COMPARATIVE DETECTION OF *ACANTHAMOEBA* FROM BORE-HOLE WATER IN EGOR LGA, EDO STATE, NIGERIA USING TWO METHODS

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O.D. (ABSU)

A THESIS WRITTEN IN THE DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY AND SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE (M.Sc) IN PARASITOLOGY OF THE UNIVERSITY OF BENIN, BENIN CITY

APRIL, 2024

CERTIFICATION

This is to certify that the project work was successfully carried out by Okoro Chiemela Chidozie in the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, under my supervision.

Date:____/_____/2024

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Prof. M.O. Omoigberale

Head of Department

CERTIFICATION OF THESIS

We, the undersigned attest and declare that the thesis of Okoro Chiemela Chidozie, Titled: **Comparative Detection of *Acanthamoeba* from Bore-hole Water in Egor LGA, Edo State, Nigeria using two methods** has successfully passed the anti-plagiarism test and does not violate any copyright regulations.

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Prof. (Mrs). A.O. Awharitoma

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Prof. M.O. Omoigberale

Head of Department

DEDICATION

I dedicate this thesis work to my mother, Mrs Patience Abraham and my lovely wife, Mrs Faith Uloaku Okoro, and to myself for remaining diligent and determined during these past rigorous three years.

ACKNOWLEDGEMENT

The completion of this thesis was possible, by far due to the immense contributions of several personages. First and foremost, I would like to acknowledge God almighty for the strength and perfect health to see this project through; my dogged mother Mrs Patience Abraham and my lovely wife, Mrs Faith Okoro for their support, love and care; my amiable supervisor, Prof. (Mrs) A.O. Awharitoma, who was always open to questions and making available resource materials that would enrich the body work of this project, thank you for your time, sincerity, and invaluable contributions. I would like to appreciate the effort of Dr. Abraham Ogofure for his immeasurable assistance that made this research came to fruition. My appreciation would also go to some academic staff of the Department of Animal and Environmental Biology who offer help at various times that I needed it. May our good Lord bless you all.

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ABSTRACT

Acanthamoeba, a free-living *Amoeba* commonly found in water, a category of protozoans which are pervasive and can be found in diverse environments, including, sewage water, air, soil, dust, and sediments. One of the vital issues in water pollution is the occurrence of pathogenic amoebae in tap or drinking water. The aim of the study was to compare the detection of *Acanthamoeba* from bore-hole water source and tap water in Egor Local Government within Benin metropolis using two methods.

This observational, prospective cross-sectional study collected 52 water samples from various locations within the Egor Local Government Area (26 samples each from bore-hole water source and tap water). Samples were processed on the same day of collection. For *Acanthamoeba* detection, 500 mL of water sample was passed through a 0.45 µm cellulose nitrate membrane filter. Prepared non-nutrient agar plates were seeded with heat-killed *Escherichia coli*. Cultures were incubated at 30℃ for amoebic growth and examined microsopically. The study also employed centrifugation, and sediments were microscopically viewed under iodine-stained slides.

Results showed a significant difference in the occurrence of *Acanthamoeba* species ($p = 0.007$) in bore-hole water and tap water when using the culturing method. However, there was no significant difference ($p = 0.277$) between samples of bore-hole water and tap water obtained through centrifugation. This suggests that an isolation method can influence the detection of *Acanthamoeba* species. This research highlights the substantial prevalence of *Acanthamoeba* species in bore-hole water sources within Benin City, with potential implications for water quality and safety. The discrepancy in results between culture and centrifugation methods underscores the need for careful consideration when choosing a detection method. The high prevalence of *Acanthamoeba* in bore-hole water may have a link to the nature of the water facility. Further studies are necessary to investigate the reasons behind the methodological differences and their implications for water quality and safety in Benin City.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Advancements in technology and human activities, including the development of recreational facilities and industrial operations, have brought to light the issue of microbial contamination in water. The detrimental impact on the safety of the water supply has been colossal, as noted by (Oyedum *et al*., 2016). Water remains one of the most precious and fundamental natural resources, constituting 70% of what is essential for the survival of all living beings, especially humans. Potable water is the term used to refer to water suitable for human consumption. Water can be utilized for various purposes by users without any risk of contracting water-borne diseases. Water supply to end-users requires treatment to ensure the reduction of microbial load to a safe level (Oyedum *et al*., 2016).

