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LIMAX AMOEBAE IN FIVE SWIMMING POOLS IN STOCKHOLM

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Abstract. Five indoor swimming pools in Stockholm were inspected for the presence of potentially pathogenic limax amoebae. The highest concentration found was 700 amoebae in 1 litre of water in the pool. Only one strain of *Naegleria gruberi* was isolated. Other isolates were members of the genera *Flabellula*, *Hartmannella* and *Acanthamoeba*. In animal experiments 33 amoeba clones proved to be nonpathogenic. This, however, does not exclude the possibility of an outbreak of primary amoebic meningoencephalitis in the visitors of swimming pools. Conditions favourable for the multiplication of amoebae were found in all pools independent on the technology of water treatment.

Since 1965, about 60 cases of primary amoebic meningoencephalitis (PAME) of men have been registered in different parts of the world (Červa 1970). The causative agent of this disease — amoebae of the genus *Naegleria* — belongs to the group of limax amoebae known as free-living organisms in soil and water. Most of the deceased persons became infected by bathing outdoors. Surprising was the occurrence of PAME found in 16 visitors of an indoor swimming pool in Northern Bohemia (Červa et al. 1968) and in 3 persons in Antwerp — Belgium (Jadin et al. 1971). In spite of the sufficiently high levels of free chlorine used for disinfection, populations of limax amoebae were unexpectedly high in the water, and on all inside surfaces of the pool and in the recirculation system (Červa 1971). Later, a similar high incidence was found in Czechoslovakia in several other indoor swimming pools with chlorinated water. During a short stay in Stockholm (September 1969) we investigated the state of limax amoebae populations in some of the indoor swimming pools of the Swedish capital. A short survey of the results is given in this paper.

MATERIAL AND METHODS

Five swimming pools with different systems of water technology were chosen for investigatio n The principal data on these pools are given in Table 1. These were obtained from management technicians, but could not be verified for lack of time.

The exact incidence of limax amoebae was determined in the superficial water layer in the centre of all examined swimming pools. The method of culture titration on agar plates was used for this purpose. A detailed description of all procedures and culture methods is given in an earlier paper (Červa 1971). Inoculated plates were incubated at 37 °C and only strains capable of growth at this temperature were considered.

Table 1. Technical data on the investigated swimming pools.

Swimming pool	Dimension in meters	Volume in m³	System of water technology	Free chlorine level	pH
Liljeholms 1929	16.3 × 6	243	chlorine-sand Na_2CO_3	2.5 mg/l	8.8—9
Forsgrenska 1939	33.3 × 12	1800	intermit. sedim. rapid filtration sand filters chlorination	0.4—0.6 mg/l	7.1—7.3
Åkeshov 1959	25 × 12.5	760	celit filters with MgO chlorination	2.0 mg/l	9.2
Eriksdals 1964	50 × 17	1430	intermit. sedim. sand filters chlorination	0.6 mg/l	7.4
Gymnast. Inst. 1968	25 × 12.5	800	intermit. sedim. sand filters rapid filtration chlorination	0.4 mg/l	7.3—7.6

Also the bottom, walls and wooden steps of the swimming pools were tested for the presence of amoebae by means of cotton swabs on skewers. Each swab with the scooped sample was broken off the skewer using sterile pincers, and pressed into the agar layer in a Petri dish. Only approximate quantitative data could be obtained by this method. The findings were marked with crosses from 1 to 3 in number.

Results presented in this paper were obtained from a single collection of samples. For the identification of the species in some of the isolates we tried to utilize the criteria proposed by Page (1967 *a*, b, 1968). Pathogenicity tests were accomplished in mice (weight 10 to 13 g) inoculated intracerebrally with a suspension of amoeba trophozoites washed off the surface of the agar plates by means of 3 ml of sterile saline, and concentrated by gentle centrifugation to the volume of 0.5 ml.

RESULTS

Table 2 shows the results of our short study. These were most favourable in culture of water from the two oldest swimming pools. The level of free chlorine and pH value were exteremely high in the one pool, in the other the classical system of water treatment with practically neutral pH value was used. The least favourable were those from the Åkeshovbadet with its unusual celit-filtration and high chlorine and pH value.

Table 2. Findings of limax amoebae in the swimming pools under study

Swimming pool	Number of amoebae per 1 litre	Incidence of amoebae		
		bottom	walls	wooden steps
Liljeholms	10	+ + +	+	+ + +
Forsgrenska	10	0	0	+ + +
Åkeshov	700	0	+	+ + +
Eriksdals	120	+	0	+ + +
Gymn. Inst.	100	0	+ +	+ + +

In all the pools under study rich populations of amoebae were found on the macerated surface of the hardwood steps leading into the pools. Strangely enough these hardwood steps were used also in the new swimming pools. They seem to be the main source of amoebae incidence in the inside equipment of the pools.

