disease prevention news

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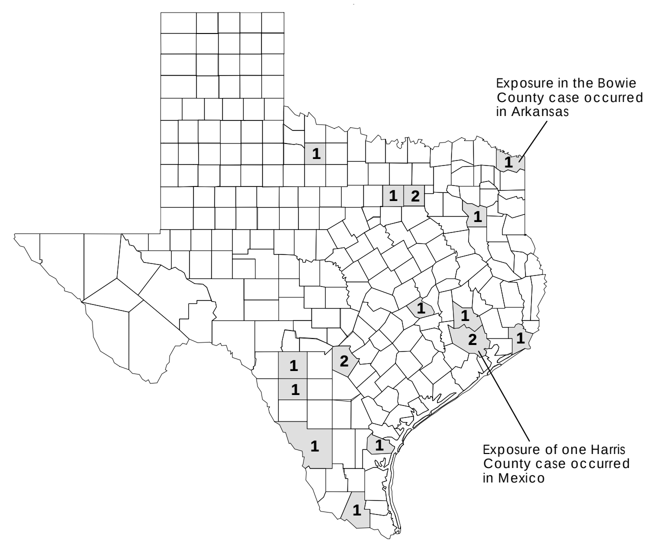
Primary Amebic Meningoencephalitis

Primary amebic meningoencephalitis (PAM) is a fulminant, purulent infection of the grey matter of the brain. Though rare, it is almost always fatal. The causative organism, *Naegleria fowleri,* is a ubiquitous, free-living ameba that thrives in warm, fresh water, particularly if it is stagnant or slow moving.

The pathogenic potential of the organism in the human host was first described in 1965; the first reported case of PAM in Texas occurred in 1972. From 1972 through 1994, 18 cases were reported from 15 counties (Figure 1). All of the patients died.

Figure 1. Counties Reporting Primary Amebic Meningoencephalitis, 1972-1994



Most PAM patients have a history of exposure to open, fresh water within a few days prior to onset of symptoms. Improperly maintained swimming pools also have been a source of infection. Another hot Texas summer is well underway, and with it the increased risk of contracting this disease. Figure 2 shows the seasonal distribution of Texas cases, 1972 through 1994.

In July of last year, a 13-year-old Hispanic boy from Laredo died shortly after he was hospitalized with PAM. The case report, which includes summary information regarding the disease and its causative organism, was published in the September 5, 1994, issue of *Disease Prevention News.*

This report provides a review of the laboratory procedures used to confirm the PAM diagnosis and establish the most probable source of infection for the Laredo case. Basic prevention recommendations also are described, including guidelines for proper swimming pool maintenance.

Laboratory Confirmation

The following anecdotal account provides an in-depth description of the standard procedures used in the Medical Parasitology Section of the Texas Department of Health (TDH) Bureau of Laboratories to confirm the presence of free-living amebae in human and environmental samples.

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Hedgehog Alert
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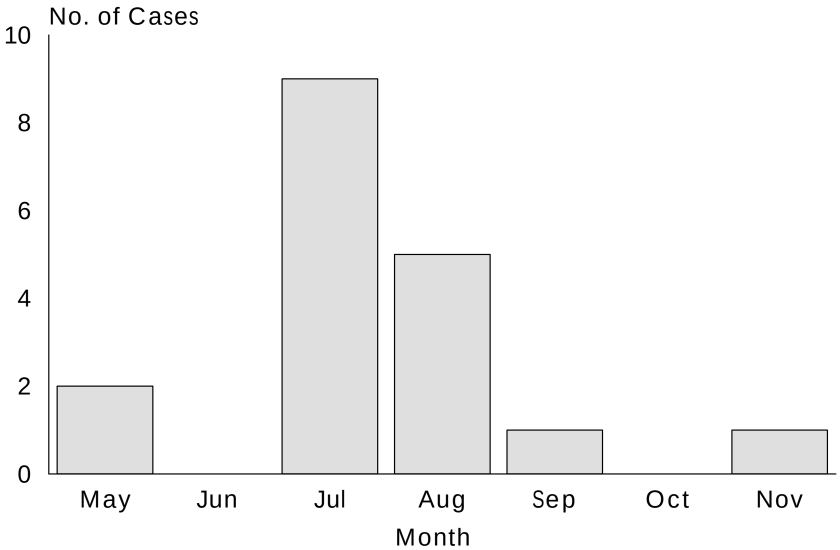
On July 26, 1994, laboratory staff of a hospital in San Antonio, Texas, contacted the TDH Medical Parasitology Section regarding a possible case of primary amebic meningoencephalitis (PAM). Since hospital laboratory reports contained documentation of amebae observed in the patient's cerebrospinal fluid (CSF), arrangements were made to forward the remaining CSF to Austin for confirmation.

