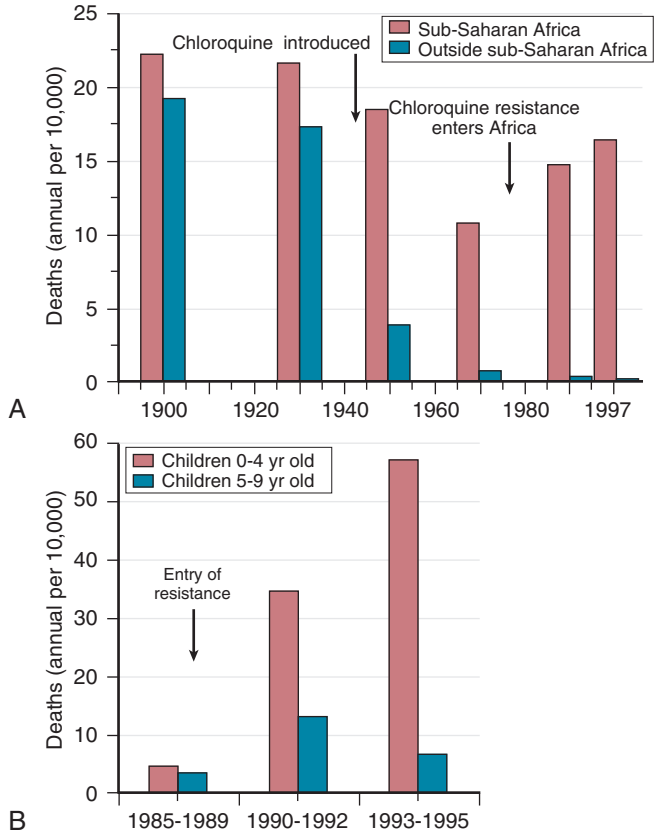


FIG. 274.1 Malaria death rates after the introduction of chloroquine and subsequent evolution of chloroquine-resistant *Plasmodium falciparum*. (A) Malaria death rates in the 20th century. Dramatic reductions in mortality were achieved outside sub-Saharan Africa. In Africa, mortality rates declined after the introduction of chloroquine but rose again after the spread of chloroquine resistance across the sub-Saharan region. (B) Rise in mortality among children in the village of Mlomp, Senegal. Increased death rates were observed after chloroquine resistance entered the village, chiefly among children younger than 5 years, the most susceptible age group in highly endemic areas. *(A modified from Carter R, Mendis KN. Evolutionary and historical aspects of the burden of malaria.* Clin Microbiol Rev. *2002;15:564–594. B modified from Trape JF, Pison G, Preziosi MP, et al. Impact of chloroquine resistance on malaria mortality.* C R Acad Sci III. *1998;321:689–697.)*

malaria resurged and death rates increased. The impact of chloroquine resistance was especially evident in young children, who do not have the partially protective immunity to malaria that usually develops after repeated episodes of the illness (Fig. 274.1B).[12,13] In the absence of a fully effective vaccine, success against malaria in Africa will continue to depend on effective drugs, such as artemisinin-based combination therapies (ACTs), that are reliable, affordable, and readily available. Although increased international support and funding for prevention, control, and elimination reinvigorated the efforts of malaria control programs, decreasing the incidence of malaria by an estimated 18% globally between 2010 and 2016, the number of cases and deaths have plateaued in the past several years.[2] Continued progress against the disease will require strengthened community commitments and health infrastructures, supported by new and improved drugs, advances in diagnostics, more effective vaccines, and better insecticides and vector control measures.[14]

PLASMODIUM AND ITS LIFE CYCLE

Plasmodium parasites belong to the Apicomplexa group of protozoa, which includes other pathogens, such as *Babesia, Toxoplasma,* and *Cryptosporidium* species. Apicomplexa are distinguished morphologically by the presence of a specialized complex of apical organelles (i.e., micronemes, rhoptries, and dense granules) involved in host cell invasion (see Fig. 274.4A).[15] Four *Plasmodium* species are classified as human malaria parasites: *P. falciparum, P. vivax, P. ovale,* and *P. malariae.* Some malaria parasites of other primates (e.g., *P. knowlesi, P. cynomolgi,* and *P. simium*) can also infect humans under natural conditions.[16] Indeed, with the recently appreciated extent of human infections from *P. knowlesi,* a natural pathogen of macaque monkeys, this parasite has been proposed to be a "fifth human malaria parasite" responsible for significant morbidity and mortality in Malaysia.[17–21]

In 1880, Alphonse Laveran[22] first observed malaria parasites in a human blood sample, witnessing the exflagellation of microgametes that usually emerge in the mosquito. It was eventually established that parasites in the bloodstream reproduce asexually in the haploid state (Fig. 274.2). During erythrocytic development, a small minority of parasites undergo a switch to sexual-stage development.[23] The resulting male and female gametocytes are the forms that are taken up by and infect anopheline mosquitoes, as proved by Ronald Ross and Battista Grassi[24,25] in the 1890s. Gametocytes emerge from erythrocytes in the mosquito midgut as male and female gametes that cross-fertilize to form diploid zygotes, which in turn differentiate into ookinetes that burrow through the midgut wall. Each ookinete develops into an oocyst containing up to 1000 sporozoites, which emerge and are then carried by the insect hemolymph to invade the salivary glands. These processes in the mosquito require an incubation period of about 1 to 2 weeks.

Female mosquitoes inject sporozoites into humans while probing the dermis in preparation for taking a blood meal. Shortt and Garnham[26] demonstrated in 1948 that sporozoites must first invade and replicate in hepatocytes before they can differentiate into merozoites capable of entering the intraerythrocytic cycle. The injected sporozoites typically take several hours to travel through dermal tissues and migrate across host cell barriers before they enter blood and lymphatic systems and are carried to the liver.[27] A molecular motor installed between the sporozoite plasma membrane and a double, inner membrane complex powers motility in this journey, whereas sporozoite surface proteins that are linked to this motor provide traction for gliding and crossing cellular barriers in tissue transit and invasion.[28,29]

Invasion of sporozoites into hepatocytes takes place by a coordinated series of steps, including host cell contact, signaling events with discharge of calcium, release of ligands and processing molecules from apical organelles, and active entry of the sporozoite into an induced parasitophorous vacuole in the hepatocyte cytoplasm. The host tetraspanin molecule CD81 is important for sporozoite entry into hepatocytes.[30,31] Two studies have shown that the class B type 1 scavenger receptor SR-B1, a known coreceptor with CD81 for invasion of hepatocytes by hepatitis C virus, also promotes efficient *Plasmodium* infection of hepatocytes.[32,33] A likely role for SR-B1 is the organization of CD81 into tetraspanin-enriched microdomains that are preferred membrane areas for sporozoite entry.[32] Because SR-B1 is vital in providing cholesterol to the hepatocyte by high-density lipoprotein (HDL)–cholesteryl ester uptake, its exploitation by *Plasmodium* and hepatitis C virus represents the evolutionary selection of a dependable invasion pathway by these pathogens. SR-B1's role in HDL–cholesteryl ester uptake and activation of the liver fatty acid–binding protein (L-FABP) carrier also supports the transformation and massive growth requirements of a parasite inside its host hepatocyte. Individual infected hepatocytes support the development of 10,000 to 30,000 merozoites, a process that is not associated with symptoms. All *P. falciparum* and *P. malariae* parasites complete their liver-stage development in about 1 to 2 weeks.[34] *P. vivax* and *P. ovale* liver stages also can develop promptly or can remain latent as hypnozoites in the liver for months to years before emerging to produce relapses of malaria (see Fig. 274.2).