In successive laboratory studies 33 clones were obtained from amoebae isolates. The proportion of the species composition of isolates from free water and of sessile forms and cultured from the cotton swabs is given in Table 3.

Table 3. Species composition of amoebae isolates

Species	Number of isolates	
	Water samples	Cotton swabs
Flabellula mira	5	1
Flabellula platypodia	3	0
Acanthamoeba polyphaga	2	0
Hartmannella vermiformis	2	3
Hartmannella exudans	5	1
Hartmannella limacoides	2	0
Naegleria gruberi	0	1
Unidentified species	5	3
Total	24	9

Table 4 shows the distribution of the species identified in the different swimming pools. In comparison with the species found in the swimming pool in Ústí—Czechoslovakia (Červa 1971) there is a striking difference in the frequency of the genus *Flabellula* and *Acanthamoeba*. Floating forms of *Flabellula* belong to the most common findings in the Stockholm swimming pools. It is noteworthy that the only strain of *Naegleria gruberi* was isolated from the Forsgrenskabadet which is one of the best kept pools.

In animal experiments none of the tested isolates showed signs of pathogenicity.

Table 4. Amoeba species identified in Stockholm swimming pools.

Species	Liljeholms	Forsgrenska	Åkeshov	Eriksdals	Gymnast. Inst.	Total
F. mira				4	2	6
F. platypodia			3			3
A. polyphaga			2			2
H. vermiformis	1		3	1		5
H. exudans	1		5			6
H. limacoides			2			2
N. gruberi		1				1
Unidentified	1		3	3	1	8
Total	3	1	18	8	3	33

DISCUSSION

Considering the fact that the quality of water in swimming pools should practically be the same as that of drinking water the high incidence of limax amoebae in these sites is most undesirable. In addition this study deals with amoeba strains only fulfilling the main condition of potential pathogenicity, i.e., those capable of growth at 37 °C. The actual total population of amoebae must be considerably more numerous. This indicates that the density of the bacterial flora — a necessary food substrate for amoebae — is extremely high. Bacteriological culture methods used nowedays as indicators in swimming pool supervision are not reliable as regards the configuration of the water biocenosis. Our results offer further evidence that water treatment systems including those used in the five swimming pools in Stockholm are incapable of preventing the incidence and multiplication of limax amoeba populations. Favourable results obtained in the Forsgrenskabadet seem to indicate that the classical system of water treatment may be as effective or even better than its recent modifications. Many other factors, however, influence the quality of water. The marked differences in finding in the Liljeholms- and Åkeshovbadet may prove that high levels of free chlorine with high values of pH cannot control sufficiently the multiplication of amoebae. As regards the Liljeholmsbadet the primitive system — sand, chlorine, natrium bicarbonicum — necessarily increases the concentration of salts in the water. This appears to be harmful not only to the amoebae but perhaps also to the visitors of the pool.

Sessile populations of amoebae on suitable substrates develop without any direct connection with conditions in free water as found in our earlier study (Červa 1971). All porous materials of the inside equipment of the pools should, therefore, be exchanged for other nonporous materials. A system of rigorous mechanical cleaning of the bottom and walls should be developed to avoid the formation of biological coatings.

Further detailed studies on the biocenosis in swimming pools are necessary to find adequate methods to destroy or control the populations of limax amoebae in these institutions. This is, at present, the only way how to prevent the danger of a further incidence of primary amoebic meningoencephalitis.

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АМЕБЫ ТИПА LIMAX ОБНАРУЖЕННЫЕ В ПЯТИ ПЛАВАТЕЛЬНЫХ БАССЕЙНАХ Г. СТОКГОЛЬМ

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Резюме. Обследовано пять закрытых плавательных бассейнов в г. Стокгольм на наличие потенциально патогенных амеб типа limax. Обнаружена самая высокая концентрация 700 амеб в 1 литре воды бассейна. Выделен 1 штамм *Naegleria gruberi*, затем амебы родов *Flabellula*, *Hartmannella* и *Acanthamoeba*. На подопытных животных 33 амебных клона оказались безвредными для человека. Однако, этот факт не исключает возможность вспышки первичного амебного менинго-энцефалита среди посетителей плавательных бассейнов. Во всех бассейнах обнаружены благоприятные условия для размножения амеб независимо от техники гигиенической обработки воды.

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