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Histological studies of selected organs of mice experimentally infected with *Acanthamoeba* spp.

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Histological studies of the brain, lungs, liver, kidneys, heart, and the spleen were carried out in mice previously infected with 6 pathogenic strains of free-living amoebae of the genus Acanthamoeba. *The potential virulence of the strains studied was determined on the basis of re-isolation of the amoebae from the organs of the inoculated animals and by the extent of the histopathological changes inflicted. The most virulent was strain AD16, affecting all organs of the inoculated mice, while the least virulent was strain AD148 re-isolated from the brain of a single mouse. The extent of the changes in the brain depended upon the amoebae strain, while in the remaining organs it also depended upon the duration of the infection.*

Key words: histopathology, brain, lung, kidney, liver, heart, spleen, *Acanthamoeba*, virulent strains

INTRODUCTION

Infections caused by virulent strains of free-living amoebae constitute a current medical problem. The amoebae, particularly those of the genera *Acanthamoeba* and *Naegleria*, commonly occur in all kinds of water bodies, as well as in soil, and their resting stages can be distributed at great distances together with atmospheric dust particles [25, 26]. The first human cases to be described prompted intensive studies on the epidemiological risk and an improvement in the diagnostic methods and the markers of the virulence traits of these amoebae. In the 1960s, in close proximity to the Polish border, fatal infections with *Naegleria fowleri* were diagnosed in young persons in the town of Usti nad Labem in the former Czechoslovakia [3]. The source of infection was the water in an indoor swimming pool. In Poland only cases of ocular acanthamoebosis (*acanthamoeba keratitis*) have been described [9, 16, 24]. It is very likely that the infection of other organs remains undetected. Studies on the occurrence of amphizoic amoebae in the natural environment have been carried out in the vicinities of the cities of Poznań [12–14, 22], Lublin [7, 8, 33], Gdańsk [1] and, most recently, Szczecin [10]. These studies have confirmed the ubiquity of amoebae in both natural and man-made bodies of water.

Histopathological studies on experimentally-induced cerebral and extra-cerebral infections caused by free-living amoebae have hitherto been very rare and have not provided any explicit account of their pathogenicity.

The aim of the present paper was assessment of the histopathological changes in the organs of mice in the course of experimental infection with 6 isolates of *Acanthamoeba* spp. from natural and artificial bodies of water within the city limits of Szczecin.

MATERIAL AND METHODS

The material for the present study were the organs of mice, experimentally infected with 6 strains of pathogenic amoebae of the genus *Acanthamoeba* that had been isolated earlier from natural and artificial bodies of water situated in the city of Szczecin [10].

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The virulence of the amoebae was determined on the basis of their ability to infect laboratory mice. Two-week-old Swiss mice were inoculated intra-nasally with 3 μl of suspension containing 10–20 thousand of the amoebae. Control animals were given the same volume of physiological solution. After inoculation the mice were monitored constantly.

Between the 3rd and the 14th days post infection (dpi), depending on the presence of clinical signs of infection (limited mobility, dejection, tail chasing, or emaciation), the animals were sacrificed with a peritoneal overdose of pentobarbitone (2 ml/kg body weight) and subsequently necropsied. Dissected fragments of the brain, lungs, liver, kidneys and heart were taken for histopathological study. Paraffin 5-μm sections were stained with haematoxylin and eosin. Concurrently, the fragments (5 × 5 mm) of the same organs were inoculated on NN agar and incubated at 42°C, to assess the infection intensity levels of the animals. The cultures were monitored daily by microscope for 7 days at low magnification.

RESULTS

The most virulent strain was AD 16, as this had invaded all the studied organs of the infected animals as early as 3 dpi (Table 1). All the strains of the pathogenic amoebae studied were re-isolated from the brain. The most intensive infections, however, were caused by strain AM 17. Within 6 hours of inoculation the brain samples affected by this strain had produced an abundance of trophozoites on agar plates. With regard to other strains, very few amoebae were observed within 24 hours of inoculation.

In a macroscopic assessment, the cerebral hemispheres of the inoculated animals demonstrated oedema and congestion and the extent of those changes depended on the strain and not on the duration of infection. A severe cerebral oedema, obliteration of the sulci and meningeal congestion on the entire surface area of the brain hemispheres were observed in mice necropsied 14 dpi (with strains AM 148 and AD 166) as well as in mice examined 3 dpi (with strains AM17 and AD16). On the other hand, in mice necropsied at 7 or 14 dpi (strains JG 172 and AD 148) the changes were limited to small oedema and congestion of the frontal lobes and olfactory bulbs.