Primary water sources, including surface water from rivers, lakes, reservoirs, basins, and groundwater, are crucial components of this concern (Geldreich, 1996). Worldwide investigations have been conducted to evaluate the quality of both treated and untreated water supplied to consumers. Richard *et al*. (2016), emphasized that considerable efforts have been made to prevent the ingress and dissemination of pathogens, aiming to ensure the provision of safe drinking water.

In Nigeria, public water supply is virtually nonexistent in most communities, leaving many households with dry taps and no prospect of running water (Nwaiwu *et al*., 2020). Consequently, individuals and institutions have resorted to self-help solutions by tapping into boreholes as their sole source of water supply. A simple method of extracting drinkable water from subterranean aquifers is through boreholes; the water is typically pumped into storage tanks prior to distribution (Nwaiwu *et* *al*., 2020). Furthermore, because groundwater is thought to be a purer source, there is a growing reliance on it as a result of the increasing contamination of surface water (Nwaiwu *et al*., 2020).

Over the past three decades, there has been an increase in the prevalence of pathogenic parasites contaminating drinking water, contributing to a surge in associated diseases affecting human populations (Al-Malki, 2020). Among these pathogens are free-living amoebae (FLA), a category of protozoans, which are pervasive and can be found in diverse environments, including, sewage water, air, soil, dust, and sediments. Notable genera of free-living amoebae, such as *Naegleria fowleri*, *Acanthamoeba spp.*, *Balamuthia mandrillari*, and *Sappinia diploidea*, pose a significant threat of infection to humans (Al-Malki, 2020).

Among protozoans, *Acanthamoeba* stands out due to its opportunistic behavior. Notably, certain species of *Acanthamoeba* exhibit remarkable resilience, enduring extremely low temperatures as cold as -20ºC (-4ºF). Conversely, temperatures exceeding 42.5°C (107°F) are lethal to them. A key survival strategy employed by *Acanthamoeba* involves transitioning from the motile trophozoite phase to a cystic phase in unfavorable conditions, allowing them to endure and maintain their virulence over prolonged periods (Bharathi *et al*., 2007; Sowka *et al*., 2016).

A potentially severe corneal disease known as *Acanthamoeba keratitis*, primarily caused by *A. castellani* and *A. polyphaga*, can lead to significant vision loss (Sowka *et al*., 2016). Several risk factors are associated with *Acanthamoeba keratitis*, with the predominant factor in developed nations being the use of contact lenses (Bharathi *et al*., 2007). This ocular infection poses a considerable treatment challenge, requiring an average of more than five months of treatment due to the resistance of the cysts to most antimicrobial agents (Al-Herrawy *et al*., 2017). Currently, there are 25 identified species of *Acanthamoeba* that can cause infections in humans, including *A. polyphaga*, *A. hatchetti*, *A. healyi*, *A. divionensis*, *A. culbertsoni*, *A. astronyxis*, *A. rhysodes*, and *A. lenticulata* (Al-Herrawy *et al*., 2017).

1.2 Protozoa

The majority of single-celled organisms without cell membrane are classified as protozoa. The invention of the microscope in the 1600s allowed for the investigation of these microscopic organisms, which are invisible to the human eye. The work was pioneered by Antonio van Leeuwenhoek (1632–1723). Pinocytosis, or the process of ingesting liquids or particles by invaginating the plasma membrane, is how protozoa obtain nutrients. Taking in larger particles through phagocytosis may require particular interactions (Khan, 2006). Binary fission is an asexual method of reproduction in protozoa. In this scenario, a parent cell divides into two daughter cells during mitosis. A parent cell that divides into multiple daughter cells is said to undergo multiple fission. It is possible for two cells to unite, exchange nuclei, and produce offspring through fission or budding. This process is known as sexual conjugation. These special characteristics give parasitic protozoa the ability to adapt to new settings, fend off host immune responses, and overcome other difficulties (Lucius and Roberts, 2017).

Parasitic protozoa typically possess relatively compact genomes. It is not an inherent feature but often a consequence of their parasitic lifestyle, which permits a reduction in metabolic pathways due to their reliance on the host organism (a phenomenon known as "reductive evolution"). There is no strict correlation between genome size and parasitism, as some parasites possess large genomes. For instance, the genome of *Trichomonas vaginalis* size, 176Mb and 59,681 protein-coding genes, ranks among the larger protozoan genomes. Nevertheless, it pales in comparison to others, like the genome of the free-living *Entamoeba proteus*, which boasts a massive size of 290,000Mb (Lucius and Roberts, 2017). Protozoa also defy easy categorization, with many Apicomplexa containing an endosymbiotic organelle of plant origin called the apicoplast, which imparts some traits of plant origin to these organisms (Lucius and Roberts, 2017).