Once a merozoite[35] egresses by protease activity from its host hepatocyte (or from its host erythrocyte in the bloodstream cycle),[36] it engages loosely with an uninfected erythrocyte and then reorients so that its apical end faces the cell surface.[37] The merozoite then drives itself into the erythrocyte through a ring-shaped, electron-dense junction that moves from the front to the back end of the merozoite by the power of an actin-myosin motor.[37] An envelope of invaginated membrane surrounds the merozoite as it enters, forming the parasitophorous vacuole once invasion is complete.[38] These steps of invasion are supported by cell-signaling events, energy-dependent migration, and discharge of contents from the rhoptries, micronemes, dense granules, and perhaps

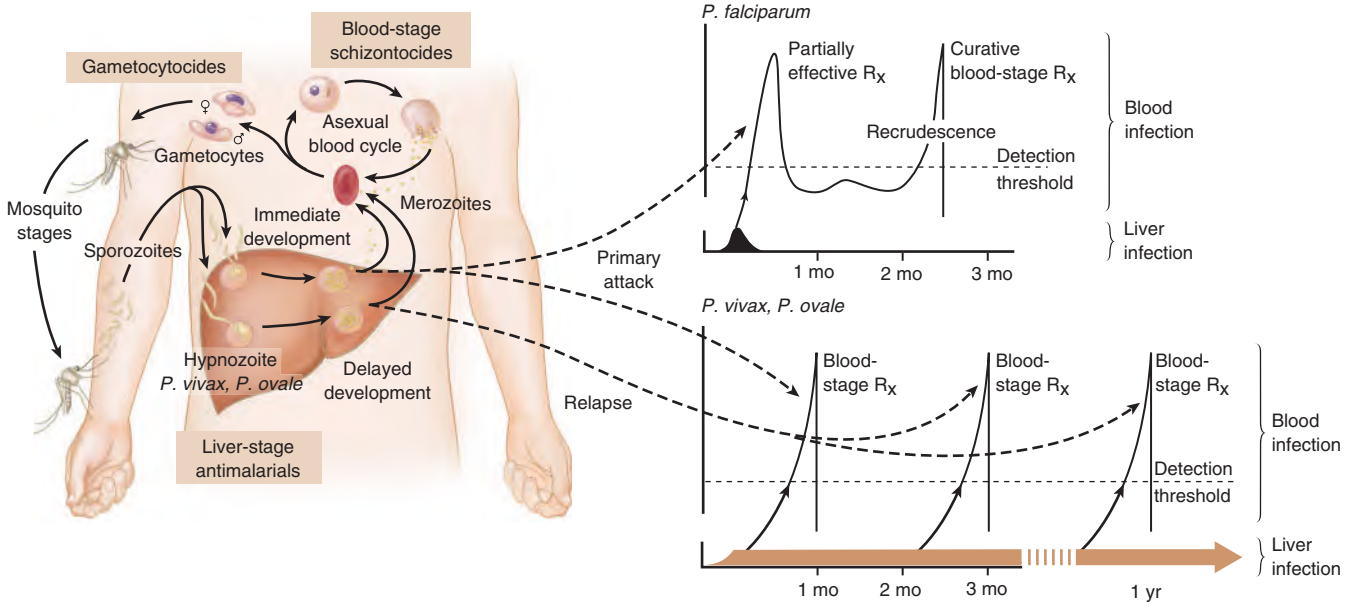


FIG. 274.2 The *Plasmodium* life cycle and disease patterns of recrudescence and relapse. Anopheline mosquitoes transmit malaria by injecting sporozoites into the human host. The sporozoites then invade hepatocytes, in which they develop into schizonts. Each infected hepatocyte ruptures to liberate 10,000 to 30,000 merozoites that invade circulating erythrocytes. Growth and development of the parasites in red cells result in subsequent waves of merozoite invasion. This asexual blood cycle repeats every 24 (*Plasmodium knowlesi*), 48 (*Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale*), or 72 (*Plasmodium malariae*) hours, leading to amplification of parasite density; paroxysms of chills, fevers, and sweats; and other manifestations of disease. Malaria symptoms are typically experienced 2 to 4 weeks after the mosquito bite. If the parasites are not cleared (e.g., patient receives partially effective therapy), recrudescence of parasitemia and malaria symptoms can occur. Eradicating parasites with an effective drug regimen cures malaria. Some *P. vivax* and *P. ovale* parasites can postpone their development in the liver, persisting as latent forms called hypnozoites. Hypnozoites are not eradicated by standard therapy (e.g., chloroquine) directed against blood stages. Resumption of hypnozoite development months to years after initial infection can lead to malaria relapse that requires an additional round of drug therapy to treat recurrent symptoms and eradicate blood-stage parasites. A course of primaquine can prevent relapses of malaria, but this treatment may not always be successful because of inadequate patient compliance, interindividual variations of drug metabolism, or variable parasite responses to low or high drug doses.

other apical compartments.[39] In *P. falciparum,* invasion can be supported by multiple different interactions between parasite molecules and erythrocyte surface molecules, including glycophorins.[40,41] Recent work has demonstrated the essential role of a ligand-receptor interaction between the *P. falciparum* RH5 (PfRH5) protein and the Ok blood group antigen, basigin.[42] A number of studies have also established a dependence of *P. vivax* merozoites on interaction with erythrocyte Duffy antigen receptor for chemokines (DARC),[43,44] but this may not be an absolute requirement considering that *P. vivax* infections occur in DARC-negative populations of Africa.[45–47]

Within erythrocytes, merozoites develop from ring forms into trophozoites and then schizonts over 24 hours (*P. knowlesi*), 48 hours (*P. falciparum, P. vivax,* and *P. ovale*), or 72 hours (*P. malariae*). After breaking down their host cell membrane by enzymatic digestion, 24 to 32 merozoites enter the bloodstream and are each capable of infecting a new erythrocyte. Cycles of invasion and growth in erythrocytes produce a parasite biomass that enlarges rapidly, causing fever and leading to pathologic processes, such as erythrocyte loss (anemia), sequestration of infected erythrocytes in microvascular beds (cerebral malaria), and adverse sequelae of inflammatory cascades and cytokine release.

PATHOPHYSIOLOGY

The Malaria Paroxysm and General Considerations

Malaria presents as an acute febrile illness that is often but not always characterized by the classic malaria paroxysm: chills and rigors, followed by fever spikes up to 40°C (104°F), and then profuse sweating that can ultimately give way to extreme fatigue and sleep. Paroxysms last several hours, can occur with a regular periodicity coinciding with the synchronous rupture of blood schizonts, may alternate with relatively asymptomatic periods, and are associated with high levels of tumor necrosis factor (TNF).[48] Paroxysms can occur in 24-hour, tertian 48-hour, or quartan 72-hour cycles, or in other more complicated patterns.[49] TNF may originate from monocytes stimulated by glycosylphosphatidylinositol moieties or other substances released on schizont rupture.[50,51]

Malaria can be acutely malignant and painful or more indolent and undermining. It predisposes African children to bacteremia[52] and increases the morbidity and mortality associated with other diseases by stressing host systems and producing effects such as dehydration, anemia, and some degree of immune suppression. Malaria is tremendously debilitating to health and impedes economic development through its adverse effects on fertility, population growth, saving and investment, worker productivity, absenteeism, premature mortality, and medical costs.[53,54] A single episode of malaria has been estimated to result in a loss of 5 to 20 working days, and an agricultural family afflicted by malaria may be up to 60% less productive than a family without malaria.[55]

Plasmodium falciparum

P. falciparum malaria can be much more acute and severe than malaria caused by other *Plasmodium* species (Fig. 274.3). Although *P. vivax* can cause serious and fatal illness,[56,57] by far the largest fraction of deaths directly attributable to malaria are caused by severe complications of *P. falciparum* infection, including cerebral malaria, severe anemia, respiratory distress, renal failure, and severe malaria of pregnancy.[58–63] Important contributory factors include metabolic acidosis, hypoglycemia, and superimposed bacterial infections. Fatal *P. falciparum* infections are often associated with the failure of multiple organ systems.