The most extensive microscopic changes to the brain were observed in the animals infected with strain AM 17. Meningeal detachment was observed, particularly in the area of the frontal lobes. Numerous trophozoites, neutrophils, macrophages, plasma cells, and single multinucleate giant cells (Fig. 1) were present under the meninges. The superficial layers of the cerebral cortex featured necrotic foci with atrophy of the nerve and glia cells and minute haemorrhages. Some necrotic areas were associated with inflammatory infiltrations (Fig. 2A). Only single trophozoites and cysts, inflicting no inflammation, were encountered in the cerebral cortex (Fig. 2B).

Damage to the endothelium and delamination of the muscle fibres of some veins and arteries, as well as the presence of macrophages, neutrophils and trophozoites of the amoebae in the perivascular zone, were also observed. Damage to the molecular and granular layers of the cerebellum were detected only in a single mouse infected with strain AM 17.

Amoebae of 5 strains were re-isolated from the lungs (Table 1). Strains AD 16 and JG 172 induced higher infection intensities. As early as 6 hours following the substrate inoculation numerous amoebae were observed in the vicinity of the lung fragments. In mice infected with these strains both lungs were severely congested.

In the histological preparations the presence of amoebae was detected in the pulmonary alveoli (Fig. 3), in the perivascular spaces and in the walls and lumen of the bronchi and bronchioli. Vascular wall dam-

Table 1. Virulent properties of *Acanthamoeba* spp. strains

Strain	N	D	Number of infected mice and intensity of organ infection											
			Brain		Lung		Liver		Kidney		Heart		Spleen	
			N	I	N	I	N	I	N	I	N	I	N	I
AM 17	3	3	3	+++	3	+	–		3	+	2	+	2	+
AM 148	3	14	3	+	2	+	–		–		–		–	
AD 16	3	3	3	+	3	++	2	+	2	+	2	+	2	++
AD 148	1	14	1	+	–		–		–		–		–	
AD 166	3	14	3	+	1	+	–		–		–		–	
JG 172	3	7	2	+	3	++	3	+	3	+	–		1	+

N — number of infected mice, D — days post infection; I — intensity of infection on a scale of 0–3, (–) — absence of infection in mice

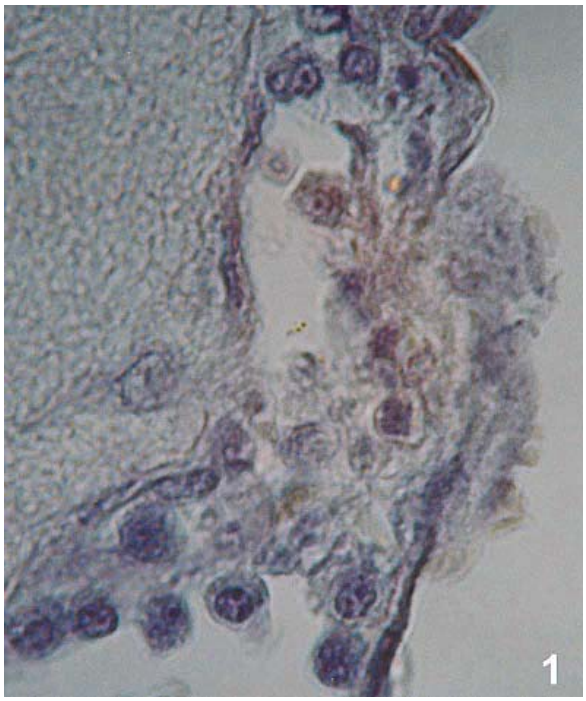

Figure 1. Exfoliation of the meninges with visible trophozoites, numerous plasma cells and macrophages. Magn. 1000×.

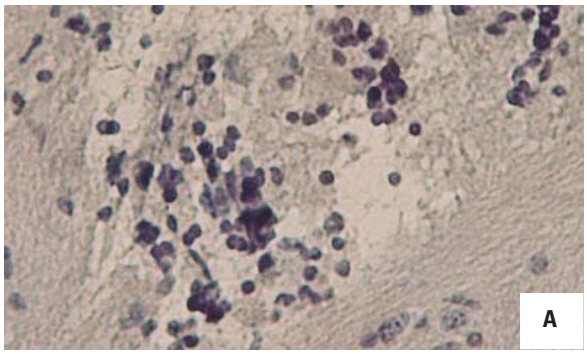

Figure 2A. Necrotic focus and inflammatory infiltration in the cerebral cortex of an infected mouse with strain AD 16 of *Acanthamoeba* spp. Magn. 400×.

