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Occurrence of *Naegleria* and *Acanthamoeba* in Aquaria

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Samples from 24 aquaria were incubated at 28, 37, and 45°C for the isolation of *Naegleria* and *Acanthamoeba*. *Naegleria* was the predominant genus (60.9%), whereas *Acanthamoeba* represented 15.5% of the isolates. No pathogenic *N. fowleri* was identified, although a high number of strains were closely related to this species. One isolate (Aq/9/1/45D) was compared with an aquarium isolate (PPMFB-6) from Australia. The Belgian isolate was found to be more related to *N. fowleri*, whereas the Australian isolate was closer to *N. gruberi*.

During recent years, free-living amoebae have been isolated from such diverse places as surface waters, swimming pools, drinking water, human and animal bodies, and the air. Amoebae were even found in a dialysis unit and in tissue cultures.

Isolates of *Naegleria* and *Acanthamoeba*, especially, have attracted much attention, because they can cause severe neurological disease in humans (for a review see ref. 4).

A few attempts have been made to examine the bacterial population in aquaria, but the search for parasites has been omitted (9). Aquaria, however, are water reservoirs present in many homes, and direct contact exists frequently. In addition, they may often have a high temperature because they contain tropical fishes, and they may therefore be a favorable breeding place for parasites, especially thermophilic amoebae such as *N. fowleri*. A few reports show that aquaria could be places for human infections. A possible relationship between *Naegleria* meningoencephalitis and aquarium contact, where no association with swimming could be established (1), has been suggested in Australia.

There has been a report on fish kills in home aquaria and in a laboratory aquarium due to amoebae which were probably *Acanthamoeba* (10). Large fish kills in lakes and fishponds have also been caused by *Acanthamoeba* (8).

All these observations taken into consideration, it was thought worthwhile to investigate the incidence of *Naegleria* and *Acanthamoeba* spp. in aquaria, which may be a source for human infection.

MATERIALS AND METHODS

During the period from October 1976 through March 1977, 24 aquaria were sampled. Half of them were located in private houses and in a laboratory, and the other half were aquaria in a zoological garden with fishes from all over the world. It is perhaps appropriate to note that the aquaria are not located in the laboratory where research is done on the amoebae. This laboratory is in a separate building, situated at the other end of town from where the samples are handled.

Sterile, 500-ml bottles were filled at the surface without stirring the water. Deposits on glass walls and deposits from the filtering systems were taken with sterile cotton swabs. The samples were processed immediately in triplicate and incubated on living *Escherichia coli* at 28, 37, and 45°C, respectively.

Samples (100 ml each) were filtered through 5.0-μm (47-mm diameter) cellulose acetate membranes, which were placed inverted on nonnutrient agar with *E. coli*. Samples (1 ml each) were poured on nonnutrient agar with *E. coli*, and the excess fluid was allowed to evaporate. Deposits were inoculated on nonnutrient agar with *E. coli* without treatment. Bacteria growing aerobically at 37°C were enumerated by the standard plate count.

Amoebae isolated at the three incubation temperatures were identified by morphological examination of trophozoites and cysts with phase-contrast and differential-interference microscopy (7). The transformation of amoebae to flagellates was examined. The genera of amoebae not belonging to *Naegleria* and *Acanthamoeba* were not identified.

To identify pathogenic *N. fowleri*, *Naegleria* isolates were transferred to an axenic medium which selectively favors its growth (2). A few *Naegleria* type strains were compared with a *Naegleria* strain isolated from an aquarium in Australia (1) and with a *Naegleria* strain isolated at 45°C during this study. They were compared in terms of their ability to grow at high temperature on bacteria and to grow axenically and their ability to destroy Vero cell monolayers (cytopathic effect [CPE]) (3). Also, the serological relationship was investigated with specific antisera (3).

