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Occurrence and Pathogenicity of *Naegleria fowleri* in Artificially Heated Waters

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The occurrence of pathogenic *Naegleria fowleri* in thermal discharges, recipient waters, and cooling towers of eight power plants located in western Pennsylvania was investigated for 2 years in conjunction with several environmental measurements. Pathogenic *N. fowleri* was detected in one cooling tower and in the discharge, receiving waters, or both of five of eight localities. The occurrence of this organism was related to elevated temperatures, but no significant correlation was found for other biological and chemical parameters. Laboratory experiments on the effect of pH on pathogenic *N. fowleri* documented 100% survival at a range from 2.1 to 8.15. Higher pH reduced or killed the amoebae. No case of human primary amoebic meningoencephalitis occurred during the study.

Naegleria fowleri is a ubiquitous, free-living amoeba found in diverse freshwater environments such as lakes, ponds, and rivers. Pathogenic strains of this species are causative agents of primary amoebic meningoencephalitis, a fulminant and usually fatal illness affecting the central nervous system. Most cases occur during the summer within 1 week after swimming in fresh or brackish warm waters. Since 1965, when this disease was first described in Australia (7), a total of 49 cases have been reported from the United States as of December 1980 (8).

Pathogenic *N. fowleri* is a thermophilic amoeba, relatively common in warm lakes. This organism has been isolated from lakes and rivers in Florida and from man-made reservoirs in Texas (10). Wellings et al. (12) have reported that over 50% of freshwater lakes in Florida yielded pathogenic *N. fowleri* when the temperature was 30°C or greater.

A new species, *N. australiensis*, pathogenic to mice has been described and characterized by DeJonckheere (4, 5). However, this amoeba does not grow at 45°C, and its pathogenicity to mice is lower than that of *N. fowleri*.

In the temperate climate the heated effluents from power plants and other industrial facilities discharging cooling waters seem to provide a suitable environment for the maintenance and reproduction of this organism. In 1974, DeJonckheere and co-workers (3) isolated pathogenic *N. fowleri* from a canal where a boy contracted primary amoebic meningoencephalitis in 1973. This canal received warm effluent from a lead and zinc factory in Belgium. This finding and additional information on the occurrence of *N. fowleri* in Virginia, Florida, Texas, and other parts of the United States and the concerns of the Pennsylvania Electric Power Company were instrumental in bringing about this study (6, 9–12).

During a 2-year period (1980 and 1981), the occurrence of pathogenic *N. fowleri* in thermal effluents, receiving waters, and cooling towers of eight power plants located in western Pennsylvania was investigated. A portion of the results obtained by the authors in 1980 was published in a preliminary study (9).

MATERIALS AND METHODS

Sampling. Water and sediment samples from eight coal-fired electrical power plants located in western Pennsylvania were collected in April, May, September, October, and November, 1980, and in April through October, 1981. The research was interrupted in summer, 1980, by a strike. Water and sediment samples were collected from discharges, receiving waters (recipients), and cooling towers (if available). On one occasion (July, 1981) samples were collected in the Presque Isle Bay area adjacent to the discharge from the Erie plant.

Cultivation of *Naegleria* isolates. A 250-ml amount of each water sample was filtered through a 1.2-μm Millipore membrane filter (Millipore Corp., Bedford, Mass.). The exposed filter was cut into halves and placed in an inverted position on nonnutrient agar seeded with *Enterobacter aerogenes* (NNAEa) (2). Bottom sediments including algae, mud, and debris were placed directly onto the middle of the same medium. The inoculated plates were sealed, inverted, and incubated at 45°C for up to 8 days. When growth was observed, the amoeba was transferred immediately onto NNAEa and again incubated for up to 8 days at 45°C to purify the isolate and partially prevent growth

TABLE 1. Chemical analyses of water samples (average values, 1981)