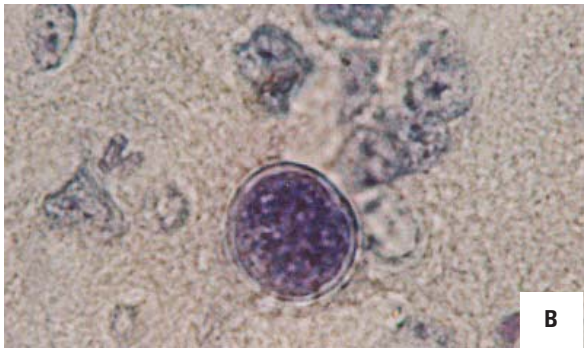

Figure 2B. A cyst of strain AD 16 of *Acanthamoeba* spp. in the cerebral cortex of an infected mouse with strain AD 16 of *Acanthamoeba* spp. Magn. 1000×.

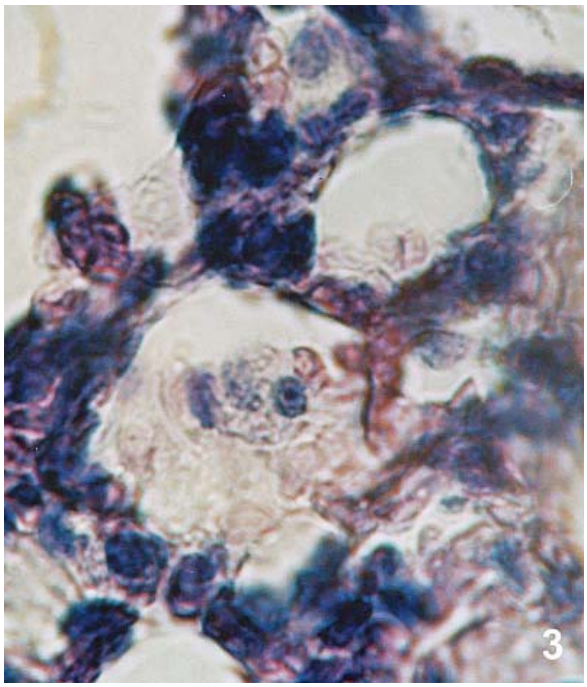

Figure 3. *Acanthamoeba* spp. trophozoites in the alveoli of an infected mouse. Magn. 1000×.

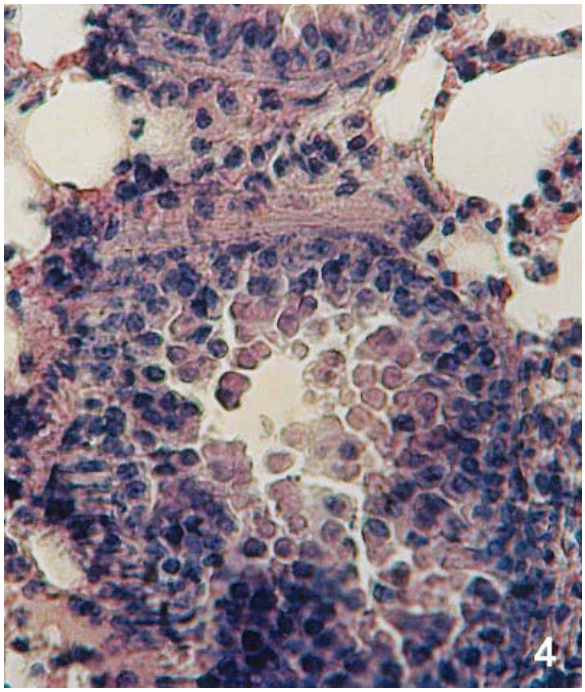

Figure 4. Hyperplasia of the epithelium of the bronchioles in the lung of a mouse infected with strain JG 172 of *Acanthamoeba* spp. Magn. 400x

age and extravasations to the parenchyma and alveolar lumen were also observed. Epithelial hyperplasia in the bronchi and bronchioli occurred in all infected mice (Fig. 4). Necrotic foci, vast abscesses and

thickening of the alveolar walls were found in mice infected with strains AM 148 and AD 166 at 14 dpi.

