

The presence of pathogenic genotypes of free-living amoebae isolated from sandboxes in children's playgrounds in the city of Poznań, Poland

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Free-living amoebae are a potential threat to human health. Species from genus *Acanthamoeba* are responsible for two dangerous diseases of human: GAE (granulomatous amoebic encephalitis) and AK (*Acanthamoeba* keratitis), but they can also cause different tissue inflammation. The *Acanthamoeba* trophozoites and cysts can be found in many environments like dust, water, soil, waste water, bottled mineral water, swimming pools, air conditioners, and many other. Currently, there are twenty genotypes of *Acanthamoeba* (T1-T20). Each genotype is considered a different species. The most frequently occurring genotype in the natural environment as well as the most frequent one in case of causing illnesses is the genotype T4.

The aim of our study was to search for potential pathogenic genotypes of *Acanthamoeba* in the sand from children's playgrounds located in the city of Poznań. Basic material was sand collected from thirteen sandboxes located in the city of Poznań. *Acanthamoeba* were cultured on agar plates in 28şC and after 3–5 days, growth of trophozoites was observed. Identification of *Acanthamoeba* species was conducted under a microscope and additionally with molecular tools (isolation of DNA and PCR). Pathogenicity of environmental samples of *Acanthamoeba* was examined through infection of two-week-old Balb/c mice. Pieces of lungs and brain from dead or infected mice were collected and re-isolated on agar plates to confirm growth of pathogenic genotypes of *Acanthamoeba*. Determination of particular pathogenic genotypes was made by a molecular method – sequencing.

There were 15 pathogenic samples isolated from examined sandboxes. Six strains of *Acanthamoeba* were obtained from the lungs, and nine from brain. All determined genotypes belonged to genotype T4. The sand on children's playground sandboxes is not free of free-living amoebae of the genus *Acanthamoeba*, including potentially pathogenic strains. The research showed that all determined genotypes were T4. This indicates that there is a possibility of getting infection with pathogenic *Acanthamoeba*. The results of this study point for necessity of broadening routine checks for potentially pathogenic amoebae in sandboxes on children's playgrounds.