Figure 2. Month of Onset of Primary Amebic Meningoencephalitis in Texas, 1972-1994



Note: The case with the Nov onset was imported from Mexico

This CSF arrived at TDH on July 28, 1994, and was allowed to settle for approximately one hour before examination. Drops of CSF were placed onto glass slides, coverslipped, and examined microscopically for the presence of amebae. A large number of organisms were seen in these preparations and also in those permanently stained to allow more detailed examination (Figures 3 & 4).

Procedures for isolating normally free-living, yet opportunistic organisms such as *Naegleria fowleri* differ from those for isolating obligate parasites such as *Entameba histolytica,* which also can cause systemic infections of the brain. Therefore, once the presence of amebae are confirmed in a CSF specimen, they must be speciated.

Naegleria fowleri and *Entameba histolytica* exhibit practically identical motility patterns. Both have blunt, lobular pseudopodia; and both exhibit directional motility (a tendency to remain mobile while extending and retracting pseudopodia). What differentiates one species from the other is the presence of contractile vacuoles. These vacuoles, which are easily seen with the microscope, serve as an excretory system for free-living opportunists such as *Naegleria.* Identification of *Naegleria* is confirmed when vacuoles are seen to close, expelling their contents, and then reopen. Obligate parasitic amebae such as *Entameba* do not have contractile vacuoles. Because the organisms seen in the CSF exhibited these vacuoles, procedures for isolating free-living amebae were initiated.

Drops of CSF sediment were inoculated onto petri dishes containing non-nutrient agar that had been seeded with a suspension of *Escherichia coli.* In nature as well as in the laboratory, this bacterium is one of many on which amebae will feed. Four duplicate cultures of the CSF specimen were placed into a moisture chamber and incubated at 35°C. After 24 hours, the inoculated surfaces of the agar plates were examined for the presence of

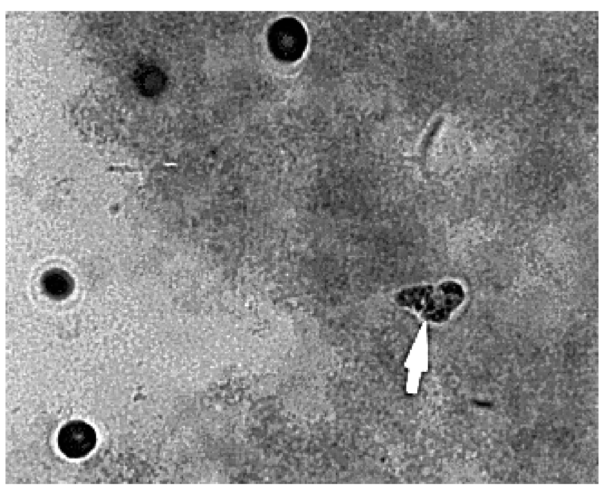


Figure 3. *Naegleria fowleri* in CSF; trophozoite with blunt, lobular pseudopodia (1000x)

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amebae. All four plates were positive, so a presumptive positive report for *Naegleria fowleri* was telephoned to the hospital.

To confirm the identification of *Naegleria*, further studies must demonstrate a stage transformation unique to this organism. *Naegleria* incorporates food more easily in its ameboid stage, yet moves toward a food source more rapidly in its flagellated stage. Confirmation studies involve harvesting amebae from the agar plates and inoculating them into a large volume of sterile-filtered water. When placed into clean water, away from a ready source of food, *Naegleria* transforms from an ameba into a biflagellated ameboflagellate. Confirmation studies done in this case were positive for *Naegleria fowleri* after forty-eight hours incubation; this result was reported to the hospital.

While the TDH confirmation studies were progressing, City of Laredo Health Department staff consulted with TDH Medical Parasitology Section staff to determine possible sources of infection. Two locations identified as most likely were the Rio Grande River and a nearby retention pond. A third possible source, a swimming pool in Laredo, also was recommended for testing.

Six one-quart cubitainers of water were collected from the Rio Grande River at the Rio Bravo pump and six from the Rio Bravo retention pond. Six one-quart cubitainers of water also were collected from the swimming pool: two each from the deep end, the middle, and the shallow end. Cubitainers were filled from near the bottom of the pool at each location. The Medical Parasitology Section received these samples August 4, 1994.