Water sample taken at:[a]	Temp (°C)			pH			Hardness (mg of $CaCO_3$/liter)			No. of samples
	Range	Avg	SD	Range	Avg	SD	Range	Avg	SD	
Conemaugh[b]										
P	21–27	23.6	±2.9	5.8–7.7	6.9	±0.7	260–960	698	±291	5
CT	11–30	24.8	±6.4	6.1–8.9	7.5	±0.9	260–840	501	±192	7
R	13–21.5	17.3	±6.0	3.9–7.0	5.5	±2.3	240–300	270	± 42	2
Erie										
D	18–35	25.6	±6.4	7.0–7.9	7.5	±0.3	102–160	129	± 23	7
R_1	15.5–33	24.1	±6.2	6.9–7.9	7.6	±0.3	94–172	122	± 27	7
R_2	18.5–34	27.4	±6.5	7.6–8.3	7.9	±0.3	102–126	118	± 11	4
Homer City										
D	30–47	40.7	±6.5	6.3–8.3	7.5	±0.8	254–724	454	±148	7
CT	28.5–34.5	30.4	±2.1	6.4–8.3	7.4	±0.8	324–840	490	±182	7
R	16–30	23.3	±4.4	4.8–7.7	6.0	±1.1	324–840	223	± 76	7
Keystone										
D	25–36.5	27.8	±4.4	5.7–8.8	7.0	±1.0	200–420	320	± 71	7
CT	13–33	27.2	±6.9	6.3–8.0	7.4	±0.7	200–612	375	±133	7
R	13.5–25	19.9	±4.5	5.5–7.0	6.7	±0.5	100–260	173	± 53	7
Seward[b]										
D	17–33	24.4	±5.1	3.8–8.4	5.7	±1.9	152–320	221	± 53	7
R	14.5–32.5	23.3	±5.4	3.6–8.4	5.6	±1.6	140–320	230	± 63	7
Shawville[b]										
D	25–38	31.5	±5.0	5.7–7.0	6.3	±0.5	122–200	166	± 25	7
R_1	22–37	30	±5.6	5.6–6.7	6.2	±0.5	114–250	163	± 44	7
R_2	26.5–36	30.2	±1.7	6.2–7.1	6.5	±0.5	112–174	152	± 35	3
Warren										
D	18–35	26.7	±6.3	6.1–8.1	7.2	±0.5	50–720	162	±246	7
R	15–35	25.3	±7.0	6.4–7.9	7.2	±0.5	60–720	157	±248	7
Williamsburg										
D	17–29	23.9	±4.5	6.9–8.1	7.5	±0.4	120–188	154	± 25	7
R	16.5–29	23.8	±5.4	6.3–8.7	7.5	±0.8	100–240	167	± 50	7

[a] Abbreviations: D, discharge; CT, cooling tower; R, recipient, P, pond.
[b] Stations affected by acid mine drainage.

of other species of amoebae.

Flagellation test. Usually after 3 to 4 days of incubation, a small piece of agar with pure amoebic culture was transferred onto a new NNAEa plate and incubated for 2 to 3 days at 37°C. A piece of agar with the amoebae from this culture was placed in a tube of sterile distilled water and incubated at 37°C. Each tube was examined hourly for 4 consecutive h with an inverted microscope at a magnification of 100×. Positive amoeboflagellate-forming strains were transferred onto a new NNAEa plate and incubated for 3 days at 37°C. Amoebae from these plates were used for mice pathogenicity tests.

Mouse pathogenicity test. A suspension of the amoebae, scraped from the plate by a sterile spreader, was prepared in sterile distilled water. The trophozoites were counted with a hemacytometer. Five weanling, white (female) Swiss Webster mice were anesthetized, and 30 μl of the amoebic suspension (7,000 to 40,000 organisms per inoculum) was instilled into their nostrils. Five control mice were instilled with sterile distilled water. The mice were fed Purina Rodent Lab Chow, provided with water ad libitum, and kept up to 16 days. Mice with severe neurological symptoms or those which died were aseptically dissected. Small sections of brain were placed on NNAEa and incubated at 40°C to reisolate the pathogenic *Naegleria* strains. The remainder of the brain was preserved in buffered formaldehyde, stained, and histopathologically examined. Reisolation of amoebae from the central nervous system tissue of mice and histopathological confirmation were considered to be the final evidence of the presence of pathogenic *Naegleria* strains in the tested water or sediment sample.

