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NAEGLERIA FOWLERI IN THERMALLY POLLUTED WATERS[1])

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Abstract. Two complexes of lakes and canals supplying water for two electric power plants, their steam condensors and an adjoining river were investigated by means of culture methods for the presence of *Naegleria fowleri* in Poland in the period from 1974 to 1980. Sixty-four strains of *N. fowleri* were isolated, 13 isolates being virulent for mice when instilled intranasally. These strains were found in the steam condensor of the power station A and in waters polluted with warm water of this plant. Pathogenic *N. fowleri* strains occurred also in an adjoining river connected with the water system of the power plant. The results show the possible role of the steam condensor A as an incubator and regular source of pollution with pathogenic amoebae for its own system of cooling waters and even the adjoining river.

Shortly after the description of the first cases of primary amoebic meningoencephalitis in man (Fowler and Carter 1965), the link between the disease and previous swimming of the victims in thermal waters was proved. This relationship became clear after discovering the pathogenic *N. fowleri* in environment. The first strains were isolated from fresh water (also tap-water), dry soil and sewage sludge (Anderson and Jamieson 1972a, b, Singh and Das 1972, Gordeeva 1973, Griffin 1973). In 1974, Kasprzak and Mazur found for the first time pathogenic *Naegleria* in samples taken from waters thermally polluted by discharges of an electric power station. Subsequently other authors found pathogenic isolates in discharges of different thermal polluting factories (DeJonckheere et al. 1975, Willaert and Stevens 1976, Wellings et al. 1977, Červa et al. 1980). DeJonckheere and co-workers conducting extended studies (1975, 1977), proved the strong link between the distribution of pathogenic *N. fowleri* and the thermally polluted waters and demonstrated the presence of the amoeba in numbers sufficient to cause a serious health problem (DeJonckheere 1978). The authors stated also that the lack of these amoebae in some thermally polluted waters in the neighbourhood of the sites of pathogenic strain isolation indicates the existence of some other factors—apart from high temperature—determining the selective proliferation of *N. fowleri*.

The finding of pathogenic *Naegleria* in discharges of thermal polluting electric power station (Kasprzak and Mazur 1974, Kasprzak et al. 1975) initiated several years' observation of the distribution of these amoebae. The aim of this investigation was to determine the frequency of *N. fowleri* presence in the waters and the relationship between the amoeba distribution and the temperature changes in the primary biologically healthy waters in Poland.

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Table 1. Isolation of *N. fowleri* from water of cooling systems of two electric power stations and a river during the period from 1974 to 1980

		1974			1975			1976			1977			1978			1979			1980		
Sampling site		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Power station A	scA	ND			ND			ND			ND			38–40	+	×	38–40	+	–	38–40	+	×
	1	ND			ND			28	–	–	31	–	–	ND			17–26	+	–	22	+	×
	2	ND			ND			27	+	–	30	+	–	15–22	+	×	17–27	+	×	20	+	×
	3	14–23	+	×	15–25	–	–	28	+	–	29	+	–	15–23	+	×	16–25	+	–	19	+	–
	4	ND			20	–	–	26	+	–	30	–	–	10–21	+	–	21	+	–	17	+	–
	5	15–19	–	–	ND			22	–	–	24	–	–	13–20	–	–	8–20	–	–	12	–	–
	6	19	–	–	ND			25	–	–	24	–	–	ND			8–20	–	–	13	–	–
	7	20	–	–	ND			24	–	–	24	–	–	15–21	+	–	8–19	+	–	14	–	–
	8	ND			ND			ND			ND			ND			9–19	+	–	15	–	–
Power station B	scB	ND			ND			ND			ND			ND			38–40	+	–	38–40	–	–
	9	ND			ND			ND			ND			ND			18–28	+	–	23	+	–
	10	23	+	–	14	–	–	27	–	–	27	–	–	13–21	+	–	18–24	+	–	18	+	–
	11	ND			ND			ND			ND			ND			10–19	+	–	17	+	–
River W	12	ND			ND			ND			ND			4–15	+	×	6–16	+	×	14	+	×