Examination of environmental samples (water) for free-living amebae follows procedures similar to those of CSF examination. After the samples are allowed to settle for one hour, the sediment is collected for examination. Direct wet smear examinations are performed prior to culturing onto agar plates. Positive cultures are harvested, and transformation studies initiated.

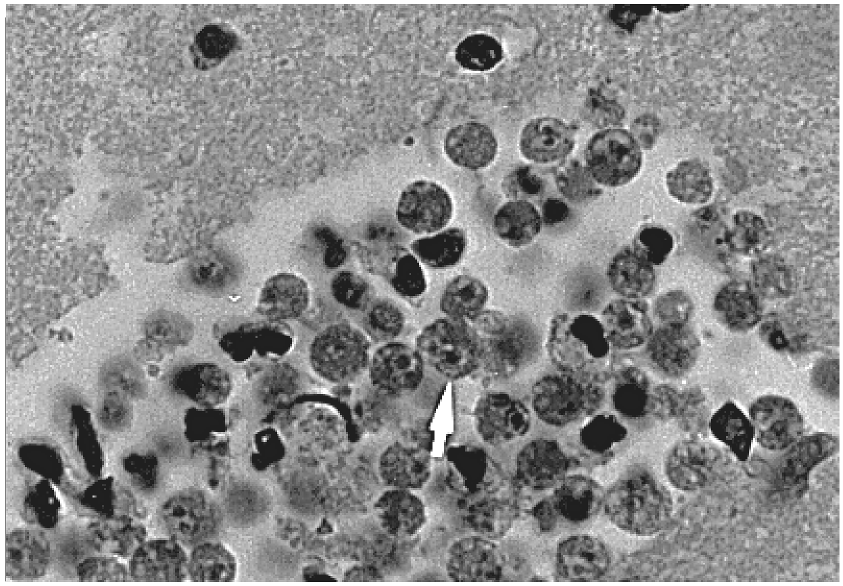

Figure 4. Numerous *Naegleria fowleri* in brain tissue (1000x)

At this point, the testing procedures for environmental samples and those for CSF differ. A presumptive identification of *Naegleria* can be made when amebae exhibiting characteristic motility, morphology, and contractile vacuoles are found in CSF. For amebae found in environmental samples, however, these same characteristics do not allow even a presumptive identification. Many different species of free-living amebae found in nature exhibit all the above characteristics, yet do not infect humans. For this reason, identification of *Naegleria* in an environmental sample requires that transformation to the biflagellate stage be observed.

Sediments of the eighteen water samples were examined microscopically. The six samples from the Rio Grande River and the six from the retention pond were all teeming with aquatic organisms, including amebae consistent in morphology with *Naegleria*. Also present were amebae morphologically consistent with *Acanthameba* spp., another free-living

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amoeba that can infect humans. The six samples from the swimming pool contained no visible organisms.

All sediments, including those from the swimming pool, were centrifuged at 800 revolutions per minute for ten minutes and the supernatant decanted. Several drops of the remaining sediment were placed onto agar plates as previously described. Each sediment was inoculated in duplicate for a total of thirty-six cultures. These cultures were examined at twenty-four-hour intervals for seven days or until adequate numbers of amebae were present to harvest for further study.

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After twenty-four hours, all twenty-four cultures of samples from the Rio Grande and the retention pond contained numerous and varied amebae. Many organisms could be identified as *Acanthameba* spp. on the basis of morphology and motility. Because many others were likely to be *Naegleria*, transformation studies were initiated. Cultures from the swimming pool water samples remained negative throughout testing.

Transformation can take place in a time period as short as two to three hours or as long as three to four days. Due to the abundant amount of food available, the transformation process for the samples from the Rio Grande and the retention pond took much longer. All transformation studies eventually were positive for *Naegleria*. Reports of these results were telephoned to the City of Laredo Health Department on August 10, 1994.

. . . swimming pool water samples remained negative throughout testing.

The same day, the Medical Parasitology Section received five cubitainers of treated water from the Rio Bravo treatment station, which is fed by the retention pond already shown to contain numerous free-living amebae. The concern was whether organisms were surviving the treatment process and finding their way into the city's distribution system.

Samples were processed and examined as previously described. All remained negative throughout testing; this result was reported on August 17, 1994.

On August 25, 1994, the City of Laredo submitted four water samples collected from Lake Casa Blanca, a lake used extensively for recreation. These samples were processed and examined as previously described; they were reported as positive for *Naegleria* and *Acanthameba* on August 31, 1994. Although positive, these samples were not as heavily populated with amebae as were the other samples.

