

Short notes

Molecular identification of *Acanthamoeba* sp. in Lake Buhi, Philippines

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ABSTRACT. *Acanthamoeba* spp. are ubiquitous in both natural and man-made environments and have been isolated in lakes, recreational pools, tap water, and air conditioning systems. Twenty surface water (SW) samples were collected from different sampling areas of Lake Buhi. Water samples were pelleted, cultured in NNA lawned with *Escherichia coli* and observed microscopically. 10% of samples (2/20) were positive for amoebic growth and were furthered tested using molecular techniques. Polymerase chain reaction showed the presence of *Acanthamoeba* sp. DNA. The presence of potentially pathogenic *Acanthamoeba* sp. poses a public health concern. The formulation of policies for proper information dissemination and control measures to avert the contraction of pathogenic FLA as well as other WBPP should be seriously considered.

Keywords: *Acanthamoeba* sp., free living amoeba, Lake Buhi, PCR

Introduction

Acanthamoeba spp. are ubiquitous in both natural and man-made environments and have been reported in lakes, recreational pools, tap water, and air conditioning systems [1]. *Acanthamoeba* is a eukaryotic pathogen that causes fatal infections like Granulomatous Amoebic Encephalitis (GAE) and *Acanthamoeba* keratitis (AK) [2,3]. The increasing popularity of contact lens use, complicated with unhygienic handling of the same has led to the increasing reports of AK worldwide [4–8]. In the Philippines, AK cases have been reported from both users and non-users of contact lenses [9,10] the latter purportedly caused by washing the face with *Acanthamoeba* contaminated water.

Lake Buhi is endemic to the smallest edible freshwater fish in the world *Mistichthys luzonensis*, and is the primary source of Nile Tilapia in the region. The lake is frequented by tourists as it is surrounded by scenic views of an extinct volcano. The lake is surrounded by dense communities with

aquaculture being the main livelihood of the working class. Finally, Lake Buhi is the main freshwater resource of the province. The aim of this study was to perform the first-ever survey of *Acanthamoeba* spp. potentially present in the lake's water, the presence of which will provide significant information relative to *Acanthamoeba* diversity and possible routes of human transmission facilitated by contaminated water resources.

Materials and Methods

Twenty surface water (SW) samples were collected from different sampling areas in Lake Buhi. 50 mL of SW was collected no deeper than 12 inches from the water's surface and stored in sterile plastic containers. Sampling areas were selected based on the presence of road access, communities near the shoreline, observed anthroponotic activities, and presence of fish cages. The samples were transported to the Department of Medical Technology, Far Eastern University-Manila and

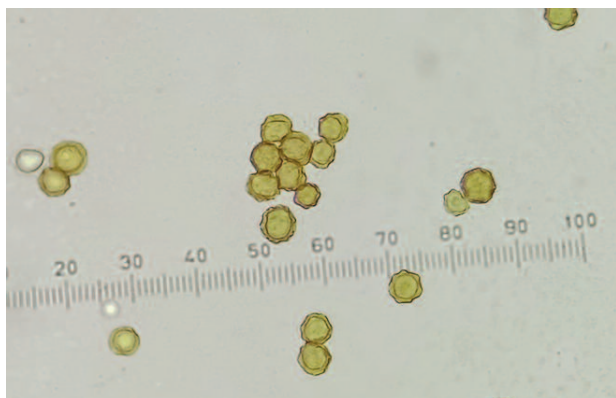


Figure 1. Cystic stages visualized through light microscopy

processed within 24 hours. The full volume of each water sample was centrifuged at 1500 g for 15 minutes. Pellets were collected and planted on non-nutrient agar lawned with live *Escherichia coli* and observed for growth for 14 days. Microscopic evaluation of amoebic growth on plates and harvesting of amoebic cysts for DNA extraction were performed following previously established protocols [11]. DNAs were made to react to polymerase chain reaction (PCR) using primer sets JDP1 5'-GGCCAGATCGTTTACCGTGAA-3' and JDP2 5'-TCTCACAAGCTGCTAGGGAGTCA-3' for cells that resemble *Acanthamoeba* spp. PCR conditions were set as follows: 95°C for 7 minutes initial denaturation, 40 cycles of denaturation at 95°C for 1 minute, annealing temperature of 55°C for 1 minute, extension at 72°C for 2 minutes and a final extension of 72°C for 15 minutes [12]. *Acanthamoeba* genotype T4 DNA was used as positive control which was provided by Prof. Patrick Scheid, and Dr. Carsten Balczun of Bundeswehr Central Hospital Koblenz, Germany.