Only in the mice infected with strains AD 16 and JG 172 were the amoebae re-isolated from the liver. It took as many as 24 hours of incubation to obtain a few trophozoites from the inoculated liver fragments of these mice. In the microscopic pictures of the livers of mice infected with strain JG 172, vast necrotic changes with inflammatory infiltrations were visible (Fig. 5). Trophozoites, whether few or numerous, occurred sporadically. In the damaged areas spaces vacated by hepatocytes were filled with blood. On the other hand, in the livers of mice infected with strain AD 16 necropsied at 3 dpi the histopathological changes were manifested in the obliteration of the lobular architecture, the presence of small inflammatory foci and the increased activity of Browicz-Kupffer cells. In the livers of mice infected with the remaining pathogenic strains subsequent incubation yielded no amoebae and only single minute inflammatory infiltrations were observed.

Three strains of amoebae were re-isolated from the kidneys: AM 17, AD 16 and JG 172. The kidneys of mice infected with strains AM 17 and AD 16 and necropsied at 3 dpi featured haemorrhages and small inflammatory foci. In mice infected with strain JG 172 and examined at 7 dpi excessive necrotic changes of renal tubules and Bowman's capsules were found (Fig. 6). In the kidneys of mice infected with the remaining strains (AM 148, AD 148, and AD 166), yielding no amoebae throughout the incubation, only small inflammatory foci were observed in the cortical part.

The substrate inoculates of the heart muscle of the infected animals yielded only 2 strains: AM 17 and AD 16. Histopathological changes in the hearts of these mice were expressed as vast haemorrhages, muscle tissue loss and small inflammatory infiltrations (Fig. 7). The heart muscles of the remaining infected mice showed no histopathological changes.

Only the spleens of mice infected with strains AM 17 and AD 16, inoculated on agar, permitted re-isolation of the amoebae. A greater intensity of infection was observed for mice infected with strain AD 16. After 24 hours of incubation there were many more trophozoites in the vicinity of fragments of this organ than in the incubation media inoculated with organs from mice infected with the remaining strains. The spleen of the infected animals did not differ from the control in the macroscopic view. In the histological preparations of this organ taken from all the infected mice, including those yielding no re-isolated amoebae, there was hyperplasia of the white pulp around the arteriolae (Fig. 8).

DISCUSSION

The present studies provide evidence of the diversified pathogenic properties of free-living amoebae. The most virulent was AD 16, since in the mice infected with this strain all the organs studied were affected. Only from the liver was strain AM 17 not re-isolated, while JG 172 was not re-isolated from the heart. Strain AD 148 demonstrated relatively weak virulence, as it infected only one mouse and the amoebae in that mouse were re-isolated only from its brain. The results now acquired indicate the neurotropic character of all the strains studied and the presence of the amoebae in the lungs, liver, kidneys, spleen and heart should be considered secondary infections. Other authors also [14, 19, 21] found the brain to be the most frequent site of the primary infection following intranasal inoculation, although some strains may have closer affinity to the lungs, without affecting the brain. It has been demonstrated that the principal invasion route is the nose, where the amoebae cross the cribriform plate and, following the olfactory nerves, reach the brain [11]. Central nervous system infections with *Acanthamoeba* may originate from primary changes to the skin or the respiratory system [4, 29]. According to Kasprzak et al. [14] some strains of free-living amoebae demonstrate a constant organ affinity during subsequent passaging through experimental animals. Other strains, however, may affect different organs or even lose their infective properties altogether.

In the present histological study the cells of the immune system, particularly macrophages or neutrophils, were present only in the vicinity of abundant amoebae. Our observations confirm the findings of other authors. Ferrante and Abell [5] and Stewart et al. [31] in their *in vitro* studies demonstrated that the presence of neutrophils and macrophages coincided with the presence of dead *Acanthamoeba* trophozoites. On the other hand, Kremer et al. [15] and Niederkorn et al. [23] observed that in the course of experimental keratitis in animals, the macrophages played the most important role in the first line of defence.

The lack of inflammatory reaction in the vicinity of single trophozoites and cysts suggests that a much higher level of antigen is needed to trigger parasite recognition by the immune system.

