

Prevalence of Potentially Pathogenic Free-Living Amoebae from *Acanthamoeba* and *Naegleria* Genera in Non-Hospital, Public, Internal Environments from the City of Santos, Brazil

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Acanthamoeba and *Naegleria* species are free-living amoebae (FLA) found in a large variety of natural habitats. The prevalence of such amoebae was determined from dust samples taken from public non-hospital internal environments with good standards of cleanliness from two campuses of the same University in the city of Santos (SP), Brazil, and where young and apparently healthy people circulate. The frequency of free-living amoebae in both campuses was 39% and 17% respectively, with predominance of the genus *Acanthamoeba*. On the campus with a much larger number of circulating individuals, the observed frequency of free-living amoebae was 2.29 times larger ($P < 0.00005$). Two trophozoite forms of *Naegleria fowleri*, are the only species of this genus known to cause primary amoebian meningoencephalitis, a rare and non-opportunistic infection. We assume that the high frequency of these organisms in different internal locations represents some kind of public health risk.

Key-Words: Free - living amoebae, *Acanthamoeba* spp., *Naegleria* spp., prevalence, dust from internal environments, Brazil.

Free-living amoebae (FLA) are unicellular protozoa widely distributed on all continents that are able to survive and replicate in the environment without a host. FLA are present in a large variety of natural habitats, including lakes, rivers, swimming pools, thermal baths, tap water, sources of running water which receive industrial refuse, sewage, humid soils, dust, as well as fluids or tissue from healthy human oropharynx [1-4]. FLA feed on microorganisms with which they can maintain an endosymbiotic relationship. The cysts of these amoebae persist for many years, permitting them to survive in unfavorable environments. The cysts are very resistant to changes in temperature, pH, chlorine, detergents and common disinfectants. Several species are able to cause infections that are considered either accidental (acute meningitis by *Naegleria* spp.), opportunistic (granulomatous meningitis, otitis and other diseases caused by *Acanthamoeba* spp.) or non-opportunistic (a serious primary amoebian meningoencephalitis caused by *Naegleria fowleri* [1,5]). FLA can be found in hospitals, dialysis units, dental premises, as well as in eye drops and contact eye lens cleaning solutions, contaminated by bacteria or yeast [1]. Subclinical infections caused by these protozoa are probably common among healthy individuals, in whom protozoa harbored in the nose and throat can, in weaker periods, behave as facultative parasites [5,6]). In Brazil, cases of human diseases caused by these amoebae have been described [7-10] derived from water [11-14] and dust from external environments [15,16]. The few reports of FLA occurring in internal environments all involve hospitals [1,18].

The present work evaluates the occurrence of free-living amoebae in dust from non-hospital internal environments, where a large number of young and apparently healthy people circulate.

Material and Methods

Sampling of Material

Dust was collected from the floors of buildings from two different campuses from the Catholic University of Santos, São Paulo, Brazil, namely D. Edílio José Soares (CDI) and Vila Nova (CVN), between May and September of 2007 with temperatures ranging from 20°C to 33°C. These campuses differ with regards to the number of people that circulate daily. On the CDI, a total of 4,000 students and faculty circulate, while on the CVN, the corresponding total is approximately 186. The samples were collected in predominantly dry areas. The floors are kept constantly clean during the day and evening activities by using different types of commercial products based on chlorine, detergents and soaps.

One hundred dust samples from the floor from internal circulating areas were randomly collected from each campus using a sterilized swab, and then transferring to screw caps tubes (200x22 mm) containing 5.0 mL of Page saline solution, used to transport the material for analysis at a single university laboratory.

Culturing

The material in Page saline solution (2.5 mM NaCl, 1 mM KH₂PO₄, 0.5 mM Na₂HPO₄, 40 mM CaCl₂.6H₂O e 20 mM MgSO₄.7H₂O) from the tubes have been transferred to tubes containing 5.0 mL of non-nutrient agar with 1% (w/v) of Difco agar in Page saline solution and 50 µL of BHI broth (Difco), inoculated with 0.1 mL of late log phase cultures of Gram-negative bacteria (*E. coli* ATCC 25922), and incubated at 30°C. The observation of trophozoites was performed every 48h for up to 20 days, by optical microscopy and 400X magnification. The positive samples were transferred to new tubes containing non-nutrient agar, in the same way described

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above. The identification of amoebae genera was based on the observation of cysts and trophozoites, according to type of movement and morphological criteria [20-22]. Formation of flagella in distilled water at 37°C and a temperature increase to 43°C made the culture media selective for *Naegleria fowleri* isolation [23].

