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Acanthamoeba spp. Found in Freshwater Fishes From Selected Areas of the Philippines - A Preliminary Report

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Acanthamoeba spp. are ubiquitous organisms that have been adapted to different types of habitats and conditions. The study of freshwater fishes has become the interest of many researchers for their ability to harbor parasitic organisms, and they also play a significant role as intermediate hosts in the transmission cycle to humans. This study examines the *Acanthamoebae* spp. present in a variety of edible freshwater fish in the Philippines. A total of 14 different fish species (six fish per species) were collected from major lakes all over the Philippines. Fish intestines were aseptically dissected, pooled, processed, and cultured in non-nutrient agar lawned with *Escherichia coli*. Culture plates were examined for 14 days to determine their response to amoebal growth. Thirty-one percent of the fish species sampled were found to be positive for amoebic growth. Genomic DNAs were extracted and examined by polymerase chain reaction (PCR) using *Acanthamoeba*-specific primers. Further sequencing of PCR amplicons confirmed the presence of four *Acanthamoeba* species (*A. mauritanensis, A. polyphaga, A. castellanii,* and *A. lenticulata*) from the culture-positive samples. This study shows the presence of *Acanthamoeba* spp. from edible freshwater fishes in the Philippines. The presence of potentially pathogenic free-living amoebae like *Acanthamoeba* in edible freshwater fish may pose a public health risk. Although the effects of direct consumption of *Acanthamoeba*-infected fish are yet to be established, the

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potential of other means of infection, as discussed previously, needs to be taken into serious perspective.

Keywords; *Acanthamoeba*, fish, Free-living-amoeba (FLA), PCR

Introduction

Acanthamoeba spp. are free-living amoebae (FLA) isolated from a wide variety of habitats worldwide (Schuster & Visvesvara, 2004). They are ubiquitous organisms found in soil and aquatic environments (fresh, brackish, seawater), sewage, beach sands, hospital and dental environments, contact lenses, and even atmospheric dust (Visvesvara et al., 2007; Goldschmidt et al., 2012). Acanthamoebiasis has been reported in a variety of animal species, including fish, due to the presence of *Acanthamoeba* spp. . in the aquatic environment. This allows for interaction with a broad range of aquatic resources, making it a primary habitat for these organisms (Schuster & Visvesvara, 2004). According to Chovanec et al. (2003), fish may act as bioindicators for ecotoxicological studies. Moreover, from a public health standpoint, it can be a potential reservoir and transmission host of several parasitic infections in humans. FLA such as *Acanthamoeba* spp. have been known to thrive as facultative parasites in certain species of fish such as *Sarotherodon aureus* (Taylor, 1977), *Astronotus ocellatus* (Laoprasert et al., 2009), *Oreochromis niloticus* (Dykova et al., 1997; Milanez et al., 2017), *Catrostomus commersoni* and *Notropis cornutus* (Franke & Mackiewicz, 1982). Despite such findings, studies concerning the diversity of possible amphizoic amoeba infecting fish have not been given enough attention, and they usually conduct studies with other eukaryotic organisms (Dykova & Lom, 2004).

The clinical significance of *Acanthamoeba* species to humans has already been established. It is the frequent cause of *Acanthamoeba* keratitis (AK), a potentially blinding infection of the cornea in non-immunocompromised individuals (Marciano-Cabral & Cabral, 2003). Consequently, it can also cause granulomatous amoebic encephalitis (GAE), a fatal brain infection (Marciano-Cabral & Cabral, 2003; Schuster & Visvesvara, 2004; Khan, 2006; Visvesvara et al., 2007). In the Philippines, the different eating habits of Filipinos in some parts of the country have proven to be a major risk factor for the transmission of fish-borne diseases and other waterborne protozoan pathogens. Several deaths in Northern and Southern Philippines due to intestinal parasites have been traced to the raw consumption of freshwater fish marinated in vinegar, such as the cases in 1963 in Luzon, 1999 in Mindanao, and 2007 in Siayan Municipality (Belizario et al., 2010). Although certain freshwater fish species in the country have been identified as reservoir/intermediate hosts for parasitic helminths, there is a knowledge gap on fish that serve as hosts for FLAs. This study attempted to investigate the possible types of *Acanthamoeba* spp. from a variety of edible freshwater fish in the Philippines. As a result, pertinent data on the isolation and identification of *Acanthamoebae* spp. were generated.

Methods

Sampling Sites, Sample Collection, Processing, and Culture

Eighty-four edible freshwater fish samples were collected from major lakes in the Philippines, namely West Pudoc, Lake Danao, Lake Sakanaw, Lake Mainit, and Lake Lanao (Figure 1). There were 14 unique edible fish species sampled (Table 1). Six samples for each fish species were collected, three of which were used for *Acanthamoeba* identification, and three were used for fish authentication. The representative samples for fish identification and authentication were preserved in plastic containers using 70% ethanol and were sent to the Institute of Biology, University of the Philippines, Diliman. Fish intestines were aseptically harvested, pooled according to fish species, processed, cultured, and subcultured following protocols (Milanez et al., 2017). Fish intestines were aseptically dissected upon arrival in the laboratory. Then, they were placed in 50 ml sterile polyethylene conical tubes with physiologic saline and were vortexed for 10 min. Intestines were removed, and the remaining fluid was centrifuged at 2000 rpm for 15 min, after which the resulting pellets were placed in previously prepared non-nutrient agar (NNA) lawned with *Escherichia coli* and were placed in an incubator set at 33 °C (Milanez et al., 2017).