

Fatal *Naegleria fowleri* Infection Acquired in Minnesota: Possible Expanded Range of a Deadly Thermophilic Organism

Sarah K. Kemble,^{1,3} Ruth Lynfield,¹ Aaron S. DeVries,¹ Dennis M. Drehner,² William F. Pomputius III,² Michael J. Beach,⁴ Govinda S. Visvesvara,⁴ Alexandre J. da Silva,⁵ Vincent R. Hill,⁴ Jonathan S. Yoder,⁴ Lihua Xiao,⁴ Kirk E. Smith,¹ and Richard Danila¹

¹Minnesota Department of Health, Saint Paul; ²Children's Hospitals and Clinics of Minnesota, Minneapolis; ³Epidemic Intelligence Service, ⁴National Center for Emerging and Zoonotic Infectious Diseases, and ⁵Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia

Background. Primary amebic meningoencephalitis (PAM), caused by the free-living ameba *Naegleria fowleri*, has historically been associated with warm freshwater exposures at lower latitudes of the United States. In August 2010, a Minnesota resident, aged 7 years, died of rapidly progressive meningoencephalitis after local freshwater exposures, with no history of travel outside the state. PAM was suspected on the basis of amebae observed in cerebrospinal fluid.

Methods. Water and sediment samples were collected at locations where the patient swam during the 2 weeks preceding illness onset. Patient and environmental samples were tested for *N. fowleri* with use of culture and real-time polymerase chain reaction (PCR); isolates were genotyped. Historic local ambient temperature data were obtained.

Results. *N. fowleri* isolated from a specimen of the patient's brain and from water and sediment samples was confirmed using PCR as *N. fowleri* genotype 3. Surface water temperatures at the times of collection of the positive environmental samples ranged from 22.1°C to 24.5°C. August 2010 average air temperature near the exposure site was 25°C, 3.6°C above normal and the third warmest for August in the Minneapolis area since 1891.

Conclusions. This first reported case of PAM acquired in Minnesota occurred 550 miles north of the previously reported northernmost case in the Americas. Clinicians should be aware that *N. fowleri*-associated PAM can occur in areas at much higher latitude than previously described. Local weather patterns and long-term climate change could impact the frequency of PAM.

Primary amebic meningoencephalitis (PAM) is a rare and typically fatal infection caused by the thermophilic, free-living ameba *Naegleria fowleri*. PAM is associated with recreational exposures (eg, swimming and diving) in warm freshwater [1, 2]. The organism is believed to cause infection by penetrating the nasal mucosa and migrating up the olfactory nerve to the brain. Infection usually progresses rapidly to coma and death.

Since 1962, all 117 cases of PAM confirmed in the United States have been limited to exposure in southern-tier states (Arizona, Arkansas, California, Florida, Georgia, Louisiana, Mississippi, Missouri, Nevada, New Mexico, North Carolina, Oklahoma, South Carolina, Texas, and Virginia) (J Yoder, unpublished data) [2]. We report the first confirmed case of PAM acquired in Minnesota, the northernmost US state in which this infection has been documented, and associated environmental data.

CASE REPORT

On 20 August 2010, a case of rapidly progressive, unexplained encephalitis in a female aged 7 years who was admitted to a local pediatric hospital was reported to the Minnesota Department of Health. Symptoms began 16 August 2010, with headache, abdominal pain, and neck

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Correspondence: Sarah K. Kemble, MD, Minnesota Department of Health, PO Box 64975, Saint Paul, MN 55164 (sarah.kemble@state.mn.us).