Results and Discussion

After 14 days of incubation, amoebic culture presented with 10% (2/20) positivity. Cystic stages visualized through light microscopy exhibited double-walled structures with wrinkled outer wall and diameter ranging from 8 to 10 μm (Fig. 1) highly suggestive of being *Acanthamoeba* spp. according to PAGE's established morphologic classification [13]. Molecular analysis of cysts harvested from positive culture plates formed distinct bands between the 400 and 500 bp regions along with *Acanthamoeba* T4 genotype positive control (Fig. 2). Lakes are important inland freshwater resources that are utilized for human washing,

recreational activities, and most importantly, consumption. Unfortunately, studies of lakes in the Philippines are at present, still fragmented [14]. More importantly, local studies on water quality relative to waterborne protozoan pathogens (WBPP) are still in its local infancy. The results of this study, however, add to the growing list of evidences of the ubiquity of potentially pathogenic free-living amoebae (FLA) such as *Vermamoeba vermiformis* from Nile Tilapia in Lake Taal [15] and *Naegleria australiensis* in Laguna de Bay which is the largest Lake in the Philippines [11]. There are two factors that possibly contribute to the presence of *Acanthamoeba* sp. in lake Buhi, these are water temperature and aquaculture. The volcanic origin of the lake and the presence of nearby active volcanoes can contribute to the relatively high temperature of the lake water which is at 33°C during sampling. Temperature plays an important role in the proliferation of virulent strains of FLA in aquatic environments [16]. The abundance of fish cages in the lake also seems to influence the persistence of *Acanthamoeba* sp. possibly by the provision of biological reservoirs as FLAs have been isolated from a variety of fish species [15,17]. Lake Buhi is home to five edible freshwater fish species which are sometimes eaten raw by the locals [18]; although the pathogenic effects of *Acanthamoeba* spp. in the gut are not yet established, this should be taken into perspective. Fish handlers and immunocompromised individuals are otherwise at risk to contract a rare type of disseminated skin infection caused by certain genotypes of *Acanthamoeba* [19]. Further, investigations on the occurrence of potentially pathogenic FLAs in natural lakes have

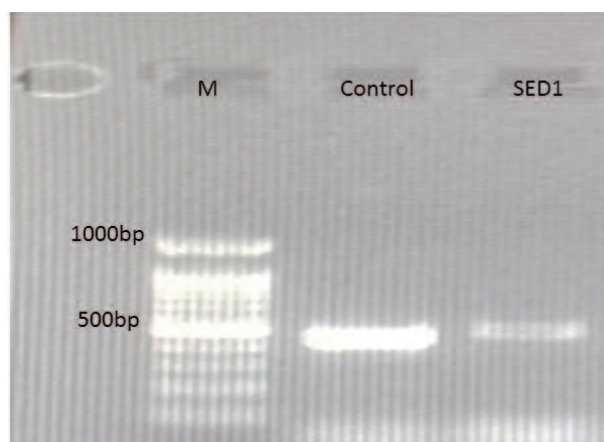


Figure 2. PCR amplicons using JDP1 and JDP2 primers in 1.5% agarose gel stained with ethidium bromide showing band formation between 400 and 500bp of isolate SED1

shown great importance in the prevention of fatal conditions acquired from contact with contaminated waters as suggested by some studies [11]. Although pathogenicity testing of the isolated *Acanthamoeba* sp. was not carried out in this study, a broad spectrum of *Acanthamoeba* spp. are considered to be pathogenic to both humans and animals, thus, making the isolation of this FLA in a natural lake frequented by humans of significant importance in a public health standpoint [3]. As mentioned previously, Lake Buhi is considered economically important to its surrounding communities, meaning that factors such as population, livestock, and soil runoffs can also be contributory to the presence of the organism. Finally, the observed anthroponotic activities and aquaculture livelihood of Lake Buhi's inhabitants is a significant public health concern for potential infections. This first report of a potentially pathogenic genotype of *Acanthamoeba* sp. from Lake Buhi is of important public health concern for immunocompetent and especially immunocompromised individuals. Effective dissemination of relative information and the formulation of control measures in preventing the contraction of this pathogenic FLA should be seriously considered.

In conclusions, the results of this study submit the first report of a potentially pathogenic *Acanthamoeba* sp. in the waters of Lake Buhi, Philippines. Water temperature, presence of aquaculture, and human interactions with water resources seemingly contribute to the presence and possible transmission of *Acanthamoeba* sp. that poses a public health concern. Formulation and implementation of policies for proper information dissemination and control measures can help avert the contraction of pathogenic FLAs as well as other waterborne protozoan pathogens.

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