Sample Maintenance

Amoebae kept in tubes containing non-nutrient agar with 1% (w/v) of Difco agar in Page saline solution at 4°C under moist conditions were maintained for several months.

Results

Potentially pathogenic free-living amoebae from the genera *Acanthamoeba* and *Naegleria* were isolated from internal environments from two campuses of the investigated university.

On the CDI campus, among 100 samples of dust analyzed, 39% were positive according to observation of cystic and trophozoite structures. 19% of samples were positive for only *Acanthamoeba*, 12% for only *Naegleria* and in 8%, the simultaneous presence of both genera was observed.

On the CVN campus, among the 100 analyzed samples and using the same observational criteria, 17% were positive. 11% were positive for only *Acanthamoeba*, 5% for only *Naegleria*, and in only 1% both genera were detected.

The presence of free-living amoebae on the CDI campus was 2.29 times larger ($p < 0.00005$) than in the CVN campus.

In two samples from different locations on the CDI campus, the flagellated form of *Naegleria fowleri* was detected.

Discussion

Species of *Acanthamoeba* and *Naegleria* are widely distributed in the environment, and can be acquired by humans by contact with contaminated water or dust by either aspiration or inhalation [24]. Giuzzi [15] evaluated the FLA prevalence in 23 dust samples from external environments and observed a high positive rate of ~86% for the genus *Acanthamoeba* and ~39% for *Naegleria*. In Brazil, several reports address FLA isolation from dust in hospitals, because of the potential pathogenicity of these organisms. SILVA & ROSA [17] collected 132 dust samples in two hospitals. The positive rate was ~46% for the *Acanthamoeba* and ~4% for *Naegleria* genus. Carlesso et al. [18] analysed 135 samples in dust and biofilms from 15 different hospital environments, and detected a 35% positive rate, with 34% with morphological aspects of amoebae from the genus *Acanthamoeba*.

In the present work, the environmental health hazard related to FLA in dust from internal non-hospital environments, in which a large number of young and apparently healthy people circulate. On both campuses a larger frequency of cysts and trophozoites from *Acanthamoeba* species than *Naegleria* species was detected, which is in accordance with expectations [4,17,18]. These results may also be a consequence of the sampling being carried out more frequently in dry places, and

also because *Acanthamoeba* is more resistant to disinfectants and environmental variations than *Naegleria* [19].

The frequency of amoebae detection was apparently similar in hospital and non-hospital environments [17,18]. Taking into account that for survival and replication FLA require to feed on microorganisms and other organic particles present on the floor [3,25,27], and that such nutrients should be scarce in the internal environments investigated because of wide-spread and constant cleaning. It is a reasonable assumption that the presence of amoebae results from the constant traffic of people transporting dust to these environments. It is possible that the statistically significant difference in frequency of positive samples for amoebae between the two campuses results from the much larger number of individuals that circulate on the CDI area (~25 times larger) than on the CVN campus.

In two of the samples isolated from different CDI campus locations, the flagellated trophozoite form of *Naegleria fowleri* was observed. This free-living amoeba can be found in thermal waters all over the world, and is the only species among the thirty of its genus that can cause primary amoebian meningo-encephalitis in humans, a rare non-opportunistic infection, that has a fast and lethal progression. Although the infection reaches the central nervous system (CNS) through the olfactory epithelium and generally affects healthy children and young adults that have swum recently in contaminated lakes and swimming pools [26,27]: it is believed also that contamination may also occur by inhalation of contaminated dust [28].

In the absence of definitive studies to the contrary, the evaluation of free-living amoebae in different internal non-hospital environments can be considered an option for health environmental surveillance.

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