The five major classes of pathogens are comprised of protozoa, fungi, intracellular parasites (viruses), prokaryotes, and multicellular pathogens (Khan, 2006). They cause illness by entering their hosts directly through the skin, genitourinary tract, respiratory tract, or oral cavity. Alternatively, they may spread covertly via rodents or insects as vectors. Their transmission also involves inanimate items like surgical instruments, towels, and contact lenses (Khan, 2006). Protozoa multiply and establish themselves within the host organism once they have penetrated the host tissue. The host tissue may get physically harmed by this. Deprivation of vital nutrients or the induction of an inflated immune response that leads to illness are other possible outcomes.

1.2.1 Pathogenic Free-Living Amoeba

Amoeba proteus is a famous example of how the study of *amoebae* began in the early days of microscopy. They comprise the most varied group among the protists. Although these organisms move in similar ways, resembling crawling amoeboids, they belong to different groups. Strong parasitic organisms such as *Entamoeba spp.*, which were identified in 1873 from a patient with bloody dysentery and were formally named *Entamoeba histolytica* in 1903, are among them (Khan, 2006). Another example is the free-living amoeba *Sappinia diploidea*, which was isolated in 1908–09 from soil and lizard excrement. In 2001, it was discovered to be the cause of granulomatous amoebic encephalitis (Khan, 2012).

Amoebae can live freely in a variety of natural settings. They can be found in soil, dust decomposing plants, airborne particles, and natural water sources like lakes, rivers, and seas. Furthermore, they have been found in the corpses of both dead and living insects, fish, amphibians, and reptiles. Apart from their natural habitats, free-living amoebae can be found in a wide range of artificial settings, such as air conditioning units, sewage pipes, tap water systems, distilled water bottles, swimming pools, dental offices, hospitals, dialysis centres, and contact lens cases. The widespread distribution of free-living amoebae implies that humans come into contact with these organisms on a daily basis (Sun and Wang, 2017).

The initial discovery of free-living amoebae dates back to 1899 when Schardinger isolated the first specimen, which he originally named *Amoeba gruberi*. In 1912, Alexeieff proposed the genus name *Naegleria*, and it was not until 1970 that Carter identified *Naegleria fowleri* as the causative agent of fatal infections in humans (Khan, 2006). The genesis of the genus *Acanthamoeba* can be traced back to 1930 when it was discovered as pollutants in eukaryotic cell cultures (Castellani, 1930; Douglas, 1930; Volkonsky, 1931). *Balamuthia*, a newly named genus, derives its name from *Balamuthia mandrillaris*, a discovery made in 1986 from the brain of a baboon that succumbed to meningoencephalitis.

These free-living creatures have drawn the interest of scientists over time because of their varied functions, especially in causing serious and occasionally fatal infections in humans. For instance, *N. fowleri* causes primary amebic meningoencephalitis (PAM), a necrotizing and hemorrhagic meningoencephalitis that affects people who have previously engaged in warm-water activities. More recently, infections in humans and animals have also been linked to *Acanthamoeba* and *Balamuthia*. These infections include granulomatous amebic encephalitis (GAE), a persistent and sneaky granulomatous disease that affects both immunocompetent and immunosuppressed people as well as animals (Martinez and Visvesvara, 2001).