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soreness. On 17 August, she experienced fever and was examined at an urgent care clinic. She was administered a dose of intramuscular penicillin for presumed streptococcal pharyngitis. The following morning, she had a witnessed tonic-clonic seizure and was evaluated and admitted to an intensive care unit. Cerebrospinal fluid (CSF) cell count and chemistries were markedly abnormal, with a white blood cell count of 8150 cells/mm^3 (90% neutrophils), red blood cell count of 800 cells/mm^3 , protein level of 461 mg/dL , and glucose level $<20 \text{ mg/dL}$. The presumptive diagnosis was bacterial meningitis, and she was treated with lorazepam, ceftriaxone, and vancomycin. Initial cranial computed tomography (CT) indicated no abnormality. The patient's condition declined rapidly, with increasing seizure frequency, and she required endotracheal intubation and mechanical ventilation. On the morning of 20 August, she had onset of myoclonic jerks accompanied by abrupt flattening of her electroencephalographic waveform and a decrease in blood pressure. Repeat cranial CT demonstrated a subtle hypodensity in the right frontal lobe consistent with focal edema. An intracranial pressure monitor was placed, and hypertonic saline, mannitol, and vasopressors were administered. Despite these attempts, neurologic evaluation and cerebral flow study were consistent with brain death, and a decision was made to withdraw life-sustaining support. Permission for autopsy was granted.

A Wright's stain of a CSF sample from the day of admission showed ameboid forms (Figure 1A). Ameboid forms were also noted on aspirate smears and cytology preps from autopsy brain tissue (Figure 1B–D). Hemorrhagic necrosis was observed in the optic and olfactory nerves, middle cerebral arteries, and right and left cerebellum.

METHODS

Medical records were reviewed and the patient's parents were interviewed for exposure history. Patient samples were collected, including CSF, CSF-inoculated sheep's blood agar plates, and brain tissue slides. Environmental data were recorded and samples were collected 1 and 2 weeks after the patient's illness onset from bodies of water where the patient swam during the 2 weeks preceding illness onset. Sampling locations were chosen at public access sites as near as possible to where the patient swam. Data recorded and specimens collected included (1) water temperature; (2) subjective assessment of water clarity, presence of algal blooms, and surrounding land features; and (3) water and sediment samples. For each collection site and date, three 150-mL samples of surface water and four 100-mL samples of sediment were collected in sterile clear plastic (IDEXX Laboratories) bottles without preservative. The Centers for Disease Control and Prevention's Water, Sanitation, and Hygiene Laboratory Team received the patient's and environmental

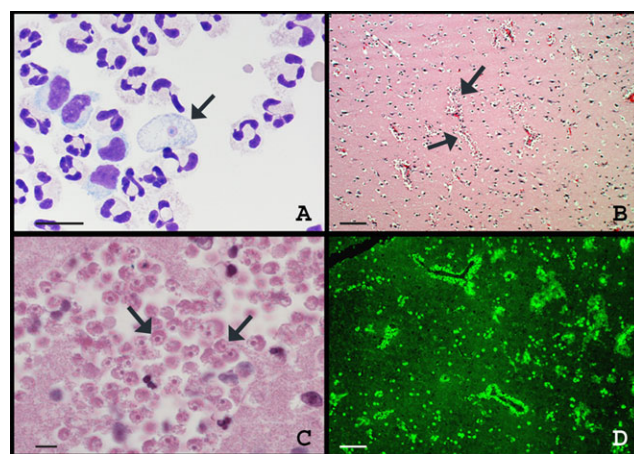


Figure 1. Patient histopathology. In a Wright's stain of cerebrospinal fluid from the patient drawn at the time of hospital admission (A, bar = 100 microns), an ameba is visible in the center of the field, indicated by the arrow, and abundant polymorphonuclear cells are present. Brain tissue stained with hematoxylin and eosin (B, bar = 10 microns and C, bar = 100 microns) demonstrates perivascular amebic infiltrates and an amebic micro-abscess with surrounding tissue necrosis. Examples of amebae are indicated by the arrows. *Naegleria fowleri*-specific immunofluorescent antibody staining (D, bar = 10 microns) highlights amebic infiltrates.

specimens and cultured them for *N. fowleri* via incubation at 44°C . Speciation was confirmed by real-time polymerase chain reaction (PCR) [3]. For the patient samples, real-time PCR was performed on an isolate cultured from the CSF-inoculated sheep's blood agar plates and was also performed directly on the patient's CSF sample. Environmental samples were combined to create composite water and sediment samples from each sample site and date. Composite samples were centrifuged, resuspended, and cultured on nonnutrient agar coated with *Escherichia coli*. Isolates from CSF and water were genotyped by sequencing of the 5.8S ribosomal RNA gene and internal transcribed spacers 1 and 2 (ITS1 and ITS2) [4]. Historic ambient temperature and precipitation data were obtained from a National Weather Service station located within 15 miles of exposure sites, and normal temperatures for the Minneapolis–Saint Paul area were obtained from the National Climatic Data Center.