Physical, chemical, and bacteriological analysis of the water samples. The temperature, pH, conductivity, hardness, total coliforms, and standard plate count bacteria incubated for 2 and 8 days at 35°C were measured in all water samples according to the methods described in *Standard Methods for the Examination of Water and Wastewater* (1). The data on temperature and coliform bacteria were correlated with

TABLE 2. Total coliform counts at localities positive for pathogenic *Naegleria* strains

Locality	Date of isolation of pathogenic *N. fowleri*	No. of pathogenic *Naegleria* isolates		Total coliforms (CFU/100 ml)[b]	
		Water	Sediment	$\bar{x}$	K
Erie R_1	June 1981	1	1	3,419	600
Erie R_1	July 1981	1	0	3,419	5,000
Erie R_1[c]	September 1980	1	0	3,540	6,000
Warren R	July 1981	0	1	2,756	900
Erie D	July 1981	1	0	2,103	800
Williamsburg D[c]	September 1980	1	0	1,048	2,800
Williamsburg[c] Spray	October 1980	1	0	ND[d]	1,800
Erie R_2	July 1981	0	1	944	1,100
Conemaugh CT	August 1981	1	0	353	800
Shawville D	August 1981	1	0	326	600

[a] Abbreviations: R, recipient; D, discharge, CT, cooling tower.
[b] $\bar{x}$, Geometric mean, all examples; K, count on the day when pathogenic *N. fowleri* was isolated.
[c] Reported by Shapiro et al. (9).
[d] ND, Not done.

the number of isolates of pathogenic *Naegleria* strains. Statistical significance was determined by the Student *t* test criterion.

Survival at various pHs. Cysts of pathogenic *N. fowleri* were examined for survival in buffered (0.2 N) waters at pHs ranging from 2.1 to 10.0 after 2 and 24 h at 37°C. A 5-ml amount of buffer was inoculated with 75,000 cysts per ml in the 2-h experiment and with 104,000 cysts per ml in the 24-h experiment. One drop (about 40 μl) of the inoculated buffer solution was placed on each of three NNAEa plates, and the plates were incubated up to 8 days at 37°C and examined for growth.

RESULTS

Field survey. The chemical analyses and temperature measurements collected in 1981 are summarized in Table 1. The pH and hardness were often variable, primarily from acid mine drainage at Seward, Shawville, Conemaugh, and Keystone.

Standard plate count bacteria were highly variable and did not follow any definite distribution pattern. Total coliform counts were usually lower at the stations with lower pH. Geometric averages of total coliform counts were found to range from 326 to 3,540 CFU/100 ml at stations from which pathogenic *Naegleria* strains were isolated (Table 2). The stations showing no pathogenic *N. fowleri* had average total coliform concentrations from 21 to 5,934 CFU/100 ml, indicating that pathogenic *Naegleria* may occur more frequently in water contaminated by elevated concentrations of total coliforms. However, this difference was not statistically significant ($P < 0.3$).

The isolation and identification of all *Naegleria* strains (pathogenic and nonpathogenic) was based on thermal separation at 45°C and formation of amoeboflagellates. A large fraction of samples showed positive growth at 45°C, indicating the presence of thermophilic amoebae. In 1980, a higher percentage of isolates with positive growth at 45°C, except for the Erie station, was obtained from water rather than from sediment samples. The isolates from the Erie and Seward power plants had higher percentages of *Naegleria* strains (samples with positive flagellation) from sediment samples than from water samples. The frequency of *Naegleria* isolates occurred in decreasing order at Keystone, Homer City, Erie, Williamsburg, Seward, Conemaugh, Warren, and Shawville (9). In 1980, pathogenic *N. fowleri* was isolated in September in sediment samples collected at the discharge from the Erie power plant. In addition, pathogenic *Naegleria* isolates were present in water from the Williamsburg station collected in September from the recipient stream affected by the heated effluent. The third pathogenic *Naegleria* isolate was obtained from the same locality in October when the water temperature was as low as 16°C (Table 2). Wellings et al. (12) isolated pathogenic *N. fowleri* from bottom sediment samples when the average water temperature was 12°C.