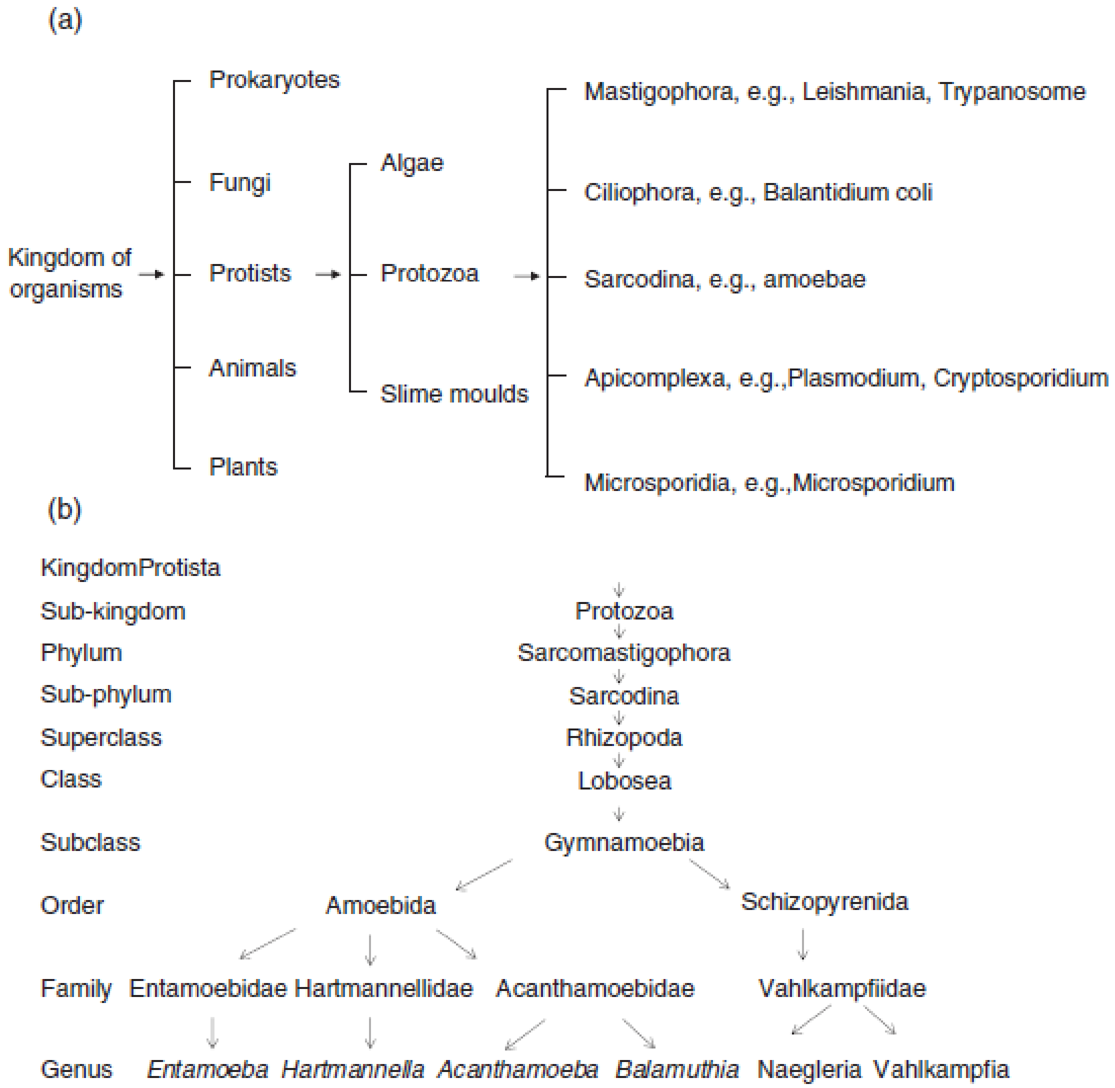

Figure 1.1: Classification scheme of free-living amoeba

1.2.2 *Acanthamoeba spp.*

Castellani found an amoeba in the *Cryptococcus pararoseus* fungus in 1930. These amoebae were spherical or oval in shape, measuring between 13.5 and 22.5 mm in diameter. They were also notable for having pseudopodia, or acanthopodia. These amoebae also had a double-walled structure in their cyst form, which had an average diameter of 9–12 mm. At first, this amoeba was identified as *Hartmannella castellanii* and placed under the *Hartmannella* category (Khan, 2006). One year later, Volkonsky separated the *Hartmannella* genus into three additional genera according to particular traits:

1. *Hartmannella*: *Amoebae* characterized by round, smooth-walled cysts.
2. *Glaeseria*: *Amoebae* characterized by nuclear division occurring within the cysts.
3. *Acanthamoeba*: *Amoebae* distinguished by the presence of pointed spindles at mitosis, double-walled cysts and an irregular outer layer.