The most common microscopic changes observed in the organs of the infected animals were blood extravasations which occurred as a consequence of damage and wall-continuity breakage of a vessel. Vascular damage caused by amoebae has also been emphasised by other authors [8, 18]. The histopatho-

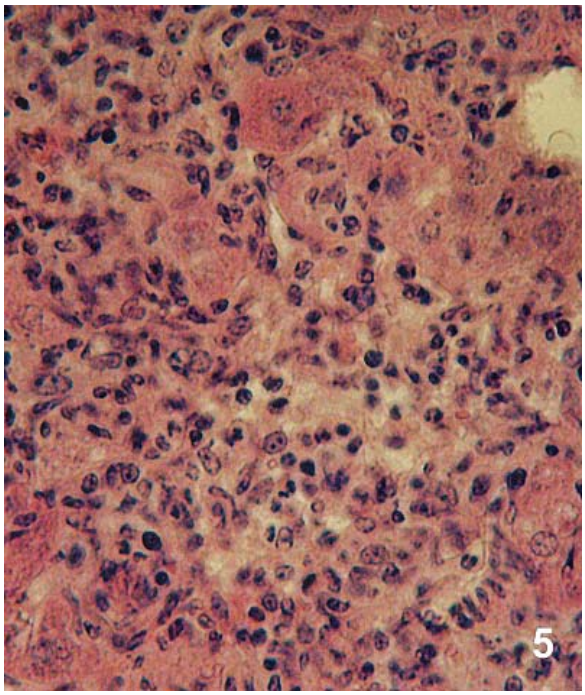

Figure 5. Extensive inflammatory infiltration in the necrotic area of the liver of mouse infected with the strain AD 16 of *Acanthamoeba* spp. Magn. 400×.

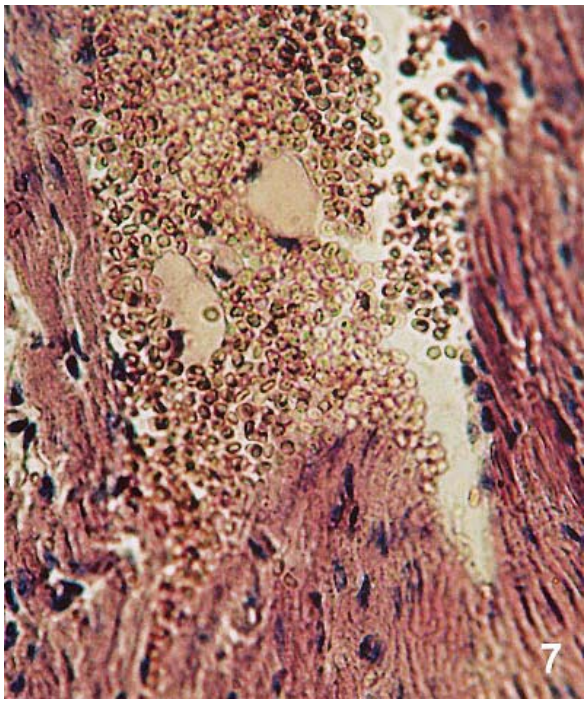

Figure 7. Hyperplasia of the white pulp in the spleen of a mouse infected with strain AD 16 of *Acanthamoeba* spp. Magn. 400×.

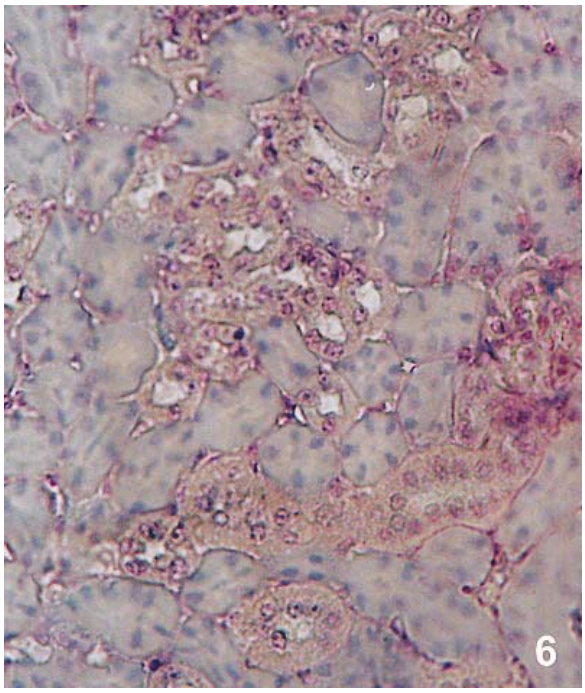

Figure 6. Necrosis of the renal tubules of a mouse infected with strain AM 17 of *Acanthamoeba* spp. Magn. 400×.