An important feature of the pathogenesis of *P. falciparum* is the ability of its mature trophozoite and schizont forms to sequester in the deep venous microvasculature. This sequestration is promoted by a number of processes: the adherence of infected erythrocytes to endothelial cells[64–66] (Fig. 274.4F and G); rosetting—the binding of infected erythrocytes to uninfected erythrocytes[66–68] (see Fig. 274.4H); reduced erythrocyte deformability[69,70] (see Fig. 274.4C and D); and platelet-mediated clumping of infected erythrocytes.[71,72] In malaria of pregnancy, infected erythrocytes accumulate within the proteoglycan matrix of placental intervillous spaces.[73] *P. falciparum*–infected erythrocytes can thus accumulate throughout the body, including the heart,[74] lung,[75] liver,[76] brain,[75,77–80] kidney,[81] intestine,[75,77] dermis,[77] bone marrow,[82] and placenta.[83] Uninfected erythrocytes, monocytes, platelets, and deposits

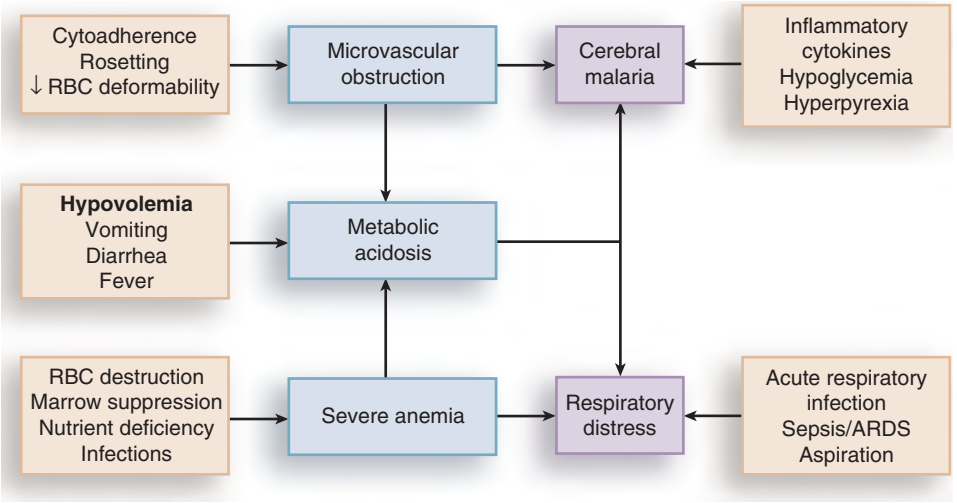


FIG. 274.3 Pathogenesis of severe *Plasmodium falciparum* malaria. Deaths from severe *P. falciparum* malaria are commonly attributable to the effects of severe anemia, cerebral malaria, and respiratory distress in young children. This schematic illustrates how multiple pathogenic events such as cytoadherence, destruction of uninfected erythrocytes, and production of inflammatory cytokines combine to produce the microvascular sequestration and metabolic acidosis that are central to the development of severe disease. *ARDS,* Acute respiratory distress syndrome; *RBC,* red blood cell.

of thrombin and fibrin are often found in association with these infected erythrocytes.[75,78,80,81]

By sequestering in microvessels, *P. falciparum* may avoid filtration and destruction by the spleen and thus multiply to high densities.[84] The survival and propagation of parasites may be enhanced when they sequester in the low-oxygen-tension environment of postcapillary venules. Attachment points to endothelium have been shown by electron microscopy to be dense protrusions, termed *knobs,* on the surface of infected erythrocytes (see Fig. 274.4D and E), where antigenically variant cytoadherence proteins (*P. falciparum* erythrocyte membrane protein 1 [PfEMP-1] variants) are anchored. Attachment at knobs (see Fig. 274.4F) supports cytoadherence in vitro and sequestration in vivo (see Fig. 274.4G).[85,86] Under flow conditions, cytoadherence events are reminiscent of leukocyte adhesion, involving distinct phases of tethering, rolling, and stable adhesion.[87]

PfEMP-1 is central to malaria pathogenesis.[88] PfEMP-1 is a family of antigenically variant proteins encoded by the multicopy *var* gene family.[89] Approximately 60 different *var* genes are present in the haploid genome of each parasite, encoding PfEMP-1 variants with unique antigenic and cytoadherence properties.[90,91] A single PfEMP-1 variant is thought to be predominantly expressed on the surface of an individual infected erythrocyte,[92] whereas all others are silenced.[93] Switches in expression between individual members of the *var* gene family occur at an estimated rate of 2% to 18% per cell per generation[94,95] and produce the antigenic variation in *P. falciparum* populations during the course of an infection. These switches may be structured to safeguard sequential expression among variant antigenic genes and thereby promote longevity of infection.[96]

The various PfEMP-1 proteins exposed on knobs have binding domains that adhere to host molecules, including CD36, intercellular adhesion molecule 1 (ICAM-1), thrombospondin, platelet endothelial cell adhesion molecule (PECAM/CD31),[87,97–100] chondroitin sulfate A (CSA),[73,101] and endothelial protein C receptor, which is involved in the deadly sequestration of parasitized erythrocytes that leads to cerebral malaria.[102–104] In addition to the association of cerebral malaria with subsets of PfEMP-1 variants that adhere to brain endothelium, other pathologic conditions have also been linked to particular PfEMP-1 variants and host receptors.[64,105–110] CD36 is an important cytoadherence receptor expressed on microvascular endothelium, as well as on monocytes and platelets, and is thought to mediate the sequestration of parasites as well as the host immune response to infection.[111] Infected erythrocytes that bind CSA expressed by syncytiotrophoblasts sequester selectively in placental tissue and are responsible for malaria of pregnancy.[73]

Some PfEMP-1 variants are also important parasite ligands in rosetting[112] because they can adhere to complement receptor 1 (CR1)[113] and blood group A antigen[114] on uninfected erythrocytes. A human CR1 polymorphism that reduces *P. falciparum* rosetting was found in one study to protect against severe malaria[115]; data from other studies of rosetting and disease severity have, in some cases, shown an association[116–120] and in others have not.[121,122] PfEMP-1 variants that bind to glycoprotein CD36 on the surface of platelets are also thought to play an important role in the platelet-mediated clumping of infected erythrocytes.[71]

P. falciparum can infect erythrocytes of all ages,[123] promoting heavy parasite burdens. High parasite densities,[124] increased parasite multiplication rates,[125] and evidence of high parasite biomass (e.g., intraleukocytic pigment, mature trophozoites, and schizonts) on peripheral blood smears are associated with increased severity of malaria and death.[126–128] Such high peripheral blood parasitemias are not observed with the severe pathology of vivax malaria. *P. vivax* parasites selectively infect reticulocytes[129,130] and accumulate as developing sexual stages (gametocytes) and mature replicative stages (schizonts) in the bone marrow and liver.