It is important to note that finding these species of amebae in fresh water lakes, ponds or rivers is to be expected. In the TDH laboratories, extensive testing over many years has confirmed that these organisms are ubiquitous. In fact, impoundments that do not test positive are considered an exception to the rule. One observer has noted, "They're more common than roaches -- just smaller so you can't see them; and fresh water is where they live."

Addendum:

During the TDH Laboratory investigation of the Laredo case, El Paso city officials became concerned about the section of Rio Grande River in their area. Testing of water from this area was completed August 30, 1994. Not surprisingly, both *Naegleria* and *Acanthameba* were found in these samples.

Acknowledgment:

Isolation and identification of both *Naegleria* and *Acanthameba* are labor-intensive tasks requiring many hours of hazardous, tedious manipulation and examination of samples. Medical Parasitology Section staff put in long hours to complete this work accurately and quickly. The efforts of Katherine von Alt and Nivea Ortiz are especially noteworthy.

Prepared by: Dale Dingley, MPH, M(ASCP), Chief, Medical Parasitology Section.

Prevention

While the likelihood of exposure to *Naegleria*, which is ubiquitous in nature, is high, the risk of infection is low. The overall probability is less than one in 100 million that a person exposed to water inhabited by *Naegleria* will become infected.

However, it is advisable to reduce the risk of infection as much as possible since the disease caused by *Naegleria*, primary amebic meningoencephalitis (PAM), is almost always fatal. While it is not practical to monitor all swimming areas for the presence of this organism, the following preventive actions are relatively effective and easy to do:

- Never swim in stagnant or polluted water.
- Hold your nose or use plugs when jumping into water.
- Swim in properly maintained pools whenever possible.
- Shower before using swimming pools.

Swimming Pool and Spa Maintenance

Natural bodies of water, especially those that are stagnant or polluted, are the most likely source of infection. However, swimming pool water was the only identified source of exposure for at least one of the 18 people who died of PAM in Texas.

Unfortunately, proper swimming pool and spa maintenance may not ensure that all *Naegleria* will be killed or removed. A study by Anderson and Jamieson in 1972 showed that even superchlorination to 10 parts per million failed to eradicate *Naegleria* from a swimming pool in Australia.

Nevertheless, proper swimming pool and spa maintenance creates an environment that is unfavorable to the growth of *Naegleria*. Removal of its primary food source, gram-negative intestinal bacilli such as *Enterobacteriaceae*, is a key factor in controlling *Naegleria* populations. Since proper pool maintenance helps control many species of algae and bacteria, including *Enterobacteriaceae*, it aids in the control of *Naegleria* as well.

Chlorination. State law requires a continuous free chlorine residual of at least 1ppm for swimming pools and at least 2ppm for spas. For control of both *Naegleria* and its food sources, up to 3ppm is recommended for swimming pools and 5ppm for spas. The higher concentrations are advisable during periods of heavy use.

Superchlorination is the term for adding an extra large dose (usually 8 to 10ppm) of chlorine to the water. Superchlorinated swimming pool water is inhospitable to the trophozoite stage of *N. fowleri*. In general, weekly superchlorinating (or "shocking") is advisable, not only to increase effectiveness against pathogens, but also to reduce the eye irritation and foul odor caused by chloramines.

Natural bodies of water, especially those that are stagnant or polluted, are the most likely source of infection.

Water Filtration. A water filtration system must be of the proper design and capacity to effectively reduce the number of bacteria and other contaminants in pool water. The more popular filtration systems for swimming pools, designed primarily for the removal of large particulates such as debris, are not adequate for filtering out bacteria or amebae. The size of *Naegleria* averages 15 microns in its vegetative or trophozoite state, and 10 microns in its encysted form. Since the bacteria upon which it feeds average 1 micron or less, they will not be removed by the most commonly used filtration systems, even when they are operating optimally.

State law requires a continuous free chlorine residual of at least 1ppm for swimming pools. . .

Though they are the least efficient, **sand filters** are probably the most widely used due to the relatively low expense and low maintenance required. Sand filter efficiency is directly proportional to

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the age of the sand, with straining capacity ranging from about 25 microns for new sand to 60 microns for sand that is a year old.

Cartridge, or **pleated-paper,** filters also are popular. The filtering capability of this type of filter ranges from 5 to 20 microns. Unlike sand, these filters can become more efficient with age as the pores get clogged and thus smaller.

