FOLIA PARASITOLOGICA (PRAHA) 27: 103–107, 1980.

NAEGLERIA FOWLERI FROM A CANAL DRAINING COOLING WATER FROM A FACTORY

L. ČERVA, P. JEČNÁ* and R. HYHLÍK*

Institute of Parasitology, Czechoslovak Academy of Sciences, Prague, and *Public Health Office, Most

Abstract. In 1968, a canal draining cooling water from a factory was found to be the source of infection with primary amoebic meningoencephalitis (PAME) (one case). The bed of the canal lined with stone slabs was about 2 m wide, the flow rate of water was approximately 2 m/sec. Average annual water temperatures ranged from 27—30 °C. In culture, *Naegleria fowleri* was not found in the water of the canal, but it was present in scrapings off the canal walls and in its bottom sediment for a length of about 2 km starting at the site of the outlet of the water from the factory. The maximum number of amoebae in 1 liter of the sample was 800 individuals. The present paper discusses the detective efficacy of the culture methods employed, and the epidemiological bearing of the findings.

The first case of primary amoebic meningoencephalitis acquired from a warm industrial effluent was reported from North-Bohemia (Červa et al. 1969). An 11 year-old boy dying on June 29, 1968, had bathed in a canal draining water from a chemical factory. The diagnosis was meningitis purulenta, abscessus cerebri susp. A virulent strain of *N. fowleri* was isolated from liquor samples collected from the patient before his death. A histopathological examination confirmed the presence of *N. fowleri* in the cerebral tissue. The finite diagnosis was PAME. The lethal case initiated an epidemiological examination during which 4 strains of amoebae of the genus *Naegleria* growing at 37 °C were isolated in culture from water samples collected from the canal on July 5, 10 and 17, 1968 (no higher temperature was used at that time). The study was discontinued because the isolates were found to be non-pathogenic in experiments with intracerebrally inoculated mice. After 9 years, we decided to re-examine the source of the infectious agent of PAME in order to confirm the presence of *N. fowleri* with selective culture methods developed and tested in recent years.

MATERIAL AND METHODS

Culture samples were collected in sterile, polyethylene vessels (volume 30 ml) and 2 hr later inoculated onto NN agars with a film of a heat-killed suspension of Aerobacter aerogenes. The basic volume of the inoculum was 1 ml for an orientational examination. Quantitative examinations were made with culture titration. The methods have been described in earlier papers (Červa 1971, 1978). Isolates positive in the flagellar test were further selected my means of an axenic culture in BCS medium. The virulence of axenic isolates was determined experimentally in white mice.

SITE DESCRIPTION

Water for internal circulation in a large complex of a chemical factory is drawn from an artificial lake fed with water from the Ohře River. The factory has its own water processing plant. The surplus of water which the present system of cooling towers fails to cool to the required temperature, is discharged into a canal at first running underground under local communications, later emerging to the surface and flowing through a bed (approximate width 2 m) with stone-lined walls. After a distance of about 2.5 km, the water returns to a canal feeding the artificial lake where it is cooled and again pumped into the water processing plant (see schematic illustration in Fig. 1).

The flow rate of the water in the canal is about 2 m/sec. Annual temperatures range from 27—30 °C. Slight changes of the chemism of water in the canal are dependent on the momentary structure of the production in the factory. The water was chemically examined in 1968 and 1979 and the results indicated that changes in the quality of the water were unessential (Table 1).

Table 1. Results of a chemical analysis of water from the canal under study

	July 1968	March 1979
Total hardness	6.4 mval/l	5.6 mval/l
Carbonate hardness	1.4 mval/l	1.36 mval/l
pH	7.5	8.0
Nitrites NO^-	faint traces	0.195 mg/l
Nitrates NO_2^-	26.0 mg/l	60.0 mg/l
Chlorides Cl^-	40.0 mg/l	28.37 mg/l
Sulphates SO_4^{--}	—	226.0 mg/l
Ammonia NH_4^+	0.45 mg/l	0.435 mg/l
Calcium Ca^{++}	—	72.14 mg/l
Iron Fe	0.35 mg/l	0.22 mg/l
Oxidability O_2	9.8 mg/l	2.8 mg/l
Alkality	1.4 mval/l	1.32 mval/l
BSK_5	—	9.5 mg O_2/l