The addition of the Greek word "acanth," meaning "spikes," to "amoeba" was made to denote the presence of spine-like structures on the surface, now recognized as acanthopodia. *Acanthamoeba amoebae* feature one or more distinct contractile vacuoles, releasing water to regulate osmotic pressure. In the cytoplasm, various vacuoles, including lysosomes, digestive vacuoles, and numerous glycogen-containing vacuoles, can be observed (Siddiqui and Khan, 2012). The composition of the plasma membrane consists of proteins (33%), phospholipids (25%), sterols (13%), and lipophosphonoglycan (29%). The primary phospholipids identified in *Acanthamoeba* include phosphatidylcholine (45%), phosphatidylethanolamine (33%), phosphatidylserine (10%), phosphoinositide (6%), and diphosphatidylglycerol (4%). Fatty acid chains in *Acanthamoeba* predominantly comprise longer polyunsaturated fatty acids (20–30%) and oleic acids (40–50%). Glycolipid levels in *Acanthamoeba* are relatively low, with glucose constituting around 60% of the sugars present in glycolipids in both whole cells and plasma membranes.

Ergosterol and 7-dehydrostigmasterol are two of the sterols found in the non-saponifiable portion of total lipids that were taken from the trophozoites of pathogenic *Acanthamoeba*. Additionally, prostaglandins are produced by *Acanthamoeba* (Siddiqui and Khan, 2012; Khan, 2006).

The trophozoite is the active form of the *Acanthamoeba* protozoa. In the right growth conditions, trophozoites can undergo binary fission, and in certain situations, they can infect humans. Trophozoites typically have an average diameter of 20 µm and a round or oval shape when viewed under a light microscope. Their diameter can range from 15 to 45 µm. Trophozoites usually move actively and undergo constant morphological changes. As a result, different trophozoite forms can be seen through a light microscope (Sun and Wang, 2017).

The trophozoites of *Acanthamoeba* have a lot of mitochondria. The T4 genotype of *A. castellanii* has a genome size of 41,591 bp for mitochondrial DNA. The single nucleus of an *Acanthamoeba* is about one-sixth the size of a trophozoite. Nevertheless, amoebae with multiple nuclei have been found. *Acanthamoeba castellanii* Neff strain, which is also of T4 genotype, has a genome of about 45 million base pairs (Mb) (Khan, 2012). *Acanthamoeba* has an average of three exons per gene, according to the analysis of coding sequences (CDS features, exon) from 200 genes. In contrast, each gene in *Dictyostelium discoideum* has 2.3 exons, whereas each gene in *Entamoeba histolytica* has 1.3 exons (Siddiqui and Khan, 2012).