Cerebral Malaria

The classic histopathologic finding of fatal cerebral falciparum malaria is the intense sequestration of infected erythrocytes in cerebral microvessels (see Fig. 274.4G), often accompanied by "ring" hemorrhages, perivascular leukocyte infiltrates, thrombin deposition, activated platelets,[79,131–135] and immunohistochemical evidence for endothelial cell activation.[78,136,137] In one autopsy series of patients who died from cerebral malaria, 94% of brain microvessels contained adherent infected erythrocytes, compared with 13% of patients who died from noncerebral malaria.[138] In another study, 7 of 31 (24%) African children who received a clinical diagnosis of cerebral malaria were found at autopsy to have nonmalarial causes of coma, underscoring the possibility that other illnesses may mimic the cerebral malaria presentation in areas where incidental parasitemia is common.[133]

Sequestration of *P. falciparum*–infected erythrocytes stimulates the local production of inflammatory cytokines, such as TNF, elevated levels of which may correlate with disease severity.[139–141] These cytokines and other inflammatory mediators also upregulate adhesion molecules, such as ICAM-1, in the cerebral microvasculature,[142,143] which may lead to further sequestration of infected and uninfected erythrocytes, leukocytes, and activated platelets.[131] Impaired nitric oxide bioavailability may also contribute to endothelial dysfunction[144] by increasing microvascular tone, endothelial cell adhesion molecule expression, cytokine production, and infected erythrocyte sequestration.[145] These processes may cause

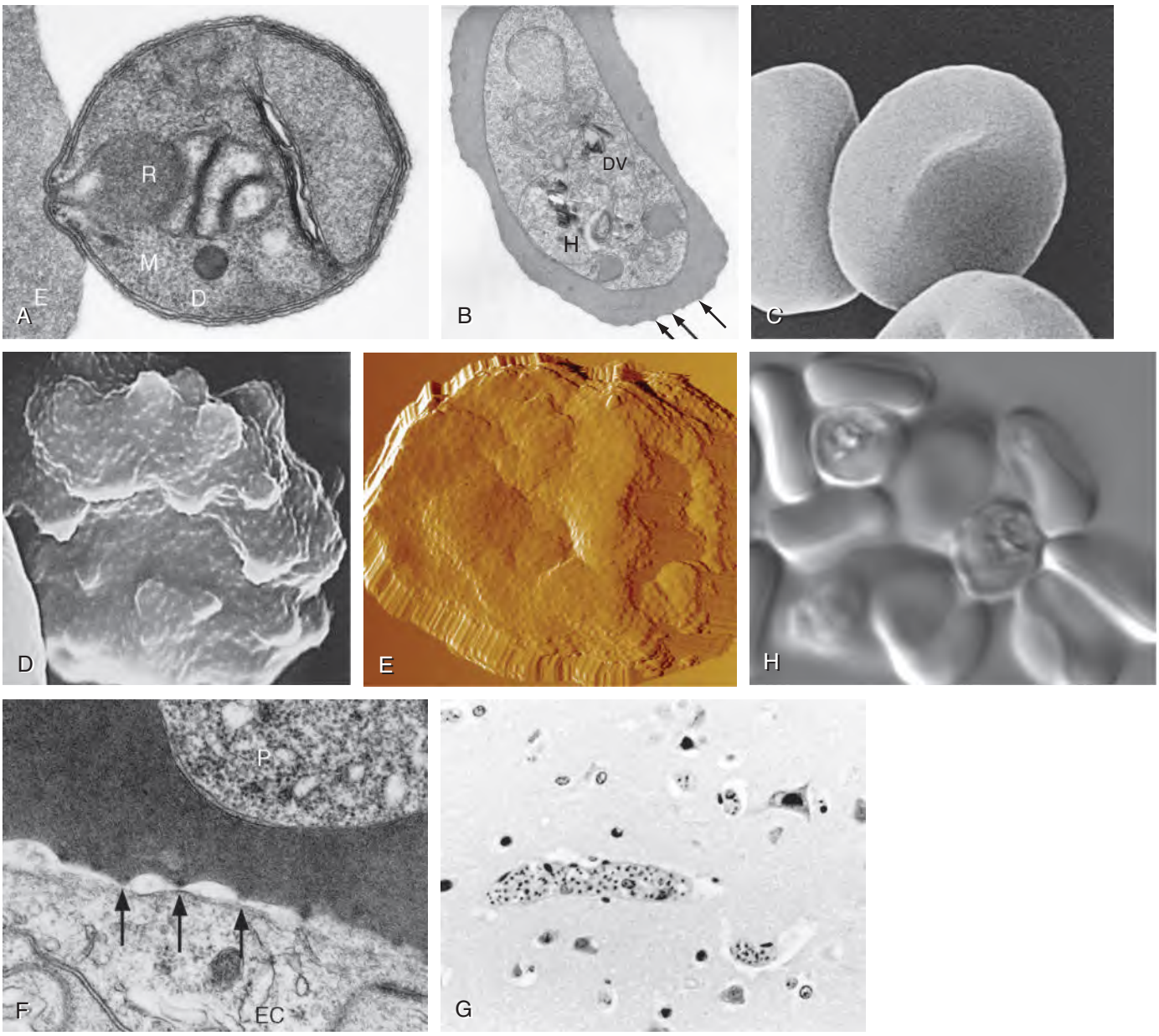


FIG. 274.4 Morphologic features of *Plasmodium* parasites. (A) Transmission electron micrograph of a *P. knowlesi* merozoite invading an erythrocyte (*E*) via its apical end, which contains rhoptries (*R*), micronemes (*M*), and dense granules (*D*). (B) Transmission electron micrograph of an intraerythrocytic *P. falciparum* trophozoite containing cytosomes, digestive vacuole (*DV*), and crystalline hemazoin (*H*). Arrows identify numerous small electron-dense protrusions, termed *knobs,* on the surface of the host erythrocyte. (C and D) Scanning electron micrographs demonstrate the effects of the malaria parasite on its host erythrocyte. Distention from the growth of the parasite *P. falciparum* converts the erythrocyte from a deformable biconcave disk (C) to a nondeformable cell (D) displaying knobs over its surface. (E) Atomic force microscopic image of the surface of a *P. falciparum*–infected erythrocyte showing numerous knob structures. (F) Transmission electron micrograph showing adherence via knobs *(arrows)* between a *P. falciparum*–infected erythrocyte (*P*) and a host endothelial cell (*EC*) of a cerebral microvessel. (G) Histologic section of brain tissue showing pigmented mature *P. falciparum* parasites sequestered in microvessels. (H) Light microscopic image of rosetting, the binding of a *P. falciparum*–infected erythrocyte to multiple uninfected erythrocytes. *(A from Fujioka H, Aikawa M. The malaria parasite and its life cycle. In: Wahlgren M, Perlmann P, eds.* Malaria: Molecular and Clinical Aspects. *Harwood Academic; 1999:19–55. B courtesy Hisashi Fujioka, Cleveland, OH. C and D from Aikawa M, Rabbege JR, Udeinya IJ, et al. Electron microscopy of knobs in* Plasmodium falciparum*–infected erythrocytes.* J Parasitol. *1983;69:435–437. E courtesy James Dvorak and Takayuki Arie, Bethesda, MD. [From Atkinson CT, Aikawa M. Ultrastructure of malaria-infected erythrocytes.* Blood Cells. *1990;16:351–368]. G courtesy Hisashi Fujioka, Cleveland, Ohio. H courtesy James Dvorak, Bethesda, MD.)*

varying degrees of functional obstruction and consequently impair local delivery of oxygen and glucose. However, obstruction by infected erythrocytes and other blood elements does not generally produce neurologic sequelae akin to those that follow the physical occlusion in thrombotic stroke, because most patients with cerebral malaria who recover can do so rapidly within 48 hours and without such consequences. Systemic sequestration of metabolically active parasites, blood cells, and platelets likely contributes to the metabolic acidosis and thrombocytopenia commonly seen in severe malaria. Metabolic acidosis, hypoglycemia, hyperpyrexia, and nonconvulsive status epilepticus can contribute significantly to the cerebral malaria presentation, as suggested by the rapid clinical improvement of some patients after fluid resuscitation, blood transfusion, dextrose infusion, fever reduction, and anticonvulsant therapy in addition to antimalarial treatment.[146–149]

Hypoglycemia

Hypoglycemia in malaria can cause coma and convulsions and thus may contribute substantially to the morbidity and mortality associated with cerebral malaria.[58] The pathophysiologic mechanisms of hypoglycemia in children and adults seem to be different. In children, insulin

levels are appropriate and hypoglycemia is associated with impaired hepatic gluconeogenesis and increased consumption of glucose by hypermetabolic peripheral tissues.[148,150–153] Large amounts of glucose are also consumed by intraerythrocytic parasites.[154] In adults, hypoglycemia is often associated with hyperinsulinemia,[155] which may result from pancreatic islet cell stimulation by parasite-derived factors or parenteral quinine or quinidine therapy, or both.[156] Depletion of liver glycogen stores after decreased food intake during the prodromal period may also contribute to hypoglycemia.