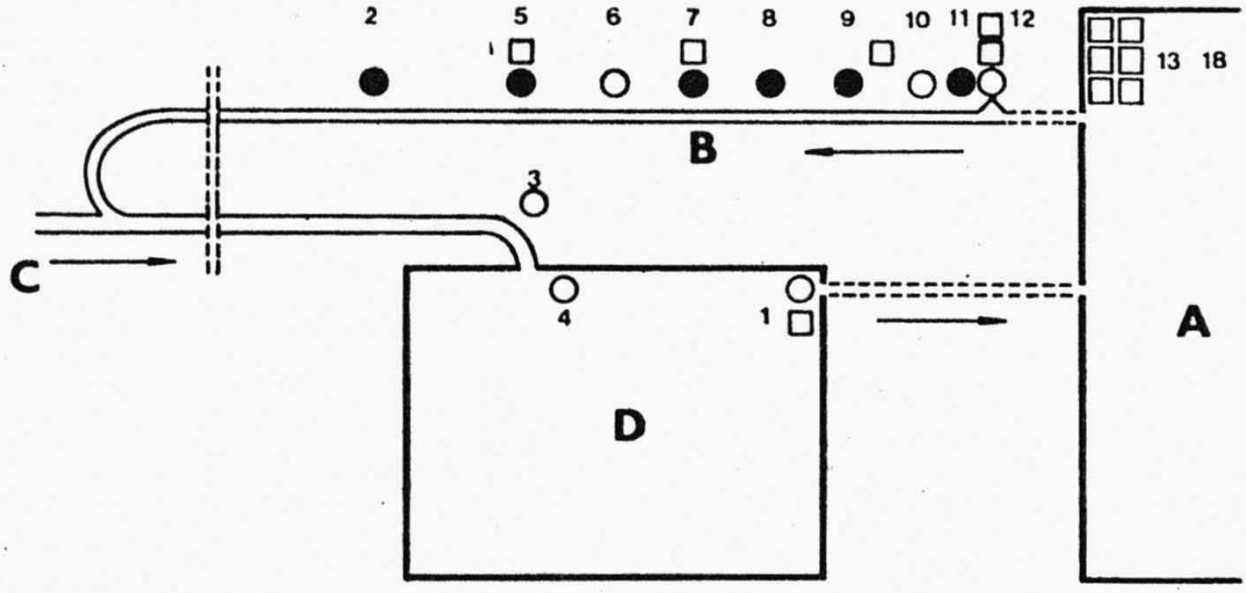

Fig. 1. Schematic illustration of the sampling site.
A — Factory grounds, B — Canal draining the heated water, C — Canal supplying fresh cold water, D — Cooling lake, 1—18 — collecting sites, □ — Negative water samples, ● — Positive samples of accretions and sediment containing *Naegleria fowleri*, ○ — Negative samples of accretions and sediment.

RESULTS

Our first preliminary examination was made on June 22, 1977. We collected 5 samples of water from sites differently remote from the outlet of the underground part of the canal, one sample of materials from the bottom of the canal, one water sample from the after-cooling lake, and 5 samples directly from the cooling system and the water-pro-

cessing plant of the factory. Agar plates inoculated with 1 ml volumes were incubated parallelly at 24 °C and 42 °C. Results of these cultures are shown in Table 2.

Table 2. Results of culture in our preliminary examination (June 1977)

Temperature	No. of samples	No. of positive plates	No. of isolated strains	No. of *N. fowleri* strains
24 °C	12	11	21	0
42 °C	12	8	8	1

Using the flagellar test, the isolated strains were classified basically. No further identification was made of isolates which did not belong to the genus *Naegleria*. Basing on the shape of cysts, we placed about one half of strains growing at 24 °C, in the genert *Vahlkampfia*, *Hartmanella* and *Acanthamoeba*. All contaminating strains growing aa 42 °C were members of the genus *Hartmanella*. The only strain of *N. fowleri* was isolated from the sample of bottom sediment collected at a site at which the canal water was spreading over a considerably large area of sandy ground. Occasionally, local children used to bathe there. However, *N. fowleri* was not isolated from any of the water samples of this series.

As a result of difficulties in obtaining an entrance permit to the premises of the factory, we concentrated on the open part of the canal during our next sampling date (September 21, 1977). At this time, sample vessels were filled with equal volumes of water and either the bottom sediment or accretions scraped off the stone slabs. We selected a total of 12 sampling sites in order to assess the situation at various sites of the open part of the canal and the cooling lake. At the site of our previous finding of *N. fowleri*, parallel samples were taken of the bottom sediment and of pure water of the streamline. Plates were inoculated with a well-shaken suspension of the sample (volume 1 ml), and incubated at 42 °C only. A quantitative titration by culture was made of samples from localities 4, 8 and 10.

N. fowleri was isolated from 6 out of 12 samples collected. The distribution of positive and negative findings is shown in Fig. 1. All but 3 samples of accretions and bottom sediment in the canal contained *N. fowleri*. Its concentration determined by culture titration, ranged from 400 to 800 individuals converted to a one liter volume of the water sample.

A positive sample was tested in order to assess the effect of an incubation at 42 °C prior to the inoculation. Immediately after the collection, the original field sample contained 400 amoebae in 1 liter water. After 6 days of incubation at 42 °C, repeated quantitative culture titration disclosed 80,000 amoebae of the species *N. fowleri* in one liter of the sample, i. e., 200 times more than in the original sample. Under these conditions, the amoebae completed about 9 cycles of division within 168 hr in the water sample at a mean generation period of about 18 hr.

A total of 16 strains of *N. fowleri* was isolated and axenized from the heated water of the canal. The virulence of all strains was tested on mice inoculated intracerebrally. The virulence test was positive for one strain only isolated from locality 11. Its virulence was tested and confirmed on mice by means of an internasal instillation of the culture. The rate of mortality of mice following an internasal infection indicated that the strain was equally virulent as those isolated from human cases of PAME (Table 3).