Although capable of removing *Naegleria* and its food sources, **diatomaceous earth (DE)** filters are not as popular due to the laborious effort required to back-flush and clean this type of system. DE filters operate at peak effeciency when clean.

All filtration equipment must be inspected and maintained regularly to ensure optimum functioning.

pH Level. The acceptable pH range for swimming pool water is 7.2 to 7.8, with the optimal level for control of organisms at the lower end. Irritation of skin and eyes of pool users is common at pH levels below 7.2, and the antimicrobial effect of chlorine is diminished at pH levels above 7.6.

Water Temperature. *Naegleria* is a thermophilic organism. Trophozoites thrive at water temperatures above 100°F, but do not multiply at temperatures below 40°F. Pool water that is maintained at temperatures below 80°F is suboptimal for the growth of *Naegleria* as well as algae. Since most pools and spas are kept at temperatures above 80°F, adequate chemical treatment and filtration is especially important.

Pool Area. The area surrounding the pool should be constructed and tended so as to minimize the amount of dirt entering the water. Overhanging structures and vegetation that allow dust, plant debris, and other contaminants to be blown or washed into pool water should be removed. Cracks and other defects in the pool floor, walls, or deck favor the growth of potentially harmful organisms and should be repaired.

For further information on proper maintenance of swimming pools and spas, please call the general sanitation office of your local or regional health department. (See DPN, Vol. 55, No. 8, for TDH Regional Office telephone numbers.) If these staff are unavailable, call the TDH General Sanitation Division central office in Austin at (512) 834-6635.

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Williams K. Aquatic Facility Operator Manual. Illinois: National Recreation and Park Association, 1991[4].

The following footnotes provide current contact information for those who wish to obtain selected materials.

1. Mosby Yearbook, Inc., 11830 Westline Industrial Dr., St. Louis, MO 63146, (800) 426-4545

2. National Swimming Pool Foundation, 10803 Gulfdale, Ste. 300, San Antonio, TX 78216, (210) 525-1227

3. TDH Audiovisual Library, 1100 W. 49th St., Austin, TX 78756, (512) 458-7260

4. National Recreation and Parks Association, 650 W. Higgins Rd., Hoffman Estates, IL 60195, (708) 843-7529x5

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Hedgehog Alert

Last March the Texas Department of Health reported the third Texas case of human infection with *Salmonella* serotype Tilene. A 14-month-old boy who resided in Colorado County became ill with fever, diarrhea, vomiting, and abdominal cramps. The Microbiological Investigation Section of the TDH Laboratory identified the causative organism as *Salmonella* Tilene. The boy's family owns one African pygmy hedgehog, which they bought from a local pet store last February. Before the toddler became ill, he occasionally handled the hedgehog. Neither the older sister, who cleans the cage, nor the parents became ill.

One of the other two Texas cases involved an eight-year-old girl from Amarillo, whose illness was diagnosed in October 1994. In March of this year, the TDH Laboratory also confirmed *Salmonella* Tilene infection in a four-year-old boy from Houston. Patient histories TDH received regarding these two cases did not include information about contact with hedgehogs.

The June 25, 1995, *Morbidity and Mortality Weekly Report*, published by the Centers for Disease Control and Prevention, contains a report on a 10-month-old girl from Washington state whose April 1994 diarrheal illness was caused by *Salmonella* Tilene infection. The family owned a dog and a breeding herd of 80 apparently healthy African pygmy hedgehogs; a stool sample from one of three hedgehogs yielded *Salmonella* Tilene. The infant's illness resolved after treatment of an upper respiratory infection with trimethoprim-sulfamethoxazole.

Salmonella spp. are found worldwide in domestic and wild animals, particularly mammals, reptiles, and birds. Although ingestion of contaminated food is the most important source of salmonellosis in humans, pets are another potential source of infection. Reported carraige rates are highest in reptiles and lowest in dogs and cats. *Salmonella* Tilene is an uncommon cause of human illness, first isolated in 1960 from a child in Senegal.

Addendum:
The Texas family has decided to keep their hedgehog, named Sonic, but be more prudent about washing their hands after handling the pet.

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USDA/FDA Foodborne Illness Database

The USDA/FDA Foodborne Illness Education Information Center has compiled a database of consumer and food worker educational materials developed by universities; private industry; and local, state, and federal agencies. The center was established as part of a national campaign to reduce the risk of foodborne illness and to increase knowledge of food-related risks from production through consumption.

These materials are available electronically through several sources, including the Internet. For more inofrmation, contact Cindy Roberts, Information Specialist, at:

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