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The Occurrence of *Acanthamoeba* and *Naegleria* from Recreational Water of Selected Hot Springs in Selangor, Malaysia

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Abstract: Many reports have been documented on the benefits of hot Spring water as balneotherapy. However, despite being one of the choices for medical purpose, many not know that hot Spring water is also a major threat to human as it is also a habitat for pathogenic microorganisms. Two of microorganisms that can live in thermal water are *Acanthamoeba* and *Naegleria* and they are capable to cause human diseases. Humans are naturally exposed to these Free Living Amoeba (FLA) when they come in contact with bodies of water as they are common in the environment. This study was conducted to detect the presence of *Acanthamoeba* and *Naegleria* and their correlation with the physical parameters of water in five selected hot Springs in Selangor and also to determine growth capability of these organisms at 28, 37 and 44°C. A total of 52 water samples were collected from all studied hot Springs and the results showed that 38 out of 52 (76%) water samples collected from all the studied hot Springs were positive for *Acanthamoeba* and *Naegleria* with Sungai Serai hot Spring recorded the highest (100%) followed by Hulu Tamu hot Spring (91.7%), Dusun Tua hot Spring (75%), Selayang hot Spring (62.5%) and Kuala Kubu Bharu hot Spring was the lowest (37.5%). A total of 52 water samples were cultivated in 312 culture plates and incubated at 28, 37 and 44°C. Out of 312 plates cultured, 104 plates were recorded positive with 96 plates contained mixture of *Acanthamoeba* and *Naegleria*, 5 plates were positive with *Acanthamoeba* only and 3 plates were positive with *Naegleria* only. Out of these 104 positive plates, the highest number (52 out of 104) were detected at 37°C followed by 28°C (44 out of 104) and 44°C (8 out of 104). Several species of *Acanthamoeba* and *Naegleria* were isolated from water samples based on their morphology, size and locomotion. Only water turbidity parameter showed significant correlation with the occurrence of *Acanthamoeba* and *Naegleria* in studied hot Springs ($p<0.01$) ($r = 0.765$). This is the first study to detect the presence of *Acanthamoeba* and *Naegleria* from hot Springs water in Malaysia. High percentage of *Acanthamoeba* and *Naegleria* showed that people who come in contact with hot Springs water bodies are highly exposed to infections.

Key words: *Acanthamoeba*, *Naegleria*, hot Springs, physical parameters of water, correlation, Malaysia

INTRODUCTION

In developing countries, waterborne diseases account for about 80% of infectious diseases and one-third of fatal infections. The major health problem caused due to the presence of microorganisms in that water itself. Free Living Amoebas (FLA) are ubiquitous in natural water environments and they are capable to cause diseases. Humans are naturally exposed to these FLA when they come in contact with bodies of water as they are common in the environment, especially in water related biotopes (Leiva *et al*., 2008). *Naegleria*, *Acanthamoeba*, *Vahlkampfia* and *Hartmannella* have been commonly found in various environment water sources throughout the world and among these genera, *Acanthamoeba*, *Naegleria fowleri* and *Balamuthia mandrillaris* are important pathogenic agents for human and animals (Schuster and Visvesvara, 2004). Many reports have been documented on the benefits of hot Spring water as balneotherapy. However, despite being one of the choices for medical purpose, many not know that hot Spring water threat is also a major concern due to reports of outbreaks in the hot Spring area throughout the world. In Malaysia, the documentation on parasitic protozoan, especially,

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FLA is very limited. Although, many countries around the world have reported cases on the deaths and threats cause by *Acanthamoeba* and *Naegleria* in Malaysia there is still lack of research conducted to detect and observe the occurrence of FLA in the environment, especially in hot Spring recreational area in Malaysia.