Numbers of sampling sites refer to the numbers in Fig. 1 (scA and scB — steam condensors of stations A, B)
A — temperature of the water (°C); B — growth of amoebae at 42 °C; C — inoculation of mice.
+ positive for *N. fowleri*; – negative for *N. fowleri*; × strain pathogenic for mice; ND — not done

MATERIAL AND METHODS

Samples of water and sand (mud) (1 : 1 ratio) placed in sterile 50 ml bottles were taken at the waterside of two complexes of lakes and canals which supply water for cooling two electric power stations and receive their warm discharges. The station A pours the thermal discharges into a large complex consisting of 5 lakes and several canals, whereas the second station (B) into one lake and several canals (Fig. 1). Although both complexes are connected, any mixing of their water is insignificant. The samples were taken from 8 sites of the station A cooling water complex (1—8) and from 3 sites of station B water complex (9—11). The samples (1 to 4 in a year) were taken during the years 1974 to 1980.

The water temperature at the sampling places ranged according to the season of the year. However, in sites nearest to the station outlets (sites Nos. 1, 2, 3, 9 and 10), the temperatures did not drop below 13 °C even during winter and were above 30 °C during summer. Therefore we recognized the water at these sites as thermally polluted.

Further samples were taken from a river (rW, site No. 12) at a distance of about 13 km from the station A outlet (Fig. 1). The river is connected with the water complex of this station. The water temperature in the river does not differ from the temperature of other fresh water. In 1979 and 1980 additional samples taken from the steam condensors of both electric power stations were investigated. The temperature of the cooling water inside the condensors ranged between 38 °C to 40 °C.

The 1 ml samples were inoculated onto non-nutrient agar (NN) previously seeded with living *Enterobacter aerogenes* (NNE) and incubated at 42 °C. The plates were examined for growth of amoebae daily for a week.

For specific identification of the isolates, morphological examination and flagellation test were used. The pathogenicity of *Naegleria* isolates was tested in Porton mice; 20,000 to 40.000 amoebae of each isolate were instilled intranasally usually in 5 weanling mice. The inocula were prepared by washing off the amoebae with distilled water from the medium. The brains of dead mice were placed on the NNE agar and incubated in 42 °C. The plates were examined for amoebae growth daily for a week. The reisolated amoebae were examined for specific identification (morphology and flagellation test).

RESULTS

Sampling for *N. fowleri* yielded thermotolerant strains in all but one water investigated (Table 1); exceptional was the site No. 5, most distant from the station A outlet (Fig. 1). In the period from 1974 to 1980, *N. fowleri* strains did not occur continuously at given sites, being found, however, most frequently in thermally polluted waters (i.e. waters with temperature ranging from 14 °C to 31 °C). The majority of *N. fowleri* strains have been isolated from sites at which the water temperature ranged between 17 °C and 21 °C (Table 2, Fig. 2). Noteworthy is the frequent isolation of *N. fowleri* from the distant river W (site No. 12). The river water had the same temperature range as other thermally non-polluted fresh waters in the whole region (i.e. from 4 °C to 16 °C at the sampling time).

We did not find a clear relationship between the frequency of amoebae isolation and the period of the year; more often the strains were found in the autumn (Table 3).

As a rule the non-pathogenic strains accompanied the pathogenic *N. fowleri*. Out of 64 strains found in 101 samples, 13 isolates were virulent for mice when given intranasally (Table 2). Depending on the strain, 40 % to 100 % of mice died after 6 to 21 days, most frequently after 7 to 14 days (Table 4). *N. fowleri* was reisolated from the brain of all dead mice. In thermally polluted waters (temperature range from 19 °C to 27 °C) the pathogenic *N. fowleri* strains were found exclusively downstream the power station A (Table 1, Fig. 1). However, the strains occurred variably at given sites. The fact of most frequent occurrence of pathogenic *N. fowleri* in the distant river (Table 2), even when the water temperature was decidedly low (5 °C—15 °C), is surprising. As previously mentioned, the river is connected with the complex of waters cooling the power station A (Fig. 1).

The pathogenic strains of *N. fowleri* were not found during winter, neither from thermal waters nor from the river. Most isolates were found in the spring.

We isolated *N. fowleri* also from samples taken from steam condensors of both power stations. However, the pathogenic isolates occurred only in steam condensor of station A (Table 1). Also in this sampling site the pathogenic strains were not found in all samples; most likely it might depend on the samples taken from different chambers of the steam condensor.