RESULTS

The location, water temperature, pH, and number of aerobic bacteria in 1 ml of each

TABLE 1. *Amoebic isolations from aquaria at 28, 37, and 45°C incubation*[a]

Aquarium	No.	Temp (°C)	pH	Bacteria/ml	Isolations at 28°C from:												Isolations at 37°C from												Isolations at 45°C from:											
					1 ml			100 ml			Solids			Solids in filter			1 ml			100 ml			Solids			Solids in filter			1 ml			100 ml			Solids			Solids in filter		
					N	A	O	N	A	O	N	A	O	N	A	O	N	A	O	N	A	O	N	A	O	N	A	O	N	A	O	N	A	O	N	A	O	N	A	O
Laboratory	1	23	7.8	21,000	+			+		+	+			+	+		+		+	+					+	+													
	2	23.5	7.6	1,900				+		+		+		+	+					+		+			+	+													
	3	25	7.6	1,500			+			+	+	+		+	+		+			+			+	+							+			+			+		
Home	4	23	6.9	270	+			+			+	+		+	+					+						+		+											
	5	26	6.7	18,000			+			+		+	+	+		+			+	+		+			+	+		+											
	6	21	7.2	ND	+			+		+	+		+	+					+	+			+			+		+											
	7	21	7.3	ND			+	+				+	+	+					+	+					+	+	+												
Laboratory	9	23	6.7	ND													+			+			+			+				+			+			+		
	10	23.5	6.7	ND																			+			+													
	11	22	7.7	ND														+			+			+			+												
	12	26	7.8	ND														+							+			+				+			+			+		
	13	16.5	7.7	ND														+			+		+	+		+													
Zoological garden	14	23.5	7.2	1,310													+				+		+			+				+						+		
	15	23	7.6	4,500															+	+						+													
	16	23	7.4	1,880													+			+			+					+											
	17	24	7.7	630													+			+				+		+											+		
	18	29.5	7.4	4,000													+			+			+			+													
	19	25.5	7.4	660															+	+			+			+											+		
	20	25	7.3	860															+			+	+			+									+				
	21	24.5	7.1	430																					+	+								+					
	22	24	7.0	8,000															+	+			+			+													
	23	24.5	7.5	1,060													+			+			+												+				
	24	23.5	7.2	1,530													+			+					+														
	25	25	6.6	380																+				+		+													
Total					3	0	3	5	0	5	4	5	3	7	4	1	9	3	8	17	2	4	12	6	7	19	5	6	3	0	0	8	0	1	5	0	0	6	0	0

[a] N, *Naegleria* sp.; A, *Acanthamoeba* sp.; O, other then *Naegleria* sp. or *Acanthamoeba* sp.; +, positive isolation; ND, not done.

aquarium are listed in Table 1.

All aquaria were found positive for amoebae at incubation temperatures of 28 and 37°C (Table 1).

At 45°C incubation, 11 aquaria (45.8%) were positive for amoebae. In the zoological garden, 66.6% of the fish tanks examined yielded amoebae at 45°C, whereas only 25% of the others were positive. The latter were all located in the same laboratory.

At 45°C, all 34 isolates except one belonged to the genus *Naegleria*. All incubation temperatures taken into consideration, *Naegleria* was predominant, with 98 of 161 plates positive (60.9%). Although *Acanthamoeba* represented 15.5% of the isolates, all other genera taken together accounted for only 23.6%.

Of 98 positives at 37°C incubation, 57 (58.2%) belonged to the genus *Naegleria* and 16 (16.3%) belonged to the genus *Acanthamoeba*. Of the 16 *Acanthamoeba* isolates at 37°C, 5 (31.2%) came from water samples, and at 28°C incubation none of the 9 *Acanthamoeba* isolates came from water samples. Apparently, *Acanthamoeba* is epiphytic rather than planktonic. This is in contrast to *Naegleria*, which has a free-swimming flagellate form. *Naegleria* was indeed found in water samples, 50% of the isolates (11 of 22) at an incubation temperature of 45°C, 45.6% (26 of 57) at 37°C, and 42.1% (8 of 19) at 28°C. This almost 50–50 proportion may be explained by the two life stages of *Naegleria*, a flagellate which will be found free-swimming in the water and an amoebic form attached to substrates.

A value of 16.3% *Acanthamoeba* positives at 37°C is more than that found in surface water, where only about 4% of the isolates belonged to the genus *Acanthamoeba* (J. F. De Jonckheere, manuscript in preparation). In the latter study, however, no samples of deposits were taken into consideration, and also, the isolation procedure differed. O'Dell (5) found *A. polyphaga* the predominant amoeba (64%) in lake bottom samples, with a peak in late September, whereas other species of amoebae remained at relatively low densities throughout the year.

