Detection of free living amoebae, Acanthamoeba and Naegleria, in swimming pools, Malaysia

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Abstract. This study reports the detection of Acanthamoeba and Naegleria species in 14 swimming pools around Petaling Jaya and Kuala Lumpur, Malaysia. Sampling was carried out at 4 sites (the platforms (P), wall (W), 1 meter from the wall (1) and middle (2)) of each swimming pool. These free living amoebae (FLA) were detected under light and inverted microscopes after being cultured on the surface of non-nutrient agar lawned with Escherichia coli. Acanthamoeba species were detected in higher number of culture plates from all sampling sites of all the swimming pools. While Naegleria, were detected in fewer culture plates at 3 sampling sites (absent at site P) of 5 swimming pools. This suggested that the thick double-walled cysts of Acanthamoeba were more resistant, thus remaining viable in the dry-hot areas of the platforms and in chlorinated water of the swimming pools whereas Naegleria cysts, that are fragile and susceptible to desiccation, preferred watery or moist areas for growth and proliferation. The prevalence of both FLA was highest at site W (76.2%), followed by site 1 (64.7%), lowest at site 2 (19.4%), and could be detected at all 3 sampling levels (top, middle and bottom) of these 3 sites. The surface of site W might act as a bio-film that accumulated all kinds of microbes providing sufficient requirement for the FLA to develop and undergo many rounds of life cycles as well as moving from top to bottom in order to graze food. Other factors such as human activities, the circulating system which was fixed at all swimming pools, blowing wind which might carry the cysts from surroundings and the swimming flagellate stage of Naegleria could also contribute to the distribution of the FLA at these sampling sites. Both FLA showed highest growth (80.4%) at room temperature (25-28°C) and lesser (70.9%) at 37°C which might be due to the overgrowth of other microbes (E. coli, fungi, algae, etc.). While at 44°C, only Acanthamoeba species could survive thus showing that our swimming pools are free from potentially pathogenic Naegleria species. However, further study is needed in order to confirm the virulence levels of these amoeba isolates.

INTRODUCTION
Free-living amoebae of the genera Acanthamoeba and Naegleria are known to cause disease in human. Infections by these amoebae are still unfamiliar to many physicians, pathologists and laboratory workers. However these amoebae have recently received more attention as a result of an increase in cases of Acanthamoeba keratitis (AK), granulomatous amoebic encephalitis (GAE), primary amoebic meningoencephalitis (PAM) and problems with effective treatment (Seidel et al., 1982; Moore et al., 1986; Schuster & Visvesvara, 1998; Kamel et al., 2003). Swimming was reported to be associated with corneal ulcer or AK due to Acanthamoeba (Mathers et al., 1996) and PAM due to Naegleria (Wiwanitkit, 2004) in healthy people. While GAE is usually found in immunosuppressive patients due to Acanthamoeba infections of the central nervous system and brain (Martinez, 1980). Both of these amoebae are ubiquitous in nature and can be found in almost all environments.