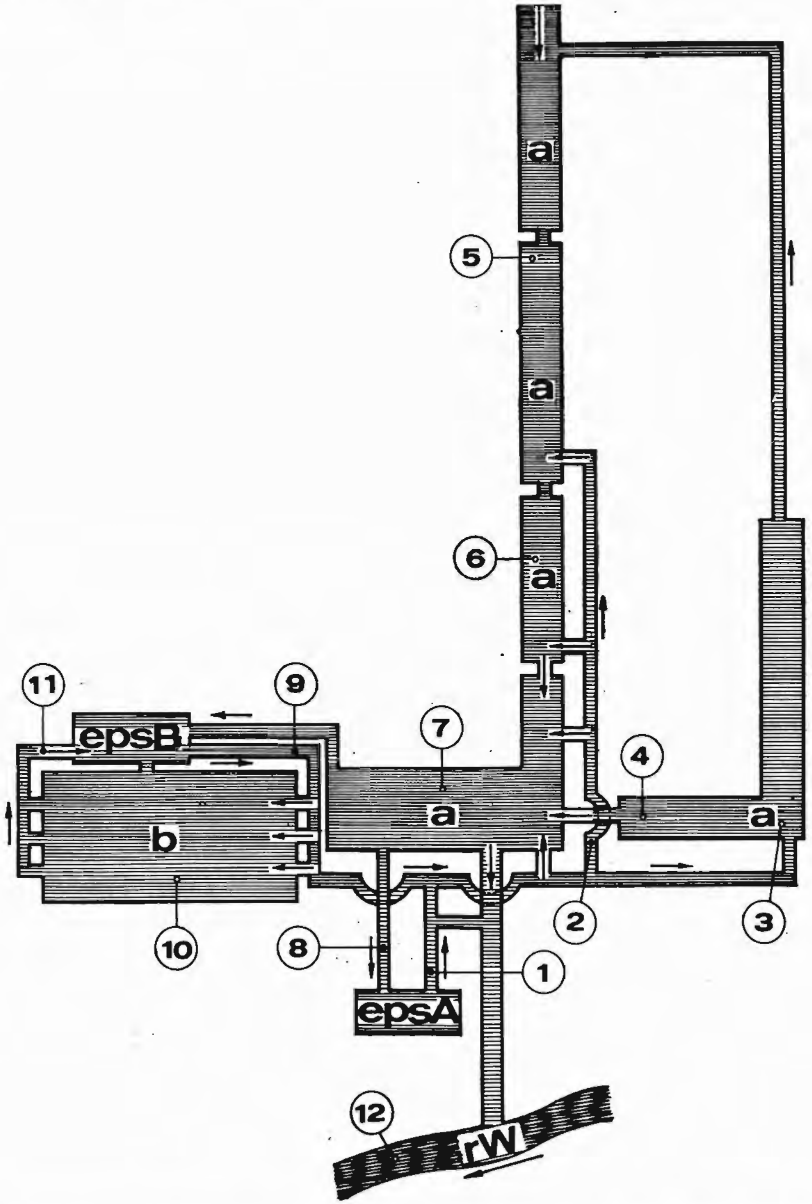

Fig. 1. Schematic illustration of sampling sites. Numbers of sampling sites refer to the numbers in Tables 1 and 2; epsA and epsB — the electric power stations A and B; rW — the river; a — the lakes in water complex of epsA; b — the lake in water complex of epsB.

Table 2. Results of *N. fowleri* isolation from samples taken in the period from 1974 to 1980

Sampling site	Water temperature (°C)	No. of samples	No. of *N. fowleri* strains	No. of pathogenic strains
scA	38—40	4	4	2
1	17—26	6	4	1
2	15—30	10	8	3
3	14—29	14	11	2
4	10—27	9	7	0
5	8—24	10	0	0
6	8—25	6	1	0
7	8—24	9	3	0
8	9—19	4	2	0
scB	38—40	2	1	0
9	18—28	4	4	0
10	13—27	11	8	0
11	10—29	4	4	0
12	4—16	8	7	5
Total		101	64	13

Numbers of sampling sites refer to the numbers in Fig. 1.
scA and scB — steam condensors of power stations A and B.