In 1981, most nonpathogenic *Naegleria* isolates were obtained from discharges having elevated temperatures, such as at Homer City, Keystone, and Seward (Table 3). A high frequency of *Naegleria* strains in the discharge waters and in the water samples and sediments collected from the cooling tower was found at Homer City. The Keystone plant had a high incidence of *Naegleria* strains in cooling tower water and benthic samples, while the Seward plant showed only a slightly elevated number of isolates in the effluent. The relationship between the elevated temperatures and the occurrence of

TABLE 3. Occurrence of thermophilic *Naegleria* strains at power plant localities in 1981

Power plant (sample)	Location[a]	*Naegleria* strains present in:[b]							% of *Naegleria* strains	% of PAM
		April	May	June	July	August	September	October		
Conemaugh (water)	CT	P	N	P	P	PAM	P	P	29	14
	R	—	P	—	—	—	—	—	0	0
	Pond	P	—	P	P	N	P	P	17	0
Conemaugh (sediment)	CT	O	O	O	O	P	N	N	29	0
	R	—	O	—	—	—	—	—	0	0
	Pond	P	—	O	P	O	P	O	0	0
Erie (water)	D	P	O	P	PAM	O	P	P	14	14
	R_1	P	O	PAM	PAM	P	O	O	29	29
	R_2	—	—	N	PAM	P	P	—	50	25
Erie (sediment)	D	P	O	P	P	O	P	P	0	0
	R_1	O	O	P	O	P	O	P	0	0
	R_2	—	—	P	PAM	P	P	—	25	25
Homer City (water)	D	P	P	O	N	N	N	N	57	0
	CT	N	N	N	N	P	P	P	57	0
	R	P	O	P	N	O	N	N	43	0
Homer City (sediment)	D	O	P	P	N	P	P	N	29	0
	CT	P	P	N	N	N	N	N	71	0
	R	P	O	P	O	N	N	P	29	0
Keystone (water)	D	N	O	O	P	O	P	P	29	0
	CT	P	P	P	N	N	N	N	57	0
	R	P	N	O	P	P	O	O	14	0
Keystone (sediment)	D	O	O	O	O	P	P	O	0	0
	CT	P	P	N	N	O	O	N	43	0
	R	P	P	O	O	O	O	O	0	0
Seward (water)	D	O	P	P	N	N	N	P	43	0
	R	O	P	O	P	N	N	P	29	0
Seward (sediment)	D	P	O	O	P	N	N	P	29	0
	R	O	O	O	O	N	N	P	29	0
Shawville (water)	D	O	P	O	O	PAM	P	O	14	14
	R_1	P	P	P	N	N	P	O	14	0
	R_2	—	O	O	—	—	P	—	0	0
Warren (water)	D	P	P	P	N	P	O	O	14	0
	R	O	P	O	O	O	O	O	14	14
Warren (sediment)	D	P	O	P	O	O	O	N	14	0
	R	P	P	P	PAM	P	P	P	14	14
Williamsburg (water)	D	O	P	O	N	N	O	P	14	0
	R	P	P	O	P	P	O	P	0	0
Williamsburg (sediment)	D	P	O	P	P	P	P	P	0	0
	R	O	P	P	O	O	P	O	0	0

[a] Abbreviations: D, discharge; CT, cooling tower; R, recipient.

[b] Abbreviations: P, positive growth at 45°C; N, nonpathogenic *Naegleria* strains; PAM, pathogenic *Naegleria* strains; O, zero; —, not done.

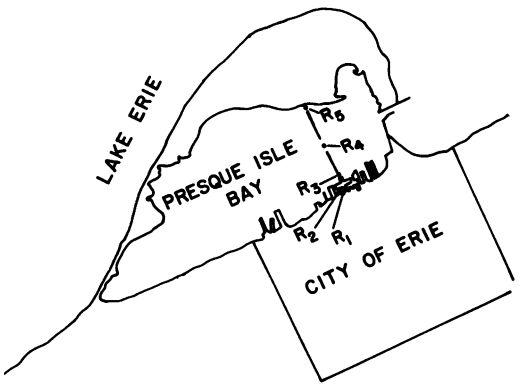

FIG. 1. Location of sampling stations in the effluent canal (R_1 and R_2) and in Presque Isle Bay (R_3 to R_5).

thermophilic (pathogenic or nonpathogenic or both) *Naegleria* strains is not always well defined, as for example, Shawville water had a higher temperature than Seward but a lower fraction of isolates. However, a statistically significant correlation between the temperature and the number of isolates established the optimum temperature for pathogenic *Naegleria* strains ranging from 27 to 35°C ($P < 0.001$).