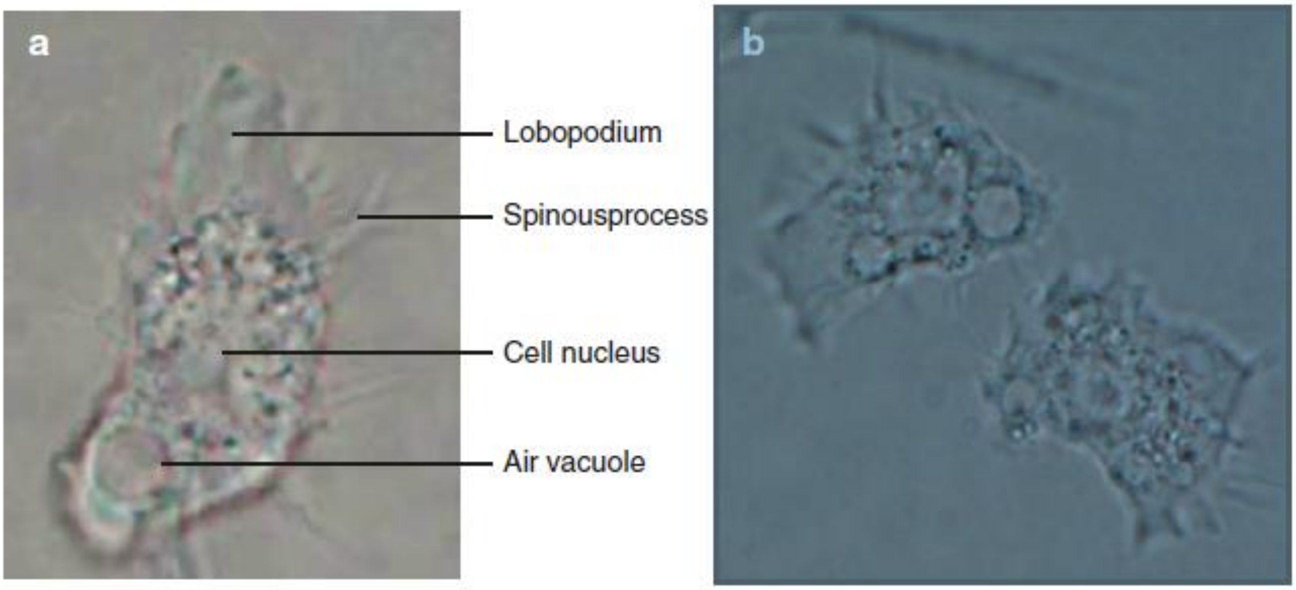

Plate 1.1. Trophozoites of *Acanthamoeba* with cell nucleus, spinous process, and air vacuole (×1000). (Sun and Wang, 2017)

1.2.3 Classification of *Acanthamoeba*

The Acanthamoebidae Family includes *Acanthamoeba*. *Balamuthia*, a different genus that was previously connected to amoebae with ambiguous relationships, has been added to this family. The genus *Balamuthia* should belong to the Acanthamoebidae family, according to molecular analysis of 16S-like rRNA genes (Marciano-Cabral and Cabral, 2003).

The spiny surface projections on trophozoites called acanthopodia make genus-level identification of *Acanthamoeba* relatively simple. Nevertheless, it has proven difficult to use morphological characteristics to identify these amoebae at the species level. Depending on the size and form of their cysts, *Acanthamoeba* species are classified into three morphological groups (I, II, and III) (Marciano-Cabral and Cabral, 2003).

Large and rounded, Group I cysts are easily distinguished from their endocyst counterparts. Group I species were divided into groups according to how big their cysts were in comparison to those of other groups. The endocysts in Group II cysts are smaller and resemble stars. Group II species can be distinguished by their variable endocyst shapes, which can be oval, polygonal, stellate, or triangular, and their wrinkled ectocyst. With endocysts and ectocysts that are not well separated, group III cysts are the smallest. Group III species usually have a round endocyst and a thin, smooth ectocyst. *Acanthamoeba* is currently classified into 17 genotypes, designated T1–T17 according to 18S ribosomal RNA sequences. Just two of these genotypes—T5 and T11, directly correlate to the *Acrocercops lenticulata* and *A. jacobsi* species that have been previously identified. The T4 genotype is linked to the majority of human infections, with the T3 and T11 genotypes following closely behind (Lucius and Roberts, 2017; Marciano-Cabral and Cabral, 2003).

Classifying *Acanthamoeba* based on cyst morphology has proven unreliable due to variations dependent on culture conditions. To differentiate between different *Acanthamoeba* species, reliance is placed on physiological, biochemical, and immunological parameters. However, the similarity in antigenic characteristics among many species makes immunological methods, such as immunofluorescence and Western blotting, unreliable for species identification. Isoenzyme electrophoresis of various enzyme systems is a method employed in comparing *Acanthamoeba* strains (Marciano-Cabral and Cabral, 2003).

Attempts are being made to establish classification at the molecular level in order to address the difficulties in classifying *Acanthamoeba* species. Certain labs have grouped *Acanthamoeba* strains using restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA. The disadvantage of this method is that it necessitates a comparatively large quantity of amoebae for analysis (Marciano-Cabral and Cabral, 2003).

The 12 sequence types (Rns genotypes), designated as T1 through T12, are integrated with the morphological groups created by Pussard and Pons (as cited by Marciano-Cabral and Cabral, 2003) in current classification schemes. Sequence types T7, T8, and T9 are included in Group I of this scheme; sequence types T3, T4, and T11 are included in Group II; and sequence types T1, T2, T5, T6, T10, and T12 are included in Group III. Sequence type 4 (T4) is home to the majority of strains that cause *keratitis*, according to studies that identify clinical isolates based on sequence types. There are still a lot of important discrepancies in the taxonomy of *Acanthamoeba* species despite these classification efforts.

1.3 Statement of problem

Acanthamoeba, a common amoebic protozoan, is responsible for various infections, including *keratitis*, granulomatous amoebic encephalitis, and disseminated granulomatous amoebic disease affecting sinuses, skin, and lungs. In its life cycle, *Acanthamoeba* exhibits two distinct forms: the active trophozoite and the dormant cyst, with the ability to transform between them under specific conditions. Typically found in contaminated soil or water sources, *Acanthamoeba* includes species such as *A. culbertsoni*, *A. triangularis*, *A. castellanii*, *A. astronyxis*, *A. mauriteniensis*, *A. griffini*, *A. lugdenensis*, *A. polyphaga*, and *A. rhysodes*, contributing to their prevalence in the environment and drinking water.