Table 3. Occurrence of *N. fowleri* according to the period of the year

Period of the year	No. of samples	No. of *N. fowleri* strains	No. of pathogenic strains
March—May	37	22	7
June—August	28	13	2
September—November	17	15	2
December—February	13	9	0

Table 4. Virulence of isolated *N. fowleri* strains for mice

Strain	No. of inoculated mice	No. of dead mice	Days from inoculation to death
1/1	5	2	9, 14
2/1	5	4	11, 15, 15, 18
2/2	5	2	10, 19
2/3	4	2	8, 12
3/1	5	5	6, 10, 10, 10, 13
3/2	5	5	6, 7, 7, 8, 8
12/1	5	3	11, 11, 12
12/2	5	5	8, 8, 8, 8, 8
12/3	5	4	8, 8, 8, 20
12/4	5	2	13, 16
12/5	5	3	9, 11, 14
scA/1	6	6	6, 7, 8, 10, 10, 10
scA/2	5	3	12, 14, 14

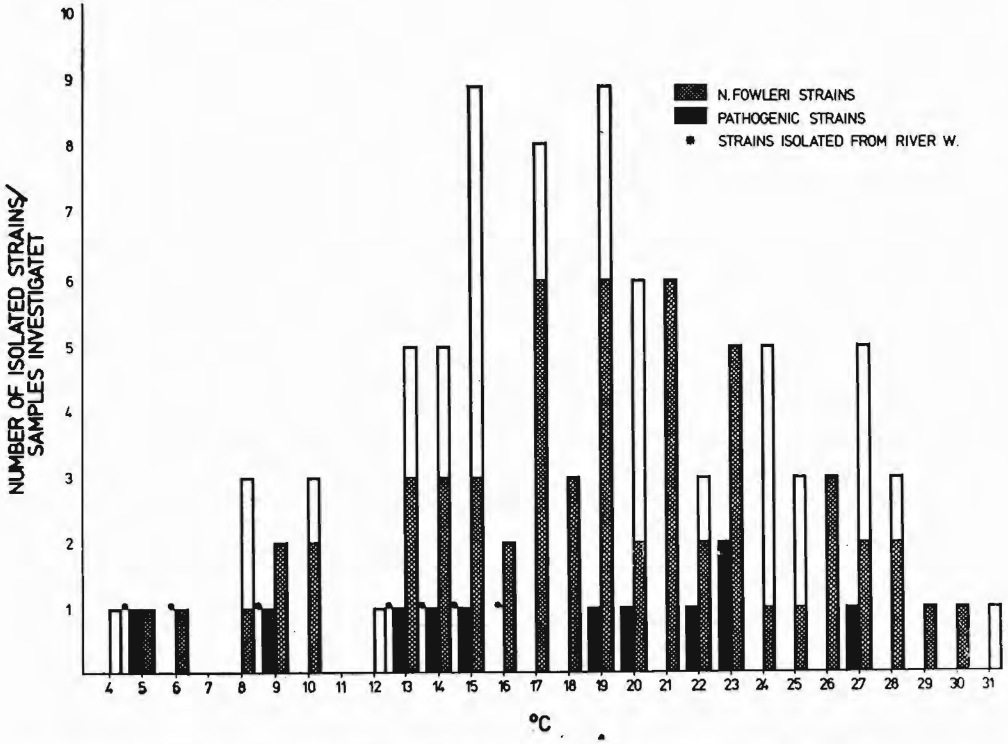

Fig. 2. Water temperature in sampling sites and number of isolated *N. fowleri* strains.

DISCUSSION

Our study demonstrated — like the investigations conducted by DeJonckheere et al. (1975, 1977), DeJonckheere (1978), Willaert and Stevens (1976), Wellings et al. (1977) and Červa et al. (in press) — that the thermotolerant *N. fowleri* strains — both pathogenic and non-pathogenic — occur in waters thermally polluted by warm industrial discharges. We found *N. fowleri* also in waters with temperature normal for our climatic region; however, these waters were connected with the thermally polluted ones. The most distant sampling place positive for amoebae yielding was about 13 km from the outlet.