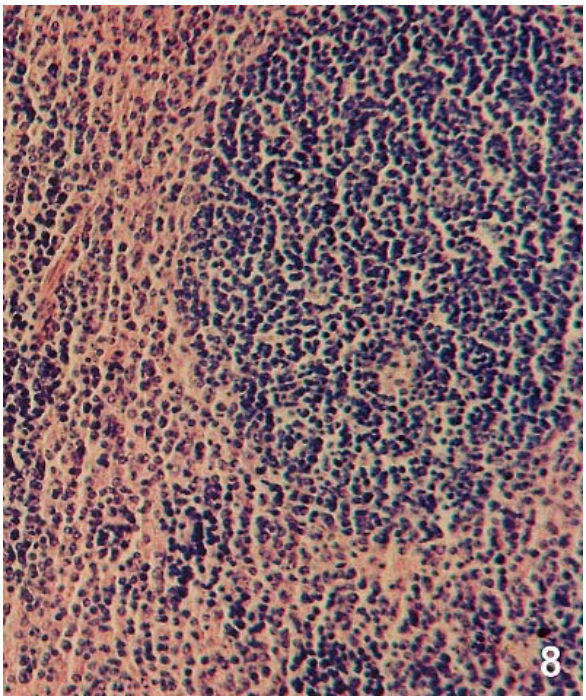

Figure 8. Extensive hemorrhage into the heart muscle of a mouse infected with strain AM 17 of *Acanthamoeba* spp. Magn. 400×.

logical changes of the infected brain observed in the present study are consistent with the observations of other researchers [7, 8, 21]. However, hyperplasia of the respiratory epithelium and necrosis of the liver and heart have not hitherto been reported among cases of extra-cerebral infection.

The results of our study and the findings of other authors indicate, on the one hand, variability in

the pathogenic properties of free-living amoebae and, on the other hand, changeability in the host resistance. It is commonly known that one aspect of the parasite-host system is a balance between the immune response of the host and the defensive mechanisms of the parasite. These relationships have been insufficiently studied in the case of *Acanthamoeba* and the human or animal organism. According to Marciano-Cabral and Cabral [18], both innate and acquired immunity play a role in the defensive host mechanisms against *Acanthamoeba*. In the light of existing knowledge, the magnitude of the symptoms and histopathological changes in the host can be a sum of many factors such as host immunocompetence on the one hand and the virulence of the parasites on the other. According to some authors studying the pathogenic properties of free-living amoebae, the intensity of the histopathological changes in the organs affected depends on the virulence of a given strain [28], while according to other authors it depends on the duration of the infection [8]. Our own observations indicate that the magnitude of cerebral changes depends on the virulence of a strain. The most virulent were strains AD 16 and AM 17. Although invasion with those strains lasted only 3 days, the changes in the brain of the animals were very extensive. On the other hand, the extent of the changes in the lungs depended on the duration of the infection. Parenchymal pneumonia ammected the entire lungs of mice with 14-day infections.

It is recommended that the malignance and virulence of the strains be tested on 2-week-old mice whose immune mechanisms are not fully developed. The fact that not all inoculated mice became infected and that the symptoms and the course of infection differ may be the result of innate immunity. Ferrante and Abell [5]. demonstrated the higher resistance of some young animals immunised with *Acanthamoeba* extract. Experimental studies on animals have demonstrated that even oral immunisation results in a high immunity against infection with parasites of the genus *Acanthamoeba* [17].

The ubiquity of the contacts of people with these amoebae can be confirmed by the results published by Èerva [2] and Niederhorn et al. [23]. These authors demonstrated that between 50 and 100% of persons examined in human populations had IgG antibodies against *Acanthamoeba* spp. This may provide a clue as to why, despite the common occurrence of these amoebae in the environment, only immunocompromised individuals acquire the infection. The relationship of rapid infection of amphizoic amoebae with immune deficiencies in those infected is known from the case descriptions of AIDS patients, transplant recipients, or patients receiving immunosuppressive therapy [6, 20, 27, 30, 32].

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