In 1981, pathogenic amoebae were isolated from four of eight power plants (Conemaugh, Erie, Shawville, Warren). Pathogenic *Naegleria* strains were detected at Shawville before by Tyndall et al. (11). The most infested locality was at Lake Erie in June and July. The effluent (D) and the discharge canal (small harbor, localities R_1 and R_2) were characterized by elevated temperatures, constant pH levels, low hardness, and intermittent occurrence of pathogenic *Naegleria* strains. However, no pathogenic *Naegleria* strains were isolated in July from the Presque Isle Bay (Lake Erie) at the R_3, R_4, and R_5 stations (for location see Fig. 1). The last two localities had low temperatures and were not substantially affected by the discharge from the power plant. This means that with the exception of the heated effluent, the water in Lake Erie was safe for swimming and bathing. Table 4 describes the results obtained from these additional stations located in the Erie Harbor.

Laboratory experiments. Cysts of pathogenic *N. fowleri* survived in buffered water for 2 h at 37°C at pHs of 2, 3.15, 4.0, 5.4, 5.7, 6.35, 6.6, 7.0, 7.5, 8.15, 8.7, 9, and 10.0. Therefore, the exposition time was extended up to 24 h. Survival was not affected by low pH, but the number of trophozoites decreased after the culturing of cysts on NNAEa at pH 8.7 and 9 and no growth was apparent at pH 10.

DISCUSSION

This study indicates that the distribution of thermophilic *Naegleria* strains is limited by season, with increasing incidence in the summer and early fall. However, the occurrence and heavy infestation of water and sediment with nonpathogenic, thermophilic *Naegleria* strains does not guarantee the presence of pathogenic strains. The localities with a high frequency of thermophilic isolates (Homer City, Seward, and Keystone) were devoid of any pathogenic *Naegleria* strains during our study period. However, on several occasions, the pathogenic strain was the only *Naegleria* isolate we obtained from a

TABLE 4. Distribution of thermophilic *Naegleria* isolates in water and sediment samples from the Lake Erie transect (July 24, 1981)

Locality[a]	Temp (°C)	pH	Hardness (mg of $CaCO_3$ per liter)	Flagellation positive		PAM positive[b]	
				Water	Sediment	Water	Sediment
Power plant discharge	35	7.8	126	+	−	+	+
Discharge canal (R_1)	33	7.9	120	+	−	+	−
Discharge canal (R_2)	34	7.9	122	+	+	+	+
Discharge canal at the lake (R_3)	35	8.4	126	+	−	−	−
Lake Erie, center (R_4)	26.5	8.2	108	−	−	−	−
Lake Erie, Presque Isle (R_5)	26	8.2	116	−	−	−	−

[a] Abbreviations: R_1, 25 m from discharge; R_2, discharge canal 70 m from discharge; R_3, discharge canal entering the lake; R_4, Presque Isle Bay, center; R_5, Presque Isle shore.

[b] PAM, Primary amoebic meningoencephalitis.

particular locality (Erie discharge and recipient, Shawville discharge, Warren recipient) (Tables 2 and 3).

It is interesting to note that pathogenic *Naegleria* strains may be present in water at relatively very low temperatures. This was documented by an isolate obtained from Williamsburg in September, 1980 (Table 2), when the water temperature was only 16°C (9). This is the first time that pathogenic *N. fowleri* was isolated from a water sample at a temperature this low. Duma (6) suggests that only those freshwater lakes whose waters may reach or exceed 30°C should be considered to be a potential medium for pathogenic *Naegleria* strains. Our data suggest that the optimum temperature for pathogenic *Naegleria* strains in heated effluents is between 27 and 35°C. On the other hand, Wellings et al. (12) isolated pathogenic *N. fowleri* from bottom sediments (but not from water samples) when the average water temperature was as low as 12°C. It is possible that in Williamsburg the pathogenic amoebae developed in the cooling water when the temperature was higher and somehow survived the change in the environment.