RESULTS

Epidemiologic Data

During the 2 weeks before illness onset, the patient swam in 2 lakes (lakes A and B) and 1 river in Washington County, Minnesota. Exposures were on 6 and 9 August off a dock on lake A, 12 August at a public beach on lake B, and 15 August off an island in the river accessed by boat. She had no history of travel outside Minnesota. Her parents recalled that during those 2 weeks, the ambient temperatures were high and the water felt

unusually warm. The patient swam for hours each time. She performed handstands under water, got water up her nose, and swallowed or aspirated water multiple times. Her parents and 2 brothers swam at the same locations, but no one else in the family became ill.

Laboratory Data

CSF bacterial culture from sheep's blood agar grew *Burkholderia cepacia* and amebae consistent with *N. fowleri*. *Naegleria fowleri* was confirmed by real-time PCR from CSF and brain tissue and from 1 of 2 composite water and 2 of 2 composite sediment samples collected on both collection days from lake A. The *N. fowleri* from CSF, brain tissue, and water samples was characterized as genotype 3. *Naegleria fowleri* was not isolated from specimens obtained at the other 2 swimming sites.

Environmental Data

Lake A's surface area is 0.21 km², and maximum depth is 17.4 m. Surface water temperature at the times of sample collection was 22.1°C on 24 August and 24.5°C on 31 August. Algal blooms were noted, and water clarity was poor, with organic matter and suspended sediment impeding visibility (Figure 2). Storm water drainage pipes fed into the lake within 30 m of the main swimming area. Other nearby features included a hospital, playing field, recreation center, and residential properties. At the other 2 swimming sites, surface water temperatures on the 2 collection days were 23.0°C and 25.5°C for lake B and 22.3°C and 24.9°C for the river. Water clarity was markedly better at these sites. Algal blooms were noted at the river but not at lake B. Lake B is located in a sparsely populated residential area. The river site is next to a small town. Minimal land development exists adjacent to these sites.

Climatologic Data

During the week preceding the patient's first swimming exposure at lake A, the mean maximum daily ambient temperature

near the exposure sites was 28°C, and the mean minimum daily temperature was 18°C. The highest maximum daily temperature during August was 32°C, which occurred on 3 August, 3 days before the patient's first exposure at lake A, and again on 9 August, the day of her second swimming exposure at this lake (Figure 3). Daily maximum and minimum temperatures exceeded normal temperatures on the majority of days during the 2 weeks before illness onset. Total precipitation exceeded 3 cm on 8 August, the day before the patient's second swimming exposure at lake A. The mean temperature during August 2010 in this area was 25°C, or 3.6°C above normal, and precipitation was 9.7 cm, or 1.8 cm above normal.

DISCUSSION

This case represents the northernmost documented occurrence of PAM in North America, approximately 550 miles north of the previous northernmost reported case in Missouri. Although the ameba is believed to be ubiquitous in water and soil in warmer climates [2], its distribution in cooler northern areas is not well understood. Therefore, it is rarely if ever considered as a possible cause of meningoencephalitis among patients whose exposures are limited to the northern United States.

Among confirmed cases of PAM, mortality exceeds 99% [5]. Whether an effective treatment exists remains unclear. In a recent review of 111 US cases during 1962–2008, only 1 patient, who was treated with intravenous and intrathecal amphotericin B, intravenous and intrathecal miconazole, and oral rifampin, survived [2, 6]. Although in vitro studies demonstrate possible efficacy of azithromycin and voriconazole and in vivo efficacy of miltefosine and chlorpromazine against experimental *N. fowleri* infection has been demonstrated in mice [7], no studies have been published regarding efficacy of treatment for human infection [1]. Although no proven treatment is known, early initiation of therapy in consultation with an infectious disease specialist should be attempted when infection is suspected.

