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PRESENCE OF VIRULENT STRAINS OF AMPHIZOIC AMOEBAE IN SWIMMING POOLS OF THE CITY OF SZCZECIN

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Abstract: The studies included 10 public indoor swimming pools and 3 public open-air swimming pools located in the city of Szczecin. In 2003, water samples were collected for detection of virulent amphizoic amoebae strains. In all pools, 16 strains of thermophilic *Acanthamoeba* spp. were isolated, 5 of which proved virulent for mice. No pathogenic strains were detected in the water sampled in the indoor swimming pools, and the virulent strains, AD 16, AD 148, AD 166, AM 17, and AM 148, were found only in the open-air swimming pools. The post-mortem studies of mice that had been inoculated with these strains revealed the amoebae invasions in brain, lungs, liver, kidneys, and spleen.

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Key words: swimming pools, amoeba, virulent *Acanthamoeba* strains.

INTRODUCTION

In the natural environment, both in waters and soil, free-living amoeba species are very common. These include amphizoic strains that are pathogenic to humans and animals. The pathogenic strains belong mainly to *Acanthamoeba* spp. and *Naegleria fowleri*, and according to most recent reports [5, 17], also to *Balamuthia mandrillaris* and *Sappinia diploidea*. Both natural and artificial bodies of water can pose a threat of infection by these protozoans for the people bathing there, and the invasion route may lead through the nasal cavity, oral mucosa, or injured skin. Due to their thermophilic properties, the risk of infection by the amphizoic amoebae is higher in heated water.

Cases of free-living amoebae invasions have been reported in Europe, the USA, Australia, Africa, and Asia [3, 10, 16, 25, 28].

Besides primary amoebic meningoencephalitis (*Naegleria fowleri*) and granulomatous encephalitis (*Acanthamoeba* spp., *Balamuthia mandrillaris*, *Sappinia diploidea*), free-living amoebae may represent an aetiological agent of pneumonia, chronic rhinosinusitis and laryngitis, and inflammatory changes of liver, kidneys, spleen, heart, cornea, and skin (*Acanthamoeba* spp.).

Studies on pathogenic properties of free-living amoebae found in natural sources of water were carried out in Poland in the area of Poznań, Gdańsk, and Lublin [1, 6, 7, 14, 20, 21, 26].

The aim of this study was to estimate the frequency of virulent amoebae strains in the water of both indoor and open-air swimming pools located in the area of Szczecin.

MATERIAL AND METHODS

In all, 72 water samples collected in 13 swimming pools, including 10 indoor pools and 3 open-air swimming pools, filled with water and available for the public during the summer. All the pools are located in the area of Szczecin.

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Each time, 1-litre water samples were collected into sterile containers, along with sediment deposited on side walls, bottoms, and outlet grids. From each indoor pool (Nos. 1–10, see Tab. 1), water was sampled 6 times during the year 2003. Open-air swimming pools were sampled 4 times during the summer (June–September 2003).

Water temperature in the indoor pools was 27–33ºC, chlorine concentration 0.3–0.5 µg/ml. In one of the indoor pools, i.e. No. 10, water was additionally conditioned with ozone, whose concentration was 0.1 mg O_3/dm^3. The water in the open-air pools was also chlorinated, and water temperature ranged during the day from 27–30ºC.

Seitz filters and fibreglass drains GF50 were used for water filtration. The drains, along with the deposit, were placed on agar medium (NN Agar) covered with inactivated *Escherichia coli* and incubated at 37ºC for approx. 72 hours. In order to isolate thermophilic strains, proliferated amoebae were passaged at 42ºC.

Taxonomic identity of genera was determined, based on morphological traits of trophozoites and cysts (in stained slides), as well as based on flagellation test. The amoebae slides were stained with trichrome according to Gomori and Wheatley, following preservation in Schaudin's fluid [13].