MATERIALS AND METHODS

Water samples were collected from the five different selected hot Springs around Selangor which are Selayang Hot Spring (SHS), Dusun Tua Hot Spring (DTHS), Sungai Serai Hot Spring (SSHS), Kuala Kubu Bharu Hot Spring (KBHS) and Hulu Tamu Hot Spring (HTHS). Water samples were collected from water surface of several sampling points (middle and sides) using 500 mL sterile Schott bottles. The physical parameters of water were measured in situ for every sampling. The parameters measured were dissolved oxygen (DO) (mg/L), pH, temperature (°C) and conductivity (ms/cm) by using SCT meters (model YSI 556) (Ithoi *et al*., 2011) while turbidity (NTU) was measured using a turbidity meter. The water samples of 500 mL each were filtered through a cellulose ester membranes (pore size, 0.45 μm; diameter, 47 mm, Millipore (Bedford, USA) held in filter holder using a vacuum pump. The trapped sediment debris on membranes was collected by scraping with sterile plastic scrapper and then rinsed with sterile distilled water before transferred into a 15 mL centrifuge tube. This mixture was then concentrated again by centrifugation at 3000 rpm for 15 min. The supernatant result from the centrifugation of the suspension was discarded using sterile pipette until 6 mL left. The resulting pellet was suspended again and 5 drops of it were transferred onto the 6 NNA plates containing the E. *coli* lawn, respectively (Ithoi *et al*., 2011). All the six plates were then sealed and two of them were subsequently incubated at 28, 37 and 44°C, respectively, for the cultivation of *Acanthamoeba* and *Naegleria* and for the testing of thermotolerance characteristics of these FLA (Ithoi *et al*., 2011). During the incubation period daily inspection of plates was done using inverted light microscope (Leiva *et al*., 2008). Identification and classification of *Acanthamoeba* and *Naegleria* were based on morphological features according to taxonomic criteria (Page, 1988). The correlation between the presence of *Acanthamoeba* and *Naegleria* with physical parameters of water was determined by analyzing data obtained using Statistical Software (SPSS) for the correlation analysis. The values of $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

From the results obtained, all the water samples collected from SSHS were found to contain *Acanthamoeba* and *Naegleria*. The second highest number of positives water samples was found from HTHS in which the percentage of positive water samples for *Acanthamoeba* and *Naegleria* was 91.7% (11 out of 12). DTHS and SHS both had more than half water samples collected were positives with *Acanthamoeba* and *Naegleria* with a percentage of 75% (6 out of 8) and 62.5% (10 out of 16), respectively. KBHS had the lowest total number of water samples that were positive for *Acanthamoeba* and *Naegleria* where only 37.5% (3 out of 8) water samples were positive for *Acanthamoeba* and *Naegleria*.

A total of 104 out of 312 (33.3%) cultured plates were positive for *Acanthamoeba* and *Naegleria* in which 96 cultured plates (30.2%) contained mixture of *Acanthamoeba* and *Naegleria* species, 5 cultured plates (1.6%) were positive for *Acanthamoeba* only and 3 cultured plates (1.0%) were positive *Naegleria* only. From the percentage obtained, only SSHS has more than half positive plates which covered 60.4%. KBHS has the lowest percentage of positive plates with only 12.5%. The highest positive plates out of the three incubation temperature was at 37°C while 44°C has the lowest positive number of plates. A total of 104 plates were cultured for each of the incubated temperature and from the result shown, the positive plates for temperature 28°C was 44 out of 104, for 37°C was 52 out of 104 and for 44°C was 8 out of 104. Only cultured plates from SHS, SSHS and HTHS water samples have the ability to grow up to temperature 44°C with HTHS has the highest number of positive plates which can survive at this temperature. The presence of *Acanthamoeba* and *Naegleria* in recreational water of hot Spring showed significantly correlated only with turbidity parameter ($p<0.01$) with correlation coefficients (r) values 0.649 and 0.765 for *Acanthamoeba* and *Naegleria*, respectively. Both amoebas did not show any significant correlation with temperature, conductivity, dissolved oxygen and pH of studied hot Springs water. Multi shaped of cysts *Acanthamoeba* are observed under the microscope such as round, triangular, pentagonal and oval shape (Fig. 1-3).

High prevalence of *Acanthamoeba* and *Naegleria* were detected from SSHS and HTHS water samples. The reason of the high prevalence of *Acanthamoeba* and *Naegleria* at these two study areas may be due to SSHS environmental nature itself compared to the other places. Brown *et al*. (1983) stated that pools with soil enclosures had high incidence of free living amoeba compared to

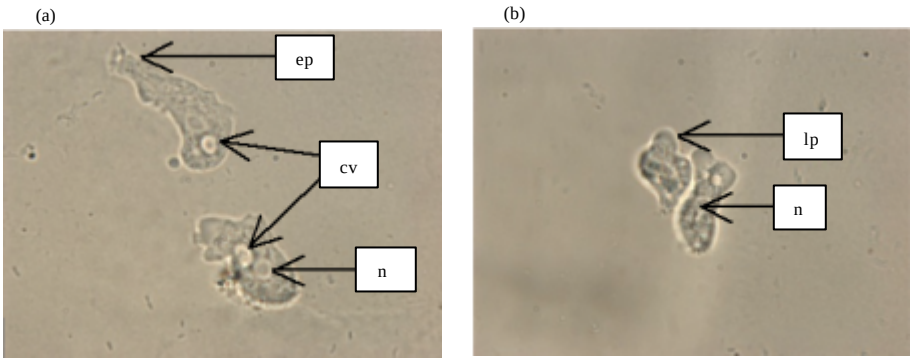

Fig. 1: Trophozoite of *Acanthamoeba* sp. (left) and *Naegleria* sp. (right) isolated from the hot Springs recreational water (1000×magnification) (cv, contractile vacuole; ep, eruptive pseudopodia; n, nucleus; lp, lobopodia)