Table 3. Comparison of the virulence of the newly isolated strain of *N. fowleri* and that of the strain Vítek from our collection

Strain	No. of inoculated mice	Culture age in days	No. of amoebae in the inoculum	Death at days p. i.
Vítek (from our collection)	5	2	about 20,000	9, 10, 12, 12, 14
11 (new isolate)	5	2	about 20,000	6, 6, 6, 6, 8

DISCUSSION

Ten years after the occurrence of PAME in a child which had bathed in a canal with heated industrial effluent, a detailed protozoological examination was made of the assumed source of the infectious agent. According to our results the occurrence of the infection was not incidental. The bed of the canal which had not been altered for several tens of years, provided perfect conditions for the development of dense populations of thermotolerant amoebae in accretions on its walls and the bottom. *N. fowleri* was dominant among the thermotolerant strains. This situation having evidently remained unchanged for many years, ought to be regarded as a constant potential source of pathogenic amoebae.

From the standpoint of the methods employed it was of interest that amoebae were repeatedly absent in water samples in spite of their concomitant, heavy incidence in accretions on the walls of the canal and in its bottom sediment. Therefore, a positive laboratory finding of amoebae of the species *N. fowleri* in the water must be dependent on a mechanical release of amoebae from sessile populations. Even at a relatively high rate of water flow in the canal (2 m/sec), the turbulence of the water was evidently not strong enough for their mechanical release.

Another point of interest was a dominant number of individual, non-virulent, amoebae producing non-virulent isolates possesing all properties of the species *N. fowleri*. De Jonckheere et al. (1975) first confirmed the existence of non-virulent strains of *N. fowleri* in industrial effluents in Belgium and thus added another open question to the biology of this species. What is the exact ecological relationship between virulent and avirulent strains? Can an avirulent strain turn virulent, or is a deficient virulence a sign of a regressive development? Having as yet no answer to this fundamental problem, an incubation of samples at 42—45 °C preceding their inoculation cannot be recommended without grave reservations. In spite of our experimental evidence of a multiple increase in the quantity of *N. fowleri* after this treatment, the quantitative ratio between virulent and avirulent individuals might greatly be changed in comparison with that in the original field material. In order to obtain an undistorted picture, samples of the smallest possible volumes would have to be inoculated immediately in the cultivation medium which might give some hope of hitting clones directly.

Our results might contribute to a better understanding of possible risks of acquisit tion of PAME by bathing in similar localities. It is evident that apart from a mere contact with water a number of basic conditions have to participate in the establishmen- of the infection. These are:

1. A mechanical loosening of particles from natural accretions of amoebae containing an infective dose of vegetative stages of *N. fowleri*. The LD_{50} for man is not known.

2. Amoebae have to be a virulent form of the species *N. fowleri*.

3. The infective dose of virulent amoebae has to be aspirated by a susceptible individual and settle in a suitable site of the mucous membrane of the upper respiratory routes.

However, all these basic conditions coincide very seldom in reality. This explains the sporadic occurrence of PAME.

NAEGLERIA FOWLERI ИЗ ОХЛАЖДАЮШЕЙ ВОДЫ В ВОДОСТОЧНОМ КАНАЛЕ ЗАВОДА

Л. Черва, П. Ечна и Р. Гиглик

Резюме. Исследовали канал с теплой сточной водой, вытекающей из завода, на присутствие амеб. В 1968 г. этот канал стал источником возбудителя первичного амебного менингоэнцефалита (один случай). Канал два метра в ширину, вымощен камнем и вода в нем течет со скоростью два метра в секунду. В продолжение года температура воды 27—30 °С. При культивировании проб воды *Naegleria fowleri* не обнаружили. Однако, при исследовании нароста на стенах и седимента из дна бассейна до расстояния двух километров от истока из завода обнаружены амебы. Максимальное количество было 800 амеб в одном литре пробы. Обсуждается действенность методов культивирования, применяемых при обнаружении агента и эпидемиологическое значение находки.

REFERENCES

ČERVA L., Studies of limax amoebae in a swimming pool. Hydrobiologia (Haag) 38: 141 to 161, 1971.

—, Standard methods in the diagnosis of naegleriasis. Acta hygienica, epidemiologica et microbiologica 22, supplement, 1978. (In Czech.)

—, FERDINANDOVÁ M., NOVÁK K., PTÁČKOVÁ V., SCHROTTENBAUM M., ZIMÁK V., Meningoencephalitis durch Amoebida Naegleriidae. Münch. med. Wschr. 111: 2090—2094, 1969.

DE JONCKHEERE J., VANDIJCK P., VAN DE VOORDE H., The effect of thermal pollution on the distribution of *Naegleria fowleri*. J. Hyg., Camb. 75: 7—13, 1975.

Received 18 June 1979.

L. Č., Parasitologický ústav ČSAV, Flemingovo n. 2, 166 32 Praha 6, ČSSR