Biological tests for virulence of the thermophilic strains were carried out through intranasal inoculations of 12–15-g inbred Swiss mice. The administered amount of 3 µl of suspension contained 10,000–20,000 amoebae. Each strain was tested on 3 mice. The control mice received physiological saline in the same quantity. Depending on the intensity of visible symptoms, 4–21 days after the inoculation, the mice were killed with intraperitoneal injection of 2 ml/kg body weight pentobarbital (Morbital) and subjected to post-mortem examination.

In order to determine the localisation of amoebae in the oculated mice, during autopsy, pieces of the brain, lungs, liver, kidneys, and spleen were take and placed on NN agar medium covered with inactivated *E. coli* bacteria and left for 48 hours at 42ºC.

The Local Commission for Experiments on Animals had approved the tests on animals carried out for this study.

RESULTS

The cultured amoebae strains and their thermal properties are presented in Table 1. The 72 collected samples of water allowed culturing of 43 strains of amoebae (59.7%) at 37ºC, of which 16 (37.2%) strains demonstrated ability to proliferate at 42ºC. Thermophilic strains had been collected in 3 indoor and 5 open-air swimming pools. In all, 9 were isolated from indoor pools, while 7 thermophilic strains were isolated from open-air swimming pools.

Based on the morphological traits and negative flagellation test, all the cultured strains were classified as *Acanthamoeba* spp.

Table 1. Isolated strains of Acanthamoeba spp. and their thermal properties.

Sampling site	Strain	Thermal tolerance	
		37ºC	42ºC
Indoor swimming-pool No. 1	OR 422	++	+++
	OR 254	+++	+++
	OR 283	++	-
	OR 210	+++	+++
Indoor swimming- pool No. 2	Mal 216	+	-
	Mal 2810	++	-
	Mal 283	+	-
	Mal 159	+	-
Indoor swimming - pool No. 3	B26 562	++	+++
	B26 2810	+++	++
	B26 159	++	-
Indoor swimming - pool No. 4	LO 262	+	++
	LO 254	++	-
	LO 523	+++	+++
Indoor swimming - pool No. 5	Dun 216	+	-
	Dun 523	+	-
	Dun 283	+++	-
	Dun 159	++	-
Indoor swimming- pool No. 6	KK 2810	+++	-
	KK 192	+++	+++
	KK 159	++	-
Indoor swimming - pool No. 7	Rad 2710	+	-
	Rad 147	++	-
Indoor swimming - pool No. 8	SDD 2810	++	-
	SDD 192	++	-
	SDD 283	+	-
	SDD 159	+++	+++
Indoor swimming - pool No. 9	SDM 192	++	-
	SDM 283	+	-
	SDM 159	++	-
Indoor swimming - pool No. 10	Wsm 192	++	-
	Wsm 283	+	-
	Wsm 159	+++	-
Open-air swimming pool No. 1	SDO 216	+	-
	SDO 217	++	+++
	SDO 167	+	-
Open-air swimming pool No. 2	AD 16	+++	+++
	AD 148	+++	+++
	AD 166	++	+++
Open-air swimming pool No. 3	AM 17	+++	+++
	AM 126	+	-
	AM 148	+++	+++
	AM 166	++	++
total		43	16

(–) no amoeba proliferation in the culture; (+) weak proliferation; (++) moderate proliferation; (+++) very intensive proliferation of the culture

Table 2. Presence of *Acanthamoeba* spp. in the organs of inoculated mice.