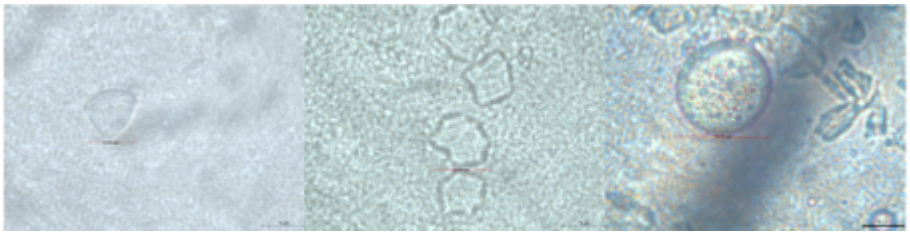

Fig. 2: Cysts of *Acanthamoeba* sp. with oval, pentagonal and round shape isolated from recreational water of studied hot Springs (1000×magnification)

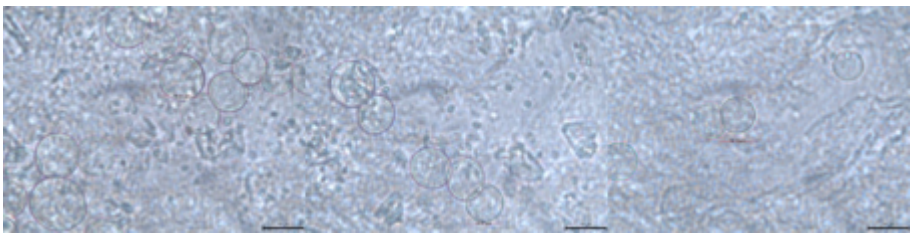

Fig. 3: Cysts of *Naegleria* sp. isolated from recreational water of studied hot Springs (1000×magnification)

those concrete line pools because concrete and tiled surrounding pool reduces the soil contamination of the water. Not only having the base floor of soil for SSHS, people are also allowed to get into the pool. When people went into the pool of that particular hot Spring they will eventually disrupt the soil base structure at the bottom which may cause the spreading of *Acanthamoeba* and *Naegleria* at that water area. Pauli *et al.* (2001) stated that the concentration of FLA in water probably depends upon the concentration of bacteria on which they feed and is closely associated with biofilms. In the studied hot Springs, bacteria that are brought from human through the bathing and swimming activity and organic compounds that present in the hot Springs may provide an ideal environment for *Acanthamoeba* and *Naegleria* growth. The hot Springs water of SSHS and HTHS probably provide an optimum growth condition for these organisms which lead to their high occurrence at these two places. Study from W-T Ji *et al.* (2014) suggested that human-derived bacteria and organic compounds in recreational hot Spring waters may provide an ideal environment for the *Acanthamoeba* growth. Warm water environment and human wastes in the recreational hot Springs water may contribute the presence of the FLA.

Acanthamoeba and *Naegleria* species from plates incubated at temperature 28 and 37°C showed good growth but not for plates incubated at temperature 44°C. The ability to survive the temperature = 37°C indicated that the strain could survive inside the mammalian body and these *Acanthamoeba* and *Naegleria* are considered potentially pathogenic (Schuster, 2002). Among the three

tested temperature, 37°C is considered the favorable temperature for these microorganisms to grow because of the high positivity of the plates could mean that they also, might favor the human body temperature which is around±37°C. High turbidity indicates that concentration of suspended solids in the water samples was high. Organic matter could promote the presence of bacteria and other microorganisms which these microorganisms later serve as food source for *Acanthamoeba* and *Naegleria*. Abundance of food supply could explain the occurrence of *Acanthamoeba* and *Naegleria* in the water.

CONCLUSION

This is the first study conducted to detect *Acanthamoeba* and *Naegleria* in hot Springs water in Malaysia and from the study, it is proved that natural hot Springs water may pose a health risk for the public health, especially, contact lens users and immunocompromised patients due to high occurrence of *Acanthamoeba* and *Naegleria* which is 76% positive waters samples.

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