Anemia

The pathophysiology of malarial anemia is multifactorial and complex.[157–159] The intravascular lysis and phagocytic removal of infected erythrocytes[160] contribute to anemia but do not account for the dramatic reductions in erythrocyte mass that can accompany acute *P. falciparum* malaria episodes. Additional processes have therefore been implicated in the pathogenesis of malarial anemia. Excess removal of uninfected erythrocytes may account for up to 90% of erythrocyte loss[161] and may be mediated by processes (e.g., oxidative stress) that accelerate the senescence and reduce the deformability of erythrocytes. The contribution of impaired erythropoietic responses to malarial anemia is significant and probably involves general processes also found in other diseases. Release of inflammatory cytokines (e.g., TNF) is associated with impaired production of erythropoietin,[162,163] decreased responsiveness of erythroid progenitor cells to adequate levels of erythropoietin,[164,165] and increased erythrophagocytic activity.[166] These pathogenic processes account for the normochromic normocytic anemia seen in malaria and explain the notable absence of a robust reticulocyte response. Although microcytosis and hypochromia are seen in malaria, these findings are often attributable to thalassemias and iron deficiency in endemic areas. Bacteremia, nutritional deficits (e.g., vitamin A and B_{12} deficiencies), concomitant infections (e.g., hookworm and *Schistosoma*), and genetic polymorphisms (glucose-6-phosphate dehydrogenase [G6PD] deficiency) have also been associated with greater degrees of anemia in malaria episodes,[167] presumably by lowering the baseline from which hemoglobin levels acutely decline. In endemic areas where chloroquine resistance is prevalent, the inability of young children to clear their parasitemias with chloroquine contributes to their higher baseline prevalence of anemia when compared with children treated with more effective drugs.[168]

Pulmonary Edema and Respiratory Distress

The most significant pulmonary manifestation directly attributable to *P. falciparum* is noncardiogenic pulmonary edema.[169,170] Sequestration of infected erythrocytes in the lungs is thought to initiate regional production of inflammatory cytokines that increase capillary permeability, leading sequentially to pulmonary edema, dyspnea, hypoxia, acute lung injury, and acute respiratory distress syndrome.[171,172] Pulmonary edema is common with severe malaria in adults but infrequent in children, and it is not associated with pleural effusion. Iatrogenic fluid overload and acute renal failure may contribute to the development or worsening of pulmonary edema. Although pulmonary edema usually occurs after other features of severe disease (e.g., coma, acute renal failure) become manifest, it may occur at any time during the clinical course, even when the patient appears to be recovering on antimalarial therapy. Dyspnea and increased respiratory rate are features of impending pulmonary edema and often precede other clinical (e.g., use of accessory muscles of respiration) and radiologic (e.g., generalized increase in interstitial markings) signs.

Pulmonary manifestations of deep breathing and respiratory distress associated with severe malaria may also arise from metabolic acidosis,[59] severe acute respiratory infections,[152] sepsis-related acute respiratory distress syndrome, aspiration (especially with diminished consciousness or convulsions), and nosocomial pneumonia. Cerebral pathologic processes may result in abnormal breathing patterns, including Cheyne-Stokes respirations and respiratory failure.[173]

Metabolic (Lactic) Acidosis

Metabolic acidosis is a common feature of severe malaria and is associated with significant lactic acidemia in up to 85% of cases. Metabolic acidosis is principally caused by reduced delivery of oxygen to tissues, from the combined effects of anemia (decreased oxygen-carrying capacity), sequestration (microvascular obstruction), and hypovolemia (reduced perfusion) resulting from fluid losses caused by fever, decreased oral intake, vomiting, and diarrhea.[174] These effects produce a shift from aerobic to anaerobic metabolism and cause lactate levels to increase.[175] The following factors may also contribute to metabolic acidosis: production of lactate by anaerobic glycolysis in sequestered parasites[176]; reduction of hepatic blood flow, leading to diminished lactate clearance[177,178]; induction of lactate production by TNF and other proinflammatory cytokines[179]; impairment of renal function[177]; and ingestion of exogenous acids (e.g., salicylate) or unknown constituents of traditional herbal remedies for fever.[180]

Malaria of Pregnancy

Placental malaria results in maternal morbidity and mortality, intrauterine growth retardation, premature delivery, low birth weight, and increased newborn mortality.[181–183] Selective accumulation of infected erythrocytes in the placenta involves their interaction with syncytiotrophoblastic CSA,[73] which is possibly complemented by interactions with other molecules, such as hyaluronic acid and immunoglobulins.[184–186] This is in contrast to the sequestration of infected erythrocytes in the systemic microvasculature, where CD36 is the major endothelial receptor. Parasites that accumulate in the placenta express PfEMP-1 variants that bind CSA[187,188] but not CD36.[189] Evidence suggests that women experiencing a malaria episode from CSA-binding parasites during their first pregnancy lack immunity to the PfEMP-1 antigenic variants presented by these strains and, despite immunity to CD36-binding variants from previous infections, are highly susceptible to the new placental infection. Placental malaria in subsequent pregnancies is typically less severe than in the first pregnancy,[190] presumably because of a woman's previous exposure to CSA-binding parasites.

Plasmodium vivax and *Plasmodium ovale*

Infections with *P. vivax* and *P. ovale* can be considered similar to each other from a clinical perspective. *P. vivax* infections can be tremendously debilitating and are sometimes associated with serious complications, including acute lung injury[191,192] and splenic pathology.[193,194] Splenic rupture has been associated with acute and chronic infections and can occur spontaneously or with minor trauma, including manual examination of the spleen. Although more commonly associated with *P. vivax* malaria, splenic rupture has been associated with all four human malaria parasites. Anemia is frequently observed as a consequence of acute or chronic infections, or as a result of repeated acute infections.[160] Suppressed erythrocyte production and hemolysis of both infected and uninfected erythrocytes have been implicated in the pathogenesis of *P. vivax* malarial anemia. Although not often fatal, *P. vivax* infections have recently been associated in Papua New Guinea and Indonesia with severe disease manifestations, including cerebral malaria, severe anemia, and respiratory distress.[56,57,195]

P. vivax merozoites selectively invade reticulocytes.[130] Because these cells account for only a small proportion of the total erythrocyte mass, parasitemias in *P. vivax* infections are usually less than 1% even when the pathology of vivax malaria is severe. *P. vivax* parasites can accumulate as developing sexual stages (gametocytes) and mature replicative stages (schizonts) in the bone marrow and liver.[196] Recent evidence suggests that *P. vivax* can cytoadhere to lung endothelial cells[197] and can cause inflammation and injury by sequestering in the pulmonary microvasculature.[198] *P. vivax* parasites may avoid splenic entrapment by increasing rather than decreasing erythrocyte deformability.[199]