It is well documented that warm water supports development of these pathogens (10, 11). However, constantly elevated and high temperatures such as those observed at Homer City discharge without any substantial changes appear to support nonpathogenic strains over the pathogens. Also, pathogenic strains of *Naegleria* are more likely to be present in effluents relatively unaffected by human activities such as mining (Table 1). On the other hand, even acid mine drainage may not eliminate the pathogen completely (Shawville).

This research shows that the probability that heated effluents may contaminate large bodies of generally colder water such as Lake Erie with pathogenic *Naegleria* strains is limited. However, smaller systems may remain contaminated as long as the temperature is elevated. Therefore, it is advisable that swimming and all water contact sports should not be permitted in heated effluents and in the sections of the recipient water bodies affected by elevated temperatures. In addition, heated effluents and water affected by thermal enrichment used for swimming should be assessed for the concentration of pathogenic *Naegleria* strains.

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LITERATURE CITED

1. **American Public Health Association.** 1980. Standard methods for the examination of water and wastewater, 15th ed. American Public Health Association, Washington D.C.
2. **Cerva, L.** 1971. Studies of limax amoebae in a swimming pool. Hydrobiologia **38**:141–161.
3. **DeJonckheere, J., P. Van Dijck, and H. Van de Voorde.** 1975. The effect of thermal pollution on the distribution of *Naegleria fowleri*. J. Hyg. **75**:7–13.
4. **DeJonckheere, J. F.** 1981. *Naegleria australiensis* sp. nov., another pathogenic *Naegleria* from water. Protistologica **17**:423–429.
5. **DeJonckheere, J. F.** 1982. Isoenzyme patterns of pathogenic and nonpathogenic *Naegleria* spp. using agarose isoelectric focusing. Ann. Microbiol. (Paris) **133**:319–342.
6. **Duma, R. J.** 1981. Study of pathogenic free-living amebas in freshwater lakes in Virginia. Project summary, report no. EPA-800151-80-037, p. 1–5. U.S. Environmental Protection Agency, Cincinnati, Ohio.
7. **Fowler, M., and R. F. Carter.** 1965. Acute pyogenic meningitis probably due to *Acanthamoeba* sp.: a preliminary report. Brit. Med. J. **2**:740–742.
8. **Martinez, A. J.** 1981. Free living amebas in human infections, p. 57. Eastern Pennsylvania Branch of the American Society for Microbiology, Philadelphia, Pa.
9. **Shapiro, M. A., M. H. Karol, G. Keleti, J. L. Sykora, and A. J. Martinez.** 1982. The role of free-living amoebae occurring in heated effluents as causative agents of human disease, p. 99–111. *In* Proceedings of International Conference on Coal Fired Power Plants and the Aquatic Environment, Copenhagen, Denmark. Water Quality Institute, Copenhagen.
10. **Stevens, A. R., R. L. Tyndall, C. C. Coutant, and E. Willaert.** 1977. Isolation of the etiological agent of primary amoebic meningoencephalitis from artificially heated waters. Appl. Environ. Microbiol. **34**:701–705.
11. **Tyndall, R. L., E. Willaert, A. R. Stevens, and C. C. Coutant.** 1978. Isolation of pathogenic *Naegleria* from artificially heated waters, p. 117–123. *In* R. M. Gerhold (ed.), Proceedings of Symposium on Microbiology of Power Plant Thermal Effluents. University of Iowa, Iowa City.
12. **Wellings, F. M., P. T. Amuso, A. L. Lewis, M. J. Farmelo, D. J. Moody, and C. L. Osikowicz.** 1979. Pathogenic *Naegleria*, distribution in nature. Report no. EPA-600/1-79-018. U.S. Environmental Protection Agency, Cincinnati, Ohio.