Developing public health messages is challenging. Persons are often exposed to *N. fowleri* in warm freshwater, but infection is extremely rare [2, 8]. Whether specific host factors (eg, recent facial trauma or an as yet unidentified intrinsic host susceptibility) increase a person's risk for infection is unknown. The amebae are commonly found in the environment, the number and distribution of amebae in the water can vary from day to day with environmental conditions [9], and the relationship between amebic concentrations and risk of human infection has yet to be defined, making it difficult to predict risk at a given location and time even if routine sampling and testing should be performed [5]. Nevertheless, some risk factors previously noted among reported cases [5] include diving, exposure to lake-bottom sediment, and prolonged time in the water. All of these exposures were present in this instance. General guidance can be



Figure 2. Surface of lake A at the time of sample collection. Of note, abundant organic matter and algal blooms were present (inset).

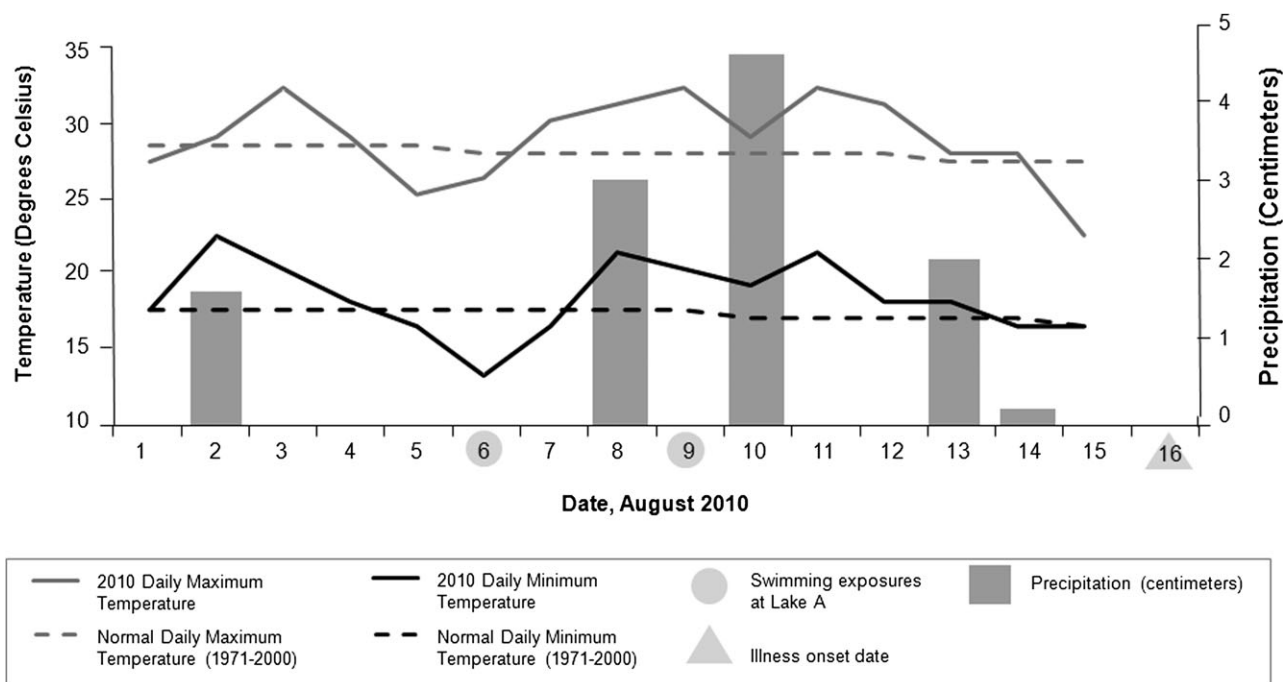


Figure 3. Ambient temperature and precipitation near case exposure sites, Minnesota, 1–15 August 2010. Reference daily normal temperatures from the Minneapolis–Saint Paul International Airport are provided for comparison. Normal temperature definitions are updated each decade by the National Climatic Data Center of the National Oceanic and Atmospheric Administration based on average temperatures over the previous 30-year period. The normal temperatures represented in this chart are based on the most currently available data collected during 1971–2000.