Plasmodium malariae

The quartan malaria of *P. malariae* usually presents with fever and paroxysms similar to those of *P. vivax* but with a 3-day rather than 2-day periodicity. *P. malariae* often establishes parasitemias that are below the level of detection by microscopy. Patients can remain infected and asymptomatic for many years before presenting with fevers, malaise, and splenomegaly decades after they have left an endemic area.[200] Chronic *P. malariae* infection can lead to nephrotic syndrome in young children living in endemic areas.[201,202] This complication has features of an immune complex–mediated glomerulonephritis.[203,204]

Plasmodium knowlesi

A large focus of human malaria caused by *P. knowlesi* occurs in Malaysia, where high case-fatality rates have been reported.[17,205] *P. knowlesi* is indistinguishable from *P. malariae* on blood smear examination, showing both immature and mature forms in the circulation. Unlike *P. malariae,* however, *P. knowlesi* replicates every 24 hours and can cause daily fever spikes and hyperparasitemias that are life threatening. In addition to hyperparasitemia, severe *P. knowlesi* malaria cases have been associated with metabolic acidosis, hepatorenal dysfunction, respiratory distress, severe anemia, and refractory hypotension.[18,205]

GENETIC RESISTANCE TO MALARIA

Fatal *P. falciparum* malaria has been a potent evolutionary force in shaping the human genome. Evidence for the natural selection of genetic polymorphisms can be found in the ethnic and geographic distributions of hemoglobin variants, thalassemias, G6PD deficiencies, erythrocyte membrane proteins, and cytokines and other mediators of inflammation and immunity.[206–215]

Hemoglobins S, C, and E

The geographic distributions of hemoglobin variants overlap considerably with those of *P. falciparum* malaria.[206,208] Case-control and longitudinal cohort studies have associated malaria protection with hemoglobin S (HbS) heterozygosity (HbAS; sickle cell trait), in which the sixth amino acid of one β-globin chain is changed from glutamate to valine.[216–220] The genetic fitness of HbS homozygosity (HbSS; sickle cell disease) is very poor in sub-Saharan Africa,[221] whereas the prevalence of HbAS individuals can be more than 25% in some areas.[222] The HbS mutation thus exists as a balanced polymorphism: the malaria protective benefit afforded to HbAS heterozygotes offsets the childhood deaths of HbSS homozygotes. The hemoglobin C (HbC) mutation is in the same sixth position as the HbS mutation but differs in that the amino acid is changed from glutamate to lysine. A number of case-control studies in West Africa have associated HbC with malaria protection.[216,223–227]

Most studies have associated HbAS protection against malaria with reductions in parasite density.[216] However, some epidemiologic studies have found similar parasite densities in HbAA and HbAS children, very high parasite densities in some HbAS children,[228] and occasional cases of severe malaria in HbAS children.[223,229] These observations are not fully explained by proposals that infected HbAS erythrocytes are more likely to sickle or support reduced parasite growth rates under conditions of low oxygen tension in microvessels,[230–233] by enhanced phagocytosis of infected HbAS erythrocytes by macrophages,[234,235] or by translocation of sickle cell erythrocyte microRNAs that may negatively regulate parasite growth in the infected host cell.[236] Other mechanisms of protection that include additional genetic or environmental factors are likely operating in HbAS children. Unlike for HbAS, malaria protection by HbC has not been associated with reduced parasite densities in vivo or significant impairment of parasite multiplication in vitro,[223,224,227,237] suggesting that HbC erythrocytes support normal invasion and development of *P. falciparum.*

In other studies of possible mechanisms of protection, freshly drawn and infected HbAS and HbAC erythrocytes were found to be impaired in their adherence to microvascular endothelial cells and monocytes,[237,238] two interactions critical to the development of severe malaria. Abnormal display of the parasite's main virulence factor and antigenically variable cytoadherence ligand, PfEMP-1, on the surface of infected erythrocytes offers one explanation for these findings. In this model of protection, reduced cytoadherence of infected HbAS and HbAC erythrocytes enables them to sequester in microvessels and achieve high parasite densities while lessening the inflammatory consequences of cytoadherence and thus reducing the chances of progressing to severe disease.[239] Evidence indicates that aberrant remodeling of host cell actin, compromised formation of knobs, and reduced PfEMP-1–mediated cytoadherence are the result of a redox imbalance inherent to hemoglobinopathic and fetal erythrocytes.[240,241] PfEMP-1–specific antibodies, which are acquired rapidly in endemic areas, may synergize with HbS and HbC to further weaken cytoadherence interactions and ameliorate disease severity.[238]

Hemoglobin E (HbE) is another hemoglobin variant characterized by the substitution of lysine for glutamate at the 26th amino acid of β-globin. This mutation also introduces an alternative splice site that reduces the amount of β-globin produced,[242] thereby conferring a β-thalassemia phenotype to HbE erythrocytes. Unlike HbS and HbC, HbE is found predominantly in Cambodia and neighboring countries in Southeast Asia. Some epidemiologic studies have found HbE to protect against malaria,[243,244] whereas others have not.[245,246] Although HbE has been suggested to decrease the multiplication rate of *P. falciparum,*[247] parasite densities do not differ between HbAA and HbE patients with malaria.[244,246]

Thalassemias

The thalassemias arise from deletion of one or more of the four genes encoding the α-globin chains or mutations or deletions in one of the two genes encoding the β-globin chains of hemoglobin. These hemoglobinopathies are generally benign in the heterozygous state and are associated with varying degrees of hypochromic microcytic anemia. Further loss of expression in the homozygous state causes severe disease and can be incompatible with life. Mutations associated with thalassemias are thus believed to exist as balanced polymorphisms in human populations; indeed, some of them (e.g., α-thalassemia) have been associated with protection from severe malaria.[216,248–250] Although *P. falciparum* development can be supported by thalassemic erythrocytes, some studies have demonstrated impaired growth, especially under conditions of oxidative stress.[235,251–253] Other studies have demonstrated that infected thalassemic erythrocytes bind increased amounts of antibody from both nonimmune and immune sera, which suggests the possibility of enhanced opsonization in vivo.[254,255] These mechanisms are difficult to reconcile with several more recent studies from Africa, which show that both heterozygous and homozygous α-thalassemias protect against severe malaria without reducing parasite densities in vivo.[225,256–258] Other evidence suggests that α-thalassemia may protect against severe malaria in Africa by the same mechanism proposed for HbS and HbC: namely, abnormal PfEMP-1/knob display, decreased cytoadherence, and reduced activation of endothelium.[259] Increased microerythrocyte counts in α-thalassemia homozygotes have been proposed to contribute to protection against severe malarial anemia in Papua New Guinea by a mechanism that reduces loss of erythrocytes and hemoglobin during *P. falciparum* infection.[260]

Hemoglobin F

Hemoglobin F (HbF, α_2/γ_2) is a normal hemoglobin variant expressed by the fetus in utero and by the infant during the first few months of life. The expression of HbF dramatically declines after the third month of life as adult hemoglobin A (HbA, α_2/β_2) replaces it. The uncommon presentation of malaria in neonates younger than 6 months led to the hypothesis that HbF contributes to malaria protection, along with maternal antibody. Proteases responsible for digesting host cell hemoglobin in the food vacuole of the parasite may work less efficiently on HbF than HbA.[261] Impaired antioxidant capacity of HbF-containing erythrocytes has also been proposed to contribute to malaria protection.[262] More recently, abnormal PfEMP-1/knob display has been associated with HbF.[263] In a new model of infant protection, the combined effects of HbF and maternal PfEMP-1–specific immunoglobulin G substantially diminish the ability of infected erythrocytes to bind and activate microvascular endothelium, thus impeding the development of malaria in infants.[239]