Strain	dpi	Brain	Lungs	Liver	Kidneys	Spleen
AD 16	3	+	+	+	+	+
AD 148	14	+	-	-	-	-
AD 166	14	+	+	-	-	-
AM 17	3	+	+	-	+	+
AM 148	14	+	+	-	-	-

dpi - days post inoculation

Biological tests carried out on mice demonstrated that 5 thermophilic strains were of pathogenic character, AD 16, AD 148, AD 166, AM 17, and AM 148 (Tab. 2). All the strains caused encephalic invasion in the inoculated animals. AD 16 was the most virulent of all. As early as 3 days post-inoculation (dpi), the mice were apathetic, did not drink or eat, and amoebae were cultured from all their dissected organs. AM 17 was a similarly virulent strain, although these amoebae were not isolated from liver. AD 166 and AM 148 strains were of lower virulence. Changes in mice behaviour were not visually detectable until approx. 14 dpi, while only brain and lungs of the dissected organs allowed isolation of amoebae. The strain AD 148 infected only the brain of the inoculated animals.

DISCUSSION

Positive results of amoeba isolation from the water of Szczecin swimming pools demonstrate that water chlorination with acceptable concentration of 0.2–0.5 µg/ml and additional ozone treatment does not destroy ubiquitous free-living amoebae. Experimental studies by many authors have demonstrated a varied sensitivity to chlorine of pathogenic and non-pathogenic strains of free-living amoebae. Pathogenic *Acanthamoeba* strains studied by De Jonckheere and Van de Voorde [4] were much more resistant to chlorine compared to those non-pathogenic. The authors have demonstrated that *A. culbertsoni* pathogenic strain showed positive growth of culture even after 3-hour contact with 40 µg/ml of chlorine. According to Rivera *et al.* [24], only chlorine concentration higher than 1.5 mg/ml effectively destroys spore forms of free-living amoebae.

Griffin [9] suggests that heating and chlorination of water eliminates other, competitive microbes, which creates better conditions for the resistant amoebae.

Permanent heating of water in indoor swimming pools is favourable for persisting thermophilic forms of amoebae, which can include strains pathogenic to humans; however, Mazur *et al.* [19] demonstrated that if kept at 4ºC for as long as 25 years, the cysts do not lose their viability and infectivity.

In the indoor swimming pools of Szczecin, 9 thermophilic strains of *Acanthamoeba* were found that did not show virulence.

The best known descriptions of invasions by *Naegleria fowleri* have come from former Czechoslovakia, where swimming-pool water was a source of invasion [3]. Studies by Kadlec *et al.* [11, 12] carried out in this particular swimming pool demonstrated that virulent amoebae persisted in cracks in the walls. Moreover, pathogenic amoebae in Europe were also detected in swimming-pool water in Sweden [2], Germany [15], and Finland [27].

In the presented studies, 7 thermophilic strains were cultured from samples collected in all the open-air bathing pools, of which 5 strains were of virulent character. Atmospheric precipitation and organic pollution transported by people from the dirt in the pool vicinity may represent factors positive for virulent strains. This confirms the view by Rivera *et al.* [23] who suggest that amoebae do not occur continuously in chlorinated water of swimming pools, but are being introduced over and over again with soil by people.

Naegleria was not found in any of the swimming pools in our city. The reason underlying the much lower frequency of *Naegleria fowleri*, according to Mazur *et al.* [19], is the considerably weaker survivability of the cysts. *Naegleria* cysts do not survive beyond 6 months, while *Acanthamoeba* cysts - can live for even more than 10 years.

Due to intranasal inoculation of the mice, encephalic and pulmonary invasions can be treated as primary, while those in liver, kidneys, and spleen – as secondary. Secondary, non-encephalic invasions in experimental mice have been also described by Mazur and Jóźwiak [20], and Mazur *et al.* [18].

Despite the fact that the investigations revealed the presence of pathogenic amoebae in natural and artificial bodies of water in the studied areas of Poland, only 2 cases of human corneitis, including one referring to a person wearing contact lenses, caused by *Acanthamoeba* invasion, have been reported so far [8, 22]. The authors indicate amoeba-polluted water as a source of infection.

CONCLUSION

The experiments demonstrated that the risk of contracting virulent strains of free-living amoebae by humans is higher in the open-air bathing pools compared to indoor swimming pools.

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