provided to the public regarding avoidance of submersion in warm freshwater bodies at times of high temperatures and low water volume, use of nose clips, and avoidance of stirring up sediment during water-related activities [5]. However, evidence for the effectiveness of these recommendations is lacking.

Naegleria fowleri is known to tolerate temperatures $\leq 45^{\circ}\text{C}$ and proliferate when ambient temperatures increase to $>30^{\circ}\text{C}$ [1]. The climatologic data reviewed show that ambient temperatures near the swimming exposure sites reached and persisted above thresholds favoring growth of the amoeba during summer 2010. Precipitation also might have played a role. In an observational study of amoebic densities in a South Carolina lake, precipitation was associated with increased amoebic counts for 1–2 days [9]. Isolation of *N. fowleri* from a lake in which the patient swam confirms the viability of the organism in Minnesota's environment.

Minnesota's temperature trends reveal an overall shift toward warmer climate for the state, with an increased rate of warming during the past 3 decades, compared with the rest of the 20th century [10]. The biggest difference is in minimum temperatures. The average monthly minimum temperature during June–August 2010 in the Minneapolis–Saint Paul area was a record high at 18.6°C . The previous record in 1939 was 18.4°C , and the 30-year average is 15.8°C [11]. Warmer winters are also a prominent part

of this trend and might lead to increased peak summer water temperatures caused by earlier thawing of winter ice cover [12], facilitating proliferation of *N. fowleri*. However, further study is required to elucidate determinants of amoebic growth in a temperate northern climate.

Although exceeding certain thresholds for ambient and water temperatures likely facilitates survival and proliferation of *N. fowleri*, this is only 1 risk factor contributing to the occurrence of PAM. On the basis of our environmental sampling, temperature alone did not predict isolation of *N. fowleri*, because the water temperature in lake A was the coolest of the 3 exposure sites. Distinguishing features of lake A included visible suspended organic matter and sediment and storm water drainage entering the lake. Certain indicators of poor water quality (eg, higher bacterial densities and increased phosphorous concentration) have been implicated in other studies as providing a favorable environment for amoebic growth [13–15], and might have played a role in proliferation of *N. fowleri* in lake A. Observations from this and other case studies suggest that the specific risk factors for the proliferation of *N. fowleri* are yet to be fully defined. Reliable methods for detection and quantification of amoebae from water sources and a larger database of environmental isolates will be necessary to better characterize these risk factors.

Burkholderia cepacia, a gram-negative bacterium typically located in water or soil, might have been introduced into the patient's CSF with *N. fowleri*. *Naegleria fowleri* has been reported to support growth of intracellular bacteria (eg, *Legionella pneumophila*) in laboratory settings, but no previous reports of clinical or environmental isolates of *N. fowleri* amoebae harboring endocytobionts exist [1]. Presence of intra-amebic bacteria at the time of infection could potentially alter the pathogenicity of the amoeba or the host immune response.

In conclusion, in the context of climate trends resulting in Minnesota's consistently warmer temperatures during the past 3 decades, this organism can no longer be assumed to be an exposure risk only in southern-tier states. Risk factors associated with this patient were consistent with those previously reported in the literature. However, patient-related risk factors are not easily modifiable because of the infrequent occurrence of the disease even in the setting of the most high-risk freshwater exposures. Elucidation of environmental factors related to proliferation and persistence of *N. fowleri* will require further targeted study. Clinicians should consider *N. fowleri* as a possible cause of meningitis or encephalitis throughout all regions of the United States among persons with recent warm freshwater exposures.

Notes

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