Glucose-6-Phosphate Dehydrogenase Deficiency

G6PD is a cytoplasmic enzyme that is essential for an erythrocyte's capacity to withstand oxidant stress, such as that exerted by the developing malaria parasite. The *G6PD* gene is located on the X chromosome and is therefore present in only one copy in males. In heterozygous females carrying a mutant gene, mosaic populations of G6PD-deficient and G6PD-normal erythrocytes are produced from hematopoietic cells that have one or the other X chromosome inactivated. G6PD deficiency is the most common enzymopathy in humans, with more than 300 allelic polymorphisms identified to date. The most common polymorphism in Africa (the A^- allele, 10%–50% enzyme activity) has been associated with malaria protection in children[264,265] and pregnant women.[266]

In a large case-control study performed in populations of West and East Africa, male hemizygotes and female heterozygotes carrying the A^- allele were reported to be 58% and 46% protected against severe malaria, respectively.[264] However, a more recent and larger study,[265] which included a reanalysis of data from the earlier study,[264] found that male hemizygotes, but not female heterozygotes, carrying the A^- allele are protected against severe malaria. These findings agree with the facts that male hemizygotes carry erythrocytes uniformly deficient in G6PD, whereas female heterozygotes carry mosaic populations of G6PD-normal and G6PD-deficient erythrocytes. Enhanced phagocytosis of infected G6PD-deficient erythrocytes has been proposed to play a role in malaria protection.[267] Although such phagocytosis is consistent with greater protection of males than females by G6PD deficiency, it does not account for the presence of similar parasite densities in G6PD-deficient males and females.[264,265] Alternative mechanisms of protection may therefore operate in vivo.

Southeast Asian Ovalocytosis and Hereditary Xerocytosis

A 27-base pair deletion in band 3 (the major anion transporter in erythrocytes) causes Southeast Asian ovalocytosis and leads to reduced membrane deformability.[268–270] These properties may be associated with reduced parasite invasion rates of ovalocytes in vitro and reduced parasitemias in heterozygous individuals.[271–274] How these findings relate mechanistically to the dramatic reduction in cerebral malaria episodes among Southeast Asian ovalocytosis heterozygotes[275] has not yet been established.

In studies of *PIEZO1* mutations and hereditary xerocytosis, cerebral malaria from *Plasmodium* infection was prevented in an engineered mouse model. Prevalence of the *PIEZO1* allele, E756del, in about one-third of Africans may reflect an association of this allele with malaria resistance.[276]

ABO Blood Groups

Blood group O antigen has been associated with protection against severe malaria[277,278] and rosetting, a phenotype of infected erythrocytes that correlates with severe disease in Africa.[116–120] A mechanism of reduced rosetting in type O erythrocytes, compared with type A, B, or AB erythrocytes, has been associated with this protective effect.[277]

Duffy Antigen Receptor for Chemokines Negativity

DARC is an erythrocyte receptor for *P. vivax* merozoite invasion.[129,130] Erythrocytes lacking DARC are resistant to invasion, but *P. vivax* infections nevertheless occur among DARC-negative individuals.[279,280] These facts are consistent with the low observed incidence of *P. vivax* malaria in regions of sub-Saharan Africa, where erythrocyte DARC negativity is highly prevalent.[43,44,281,282] The mechanism for *P. vivax* infection of DARC-negative individuals is unknown. Reduced DARC expression on erythrocytes has been identified as a protective polymorphism against *P. vivax* malaria in Papua New Guinea.[283] DARC negativity does not protect against malaria from *P. falciparum*.

ACQUIRED IMMUNITY AND ANTIGENIC VARIATION

Acquired immunity against malaria (premunition) is not a sterilizing immunity against parasitemia. Instead, it is an immunity that protects against symptomatic and severe disease without necessarily preventing the continued presence of malaria parasites in the bloodstream. It is an immunity that increases with age, cumulative number of episodes of malaria, and time spent living in an endemic area.[284] Parasitemia in individuals with premunition is usually low, whereas nonimmune individuals may develop high parasitemias (up to 80%) after infection.[285] As individuals gain the experience of numerous infections in their lifetime, they can be chronically infected yet have only mild symptoms or none at all; that is, they develop "disease-controlling" immunity. In highly endemic areas, children have multiple bouts of malaria each year and suffer greatly under its morbidity and mortality when they are young, generally less than 5 to 10 years old, depending on the transmission level. Pregnant women, especially primigravidae, are an important exception to this general rule of disease-controlling immunity from previous malaria episodes and thus can be highly susceptible to antigenically new CSA-binding parasites that sequester in the placenta (see "Malaria of Pregnancy" earlier).

Acquired immunity to malaria is believed to be short-lived without the continual stimulation of various antigenic exposures from different *P. falciparum* strains. Although individuals who reside outside an endemic area for more than a year or two can develop symptomatic or severe malaria, or both, after their return,[286] one study found evidence that acquired immunity to *P. falciparum* malaria persists after several years of nonexposure in African immigrants living in France.[287]

Neonates appear to be resistant to malaria during the first few months of life. This immunity may be conferred by transplacentally acquired maternal immunoglobulin G, although the presence of fetal hemoglobin within erythrocytes likely plays a role as well (see "Hemoglobin F" earlier).

Splenomegaly often accompanies malaria and is thought to indicate an important role of the spleen in parasite clearance.[288,289] Removal of uninfected erythrocytes by the stimulated spleen, however, may contribute to anemia. In asplenic individuals, *P. falciparum* malaria can progress extremely rapidly to high parasitemias that include mature forms not usually found circulating in the bloodstream.[84,290,291]

The immunity of endemic human populations to severe malaria is complex and not well understood.[292] Although antibodies and T-cell responses develop against a number of parasite antigens during natural infection, none of them has been found to be superior to age or parasite exposure as correlates of protective immunity.[293] Studies in which humans were infected with a single inoculum of *P. falciparum* in the use of malariotherapy[294] for tertiary syphilis showed that erythrocyte infection rose and fell in successive waves of decreasing peak parasitemia[295] (Fig. 274.5). Individuals who eventually cleared their infection were protected against subsequent reinfection by the same parasite strain but not protected against reinfection with a different *P. falciparum* strain.[296,297]

Antigen switching results in new waves of parasitemias that escape the antibody response already produced against previous waves, manifest clinically as recurrent or relapsing fevers reminiscent of those seen in *Borrelia recurrentis* relapsing fever or *Trypanosoma brucei rhodesiense* African sleeping sickness, and may not be cleared for months to years.[298,299] An important component of the eventual acquisition of disease-controlling premunition after repeated episodes of malaria is the development of an antibody repertoire that can recognize a full spectrum of PfEMP-1 variant antigens. Studies in endemic areas have shown that the ability of serum to recognize diverse heterologous parasite strains increases with age and that children tend to be infected with parasites against which they have no preexisting antibody.[300–303] The mechanisms by which variant-specific antibodies act in acquired immunity may include antibody-dependent cellular cytotoxicity, opsonization for uptake and destruction by splenic macrophages, and interference with PfEMP-1–mediated cytoadherence interactions.

EPIDEMIOLOGY OF MALARIA

Malaria occurs mostly in tropical regions of sub-Saharan Africa, Asia, Oceania, and Latin America (Fig. 274.6), but its distribution is continually changing.[304,305] The Malaria Atlas Project (https://www.map.ox.ac.uk) and the Centers for Disease Control and Prevention (CDC; https://www.cdc.gov/malaria/travelers) provide up-to-date information on the geographic distribution of malaria. Information on drug-resistant malaria is also available from the publication CDC Health Information for International Travel 2018 (https://wwwnc.cdc.gov/travel/page/yellowbook-home).