The number of *Acanthamoeba* found here in aquaria is also far less than we observed in swimming pools, where 43.6% of the isolates belonged to *Acanthamoeba* (De Jonckheere, in preparation).

In contrast to swimming pools, where only 7.3% of amoebae isolated at 37 and 45°C belonged to *Naegleria*, I found 65.3% of the isolates at these two temperatures to be *Naegleria* sp. Thus, whereas *Acanthamoeba* is more prevalent in swimming pools than in aquaria and surface water, the reverse is true for *Naegleria*.

A high proportion of *Naegleria* strains belonged to *N. gruberi* on a morphological basis, whereas others could not be given a specific name. They behaved like nonpathogenic *N. fowleri* variants (3): high titer with *N. fowleri* antisera, growth at 45°C on bacteria, CPE in Vero cell culture with formation of cysts, and nonpathogenicity for mice.

A strain isolated from a 1-ml sample at 45°C (Aq/9/1/45D) was compared with type strains of *N. fowleri* (KUL), *N. gruberi* (1518/1a, BG-6), a strain that had proven to belong to nonpathogenic *N. fowleri* variants (TS-1) (3), and a strain isolated from a fish tank in Australia and which also is shown to react at a high titer with *N. fowleri* antiserum (PPMFB-6) (1).

When cross-reacted with *N. fowleri* and *N. gruberi* antisera, strains TS-1, PPMFB-6, and Aq/9/1/45D appeared to be intermediate between *N. fowleri* and *N. gruberi* (Table 2). When tested for CPE, strains TS-1 and Aq/9/1/45D destroyed the Vero cell monolayer in 2 to 3 days with formation of cysts afterwards, as was found for nonpathogenic *N. fowleri* variants (3). Strain PPMFB-6 did not show CPE at all, like *N. gruberi* 1518/1a (Table 3). However PPMFB-6 could be grown axenically in SCGYEM after some adaptation like the other nonpathogenic test strains, except *N. gruberi* 1518/1a. Another *N. gruberi* strain, BG-6, also isolated in Australia, was cultured axenically and showed CPE but at a much longer incubation time. Thus, within *N. gruberi*, different physiological strains

TABLE 2. *Effect of indirect immunofluorescence technique on Naegleria spp.*

Amoeba isolate	Reciprocal titer		
	KUL	Aq/9/1/45D	BG-6
N. fowleri KUL	256	128	128
N. lovaniensis Aq/9/1/45D	512	1,024	32
TS-1	256	1,024	32
N. gruberi BG-6	32	1,024	1,024
PPMFB-6[a]	64	1,024	128

[a] Aquarium isolate from Australia.

TABLE 3. *Axenic growth in SCGYEM and CPE in Vero cell cultures at 37°C*[a]

Amoebae	Axenic	CPE
N. fowleri KUL	+	+
N. lovaniensis Aq/9/1/45D	+*	+*
TS-1	+*	+*
N. gruberi 1518/1a	−	−
BG-6	+*	+°
Aquarium isolate from Australia PPMFB-6	+*	−

[a] +, Positive (no cysts formed); −, negative; +*, positive with formation of cysts; +°, CPE after ±2 weeks.

exist, as was found morphologically (6). Therefore the *N. gruberi* species name is still in question. When tested at 45°C, both *N. gruberi* strains and strain PPMFB-6 failed to grow on bacteria.

Naegleria strain PPMFB-6 from an aquarium in Australia was different from strain Aq/9/1/45D from our aquarium in Belgium. Both strains are, however, nonpathogenic for mice. Although virulence could be enhanced in *N. fowleri* that had lost intranasal infectivity due to axenic cultivation, pathogenicity could not be induced in strain Aq/9/1/45D, although it is closely related to *N. fowleri* (J. F. De Jonckheere, Pathol. Biol., in press).

The genus *Naegleria* appears to enclose different intermediate strains between *N. fowleri* and *N. gruberi*. Acute virulence of a *Naegleria* isolate, together with serology of the isolate, are the criteria for *N. fowleri*. Ultrastructural differences and lectin studies of strains TS-1 and Aq/9/1/45D have resulted in the formation of a new *Naegleria* species, *N. lovaniensis* sp. nov. (Stevens, De Jonckheere, and Willaert, manuscript in preparation). Four other type strains of *N. lovaniensis* sp. nov. were isolated from water bodies also harboring pathogenic *N. fowleri*.