Most of *N. fowleri* strains were isolated from sites where the temperature of the water ranged between 17 °C to 21 °C and not in waters with highest temperature in the whole cooling water system. Similar evidence is provided by our recent study of waters thermally polluted by warm effluents from different power stations in Czechoslovakia (Červa et al., in press).

Conducting the observations for years we found that *N. fowleri* did not occur continuously in the sampling sites investigated. But we did not observe any clear correlation of amoebae distribution and the period of the year. DeJonckheere and VanDeVoorde (1977) isolated also *N. fowleri* in summer as well as in winter and expressed the opinion that heated biologically healthy waters provide a place for continuous proliferation of *N. fowleri*.

We found that about 20 % of strains isolated were virulent for mice when instilled intranasally. Of some interest is the occurrence of the pathogenic strains exclusively in samples taken from water complex receiving discharges from electric power station A (i.e. downstream the station). This observation correlates well with the demonstration of pathogenic *N. fowleri* solely in the steam condensor of station A. The difference between the two power stations (A and B) regards the time of their initiation; the station A has been working for 21 years, while the station B for 12 years. DeJonckheere and Vand De Voorde (1977) and Červa et al. (in press) also stated that the distribution of *N. fowleri* may be bound up with the cooling waters of older factories.

The finding from the steam condensor of station A shows its possible role as an incubator and regular source of pathogenic amoebae pollution for its own complex of cooling waters. One can expect that the variable occurrence of pathogenic *N. fowleri* in this environment seems to be bound up primarily with irregular discharging of the amoebae from the steam condensor as a site for their continuous growth. On the other hand, according to DeJonckheere (1978), further factors may give different chances for pathogenic *N. fowleri*, resulting in such a decrease in their number which diminishes the chances of isolation.

The most interesting finding in this study is the frequent occurrence of pathogenic strains in the distant river. As mentioned above, the electric power station A is discharging its warm effluents partly into the river but the sampling site is about 13 km distant from the station outlet. The isolation of thermotolerant pathogenic strains in samples taken in the months when the temperature of water was low suggested that the amoebae occurrence was neither a consequence of proliferation permitting conditions nor a resting form of amoebae introduced a few months earlier (DeJonckheere and Van De Voorde 1977). Also in this case one can expect the periodical discharging of amoebae from the steam condensor of station A. More rapid water stream of the navigable canal connecting the lake nearest to the outlet of station A and the river may permit the distribution of pathogenic amoebae to a distant place. Also lately we did not find favourable conditions for continuous settlement of amoebae in the environment and supposed that their distribution in discharging waters was accidental (Červa et al., in press). More extended study is actually in progress; a. o. we could not find any strains of *N. fowleri* in other neighbouring waters not receiving thermal discharges.

The results of this study, as well as our recent study (Červa et al., in press), confirm the opinion of DeJonckheere (1978) that the water temperature itself does not secure the possibility of *N. fowleri* isolation. In consequence it is difficult to predict the amoeba distribution in thermal waters and even to exclude its occurrence in non-polluted waters. However, one has to accept the opinion that thermal polluting factories are the main— if not the only one—source of contamination in our climatic zone. The distribution of pathogenic *N. fowleri* in discharges of these factories emphasizes the necessity of parasitological examinations of cases of meningoencephalitis among the population who uses waters polluted by these discharges for water sports.

NAEGLERIA FOWLERI В ВОДАХ, ПОДОГРЕВАЕМЫХ СТОКАМИ ЭЛЕКТРОСТАНЦИЙ

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Резюме. Два комплекса озер и каналов, доставляющих воду для двух электростанций, их испарительные конденсаторы и соседнюю реку обследовали на наличие *Naegleria fowleri* при помощи культивации. Обследования осуществились в Польше в 1974—1980 гг. Было

выделено 64 штаммов *N. fowleri*, в том числе 13 штаммов вирулентных для мышей при интраназальном введении. Амебы были выделены из испарительного конденсатора электростанции А и из вод, подогреваемых стоками этой электростанции. Патогенные штаммы *N. fowleri* встречались также в соседней реке, соединенной с водной системой электростанции. Результаты показывают возможную роль испарительного конденсатора А как инкубатора и регулярного источника патогенных амеб не только для своей охладительной водной системы, но и для соседней реки.

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