P. falciparum and *P. vivax* infections account for most cases of human malaria. *P. falciparum* and *P. malariae* are found worldwide. *P. vivax* is infrequent in most of sub-Saharan Africa but is common elsewhere. *P. ovale* occurs in Africa and in foci within Asia and Oceania and is often present with other *Plasmodium* species as a mixed infection.[306,307] Although most *P. knowlesi* infections of humans have been reported from Borneo and peninsular Malaysia,[17,18] cases have also been reported from other areas of Southeast Asia, including the Philippines, Singapore, Thailand, and Myanmar.[308]

Malaria is transmitted person to person by anopheline mosquitoes. Its endemicity depends upon competent mosquito vectors, a reservoir

of infected humans, and conditions that bring them into proximity. The *Anopheles gambiae* complex of species and *Anopheles funestus* transmit malaria with notoriously high efficiency and are the predominant vectors in sub-Saharan Africa, where environmental conditions favor their robust reproduction and transmission of parasites to large numbers of people. Highest levels of transmission typically occur during the wet season in endemic areas.

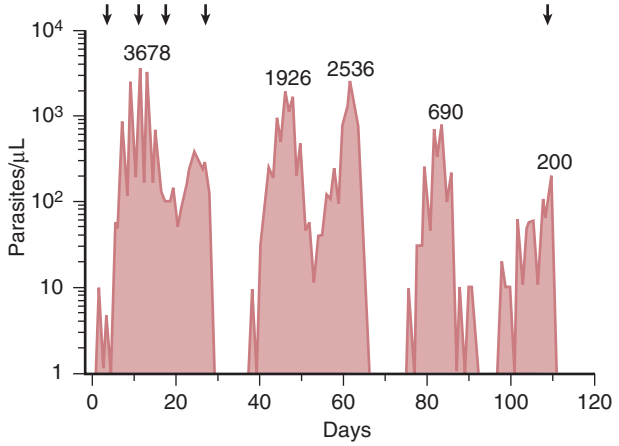


FIG. 274.5 Premunition and antigenic variation. Parasitemia waves during recrudescence in a patient infected with *Plasmodium falciparum* as malariotherapy for tertiary syphilis. Although the individual had previously been infected with *P. falciparum,* drug treatments *(arrows)* were necessary to modify the primary attack and later for radical cure. After subcurative drug treatments, four recrudescences occurred with parasite density peaks ultimately declining 10-fold. *(Modified from Collins WE, Jeffery GM. A retrospective examination of sporozoite- and trophozoite-induced infections with* Plasmodium falciparum: *development of parasitologic and clinical immunity during primary infection.* Am J Trop Med Hyg. *1999;611[suppl]:4–19.)*

Malaria epidemics can result from the movement of people with no immunity into an endemic area (e.g., nomadic traders, seasonal forest laborers, and military personnel), the breakdown of control measures in areas under previously successful management programs, or unusually heavy rainfalls that can place indigenous populations at risk for higher-than-normal transmission.[309–311] Man-made environmental alterations (e.g., agricultural and waterworks projects, damming of rivers, and deforestation) can lead to increases in malaria transmission by creating new mosquito habitats. Malaria may be imported into areas previously free of the disease as a result of immigration of populations from endemic areas (e.g., migrating workers, persons displaced by natural disasters or civil strife, and resettlement of refugees).[312–315]

In the United States, changes that include new agricultural and animal husbandry practices, improved housing with screens, water management with swamp drainage, and a radically altered landscape with urban development led to a steady decline in malaria after the mid-19th century.[316] Final pockets of transmission were removed by the mid-20th century with the help of focused water management and insecticide spraying. Malaria diagnosed in the United States today is therefore almost always acquired in a malaria-endemic country by a returning traveler or immigrant. Because of parasite or host factors, immigrants may harbor parasites for months to years and not be recognized as possible sources of transmittable infection. In 2015, the CDC received reports of 1517 confirmed cases of imported malaria.[317] *P. falciparum, P. vivax, P. ovale,* and *P. malariae* species were identified in 67%, 12%, 4%, and 3% of cases, respectively. Less than 1% of cases were infected by two species, and the infecting species was unreported or undetermined in 13% of cases.

Autochthonous transmission, although infrequent, typically occurs when parasitized individuals infect competent vectors (*Anopheles*

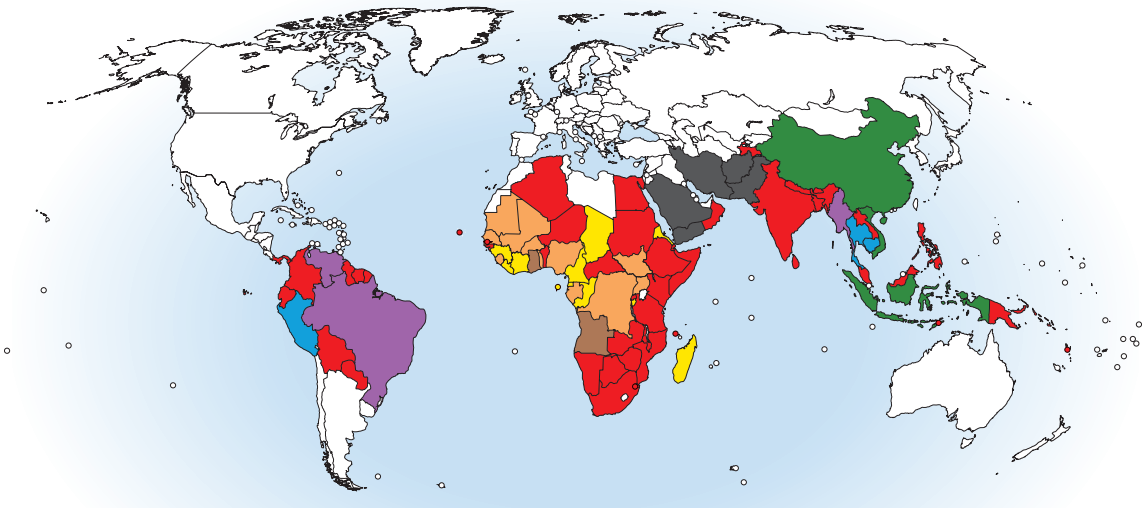


FIG. 274.6 Artemisinin-based combination therapies and the threat of artemisinin resistance. Artemisinin-based combination therapy (ACT)—the combination of an artemisinin derivative and a partner drug—is now recommended for the treatment of *Plasmodium falciparum* malaria in virtually all endemic areas. Selection of an appropriate ACT depends on the regional profile of parasite susceptibility to various partner drugs. Countries in which artemether-lumefantrine (*red*), artesunate-amodiaquine (*yellow*), artesunate-mefloquine (*blue*), dihydroartemisinin (DHA)-piperaquine (*green*), artesunate-sulfadoxine-pyrimethamine (*gray*), or some combination thereof (*orange*, *purple*, *brown*) are the first-line treatments are shown. Because of the widespread prevalence of resistance to chloroquine and sulfadoxine-pyrimethamine, these drugs are not commonly recommended as ACT partner drugs. In areas of Southeast Asia (Cambodia, Vietnam, Laos, Thailand, Myanmar, and southern China), the emergence and spread of artemisinin resistance now threatens the efficacy of all ACTs. The Centers for Disease Control and Prevention provides up-to-date information on the distribution of malaria and drug resistance at http://www.cdc.gov/malaria/travelers. *(Modified from 2018 World Health Organization listings provided by Peter Olumese.)*