Due to the high amount of *N. fowleri* variants isolated from aquaria situated in the laboratory, a few more attempts were made to isolate pathogenic *N. fowleri*. Indeed, these two *Naegleria* species have been proven to be very related in ecology, and therefore they often occur together (2). Samples (1 ml each) were therefore incubated at 45°C to prevent overgrowth of the slower growing pathogenic *N. fowleri* by nonpathogenic variants. These attempts in March and August of 1977 again yielded no pathogenic *N. fowleri*, although 37 more *Naegleria* strains were isolated.

No relationship was found between the number of bacteria and the presence of amoebae in water samples.

DISCUSSION

Although no pathogenic *N. fowleri* were identified, the high concentration of *Naegleria* closely related to *N. fowleri* in aquaria attracted my attention. Indeed, it has been found in Belgium and in other countries of Europe that these *Naegleria* spp. prefer the same niche and are therefore also frequently found together (2).

Therefore, fish tanks might indeed be sources of *Naegleria* infections, as was thought before in Australia (1). The *Naegleria* strain isolated from an aquarium in Australia was, however, found to be different from an isolate from an aquarium in Belgium, the former being more related to *N. gruberi* than to *N. fowleri*.

Also, the frequency of *Acanthamoeba* spp. in fish tanks should not be considered unimportant. *Acanthamoeba* spp. have been related to brain infections in debilitated persons and eye infections in healthy humans. *Acanthamoeba* is most probably present as a trophozoite in aquaria, whereas in swimming pools and drinking water it is probably in cyst form because of the deleterious effect of chlorine on trophozoites. Infection might therefore occur more readily when contact exists with aquaria than with chlorinated water. Moreover, although *Acanthamoeba* is the most abundant amoeba in swimming pools, the actual concentration is lower than in aquaria.

Acanthamoeba has also been shown to produce disease in fish (8, 10). In an experimental study, *Acanthamoeba*, *Vahlkampfia*, and *Naegleria* were isolated from fish tissue; the last two genera were taken only from organs in direct water contact (8). All *Acanthamoeba* isolates were identified as *A. polyphaga*. Water samples of the aquaria during the tests showed the consistent presence of *Vahlkampfia* sp. Heavy infestation of fish with *Acanthamoeba* can result in mortality as found in lakes (8) and aquaria (10).

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LITERATURE CITED

1. **Anderson, K., and A. Jamieson.** 1972. Agglutination test for the investigation of the genus *Naegleria*. Pathology 4:273-278.
2. **De Jonckheere, J.** 1977. Use of an axenic medium for differentiation between pathogenic and nonpathogenic *Naegleria fowleri* isolates. Appl. Environ. Microbiol. 33:751-757.
3. **De Jonckheere, J., and H. Van de Voorde.** 1977. Comparative study of six strains of *Naegleria* with special reference to nonpathogenic variants of *N. fowleri*. J. Protozool. 24:304-309.
4. **Griffin, J. L.** 1978. Pathogenic free-living amoebae, p. 507-549. *In* J. P. Kreier (ed.), Parasitic protozoa, vol. 2. Academic Press Inc., New York.
5. **O'Dell, W. D.** 1979. Isolation, enumeration and identification of amoebae from a Nebraska lake. J. Protozool. 26:265-269.
6. **Page, F. C.** 1975. Morphological variation in the cyst wall of *Naegleria gruberi* (Amoebida, Vahlkampfiidae). Protistologica 11:195-204.
7. **Page, F. C.** 1976. An illustrated key to fresh water and soil amoebae. Freshwater Biol. Assoc. Sci. Publ. 34:62-117.
8. **Taylor, P. W.** 1977. Isolation and experimental infection of free-living amebae in freshwater fishes. J. Parasitol. 63:232-237.
9. **Trust, T. J., and K. H. Bartlett.** 1974. Occurrence of potential pathogens in water containing ornamental fishes. Appl. Microbiol. 28:35-40.
10. **Voelker, F. A., M. R. Anver, A. E. McKee, H. W. Casey, and G. R. Brenniman.** 1977. Amebiasis in goldfish. Vet. Pathol. 14:247-255.