Acanthamoeba keratitis, a challenging ocular infection, requires over five months on average for treatment due to cysts' resistance to most antimicrobial agents(Sowka *et al.*,2016). Improper handling of contact lenses and the use of tap water for cleaning contribute to its occurrence. Additionally, *Acanthamoeba* cysts display exceptional resistance to desiccation. Considering these factors, it becomes crucial to investigate the potential public health implications associated with *Acanthamoeba* species in borehole water and utilizing two identification methods constitute a focus of this study.

1.4 Aim of Study

The aim of this study is to compare the detection of *Acanthamoeba* from bore-hole water source and tap water in Benin metropolis using two methods.

1.5 Objectives of the Study

The objectives were to:

1. investigate the presence of *Acanthamoeba* species in house-hold Bore-hole water through culture and centrifugation methods
2. determine the prevalence of *Acanthamoeba* species present in samples of house-hold Bore-hole tap water.
3. determine the prevalence of *Acanthamoeba* species present in samples of Bore-hole pipe-borne water (source).
4. Comparatively determine the mean count of *Acanthamoeba* species in culture and centrifuge methods for borehole source and tap samples.

1.6 Significance of Study

The study would:

1. provide valuable insight on the presence of *Acanthamoeba* species on house-hold Bore-hole water in Benin City,
2. help in proper education and enlightenment of the populace on the use of Bore-hole water as well as dangers, if any on improper use.
3. also provide data for further research on pipe-borne water in Nigeria.

1.7 Hypothesis

Null Hypothesis (Ho)

There is no significant difference between the presence of *Acanthamoeba* species in bore-hole water source and tap in Benin City.

CHAPTER TWO

2.0 LITERATURE REVIEW

According to Siddiqui and Khan (2012), *Acanthamoeba* can be found in a broad range of natural environments, such as freshwater lakes, hot springs, Antarctica, beaches, pond water, soil, seawater, and even the atmosphere. Additionally, isolated forms can be found in a variety of unexpected places, including distilled water containers, bottled mineral water, cooling tower discharges from factories that are thermally polluted, air conditioning units, showerheads, kitchen sprayers, ventilation ducts, humidifiers, compost, vegetables, surgical instruments, contact lenses, cases for contact lenses, pigeon droppings, freshwater fish, and living as well as dead or diseased animals (Siddiqui and Khan, 2012).

Acanthamoeba has been detected in various clinical and healthcare settings, including hospitals, physiotherapy swimming pools, dialysis units, portable and stationary eye wash stations, human nasal passages, the throat, pharyngeal swabs, lung tissues, skin lesions, human feces, corneal biopsies, maxillary sinuses, mandibular autografts, stool samples, urine from critically ill patients, cerebrospinal fluids, and brain necropsies (Siddiqui and Khan (2012). This extensive array of data underscores that *Acanthamoeba* is a pervasive environmental organism encountered in our daily lives. The presence of Anti-*Acanthamoeba* antibodies in over 85% of individuals from diverse backgrounds in London and nearly 100% of healthy populations in New Zealand provides compelling evidence of its ubiquitous nature.

2.1 Life Cycle

Acanthamoeba has two stages in its life cycle: a vegetative trophozoite stage. In the trophozoite stage (named after the Greek word "tropho," which means "to nourish"), *Acanthamoeba* consumes other microorganisms like bacteria, algae, and yeast along with organic particles. Trophozoites go through mitotic division when the right circumstances are met, which include a sufficient supply of food, a neutral pH, a temperature of about 30°C, and an osmotic pressure of 50–80 mOsmol. On the surface of the amoeba, transient structures called food cups form, which are utilised for consuming yeast and bacteria.

There is not a separate flagellated stage in the *Acanthamoeba* life cycle (CDC, 2019). Acanthopodia, or spine-like structures, are surface features of trophozoites. These acanthopodia probably play a role in adhering to inert or biological surfaces, promoting cellular motility, or catching prey. Trophozoites usually have one nucleus, which is about one-sixth of the total size of the trophozoite. (Cabral and Martinez, 2003).

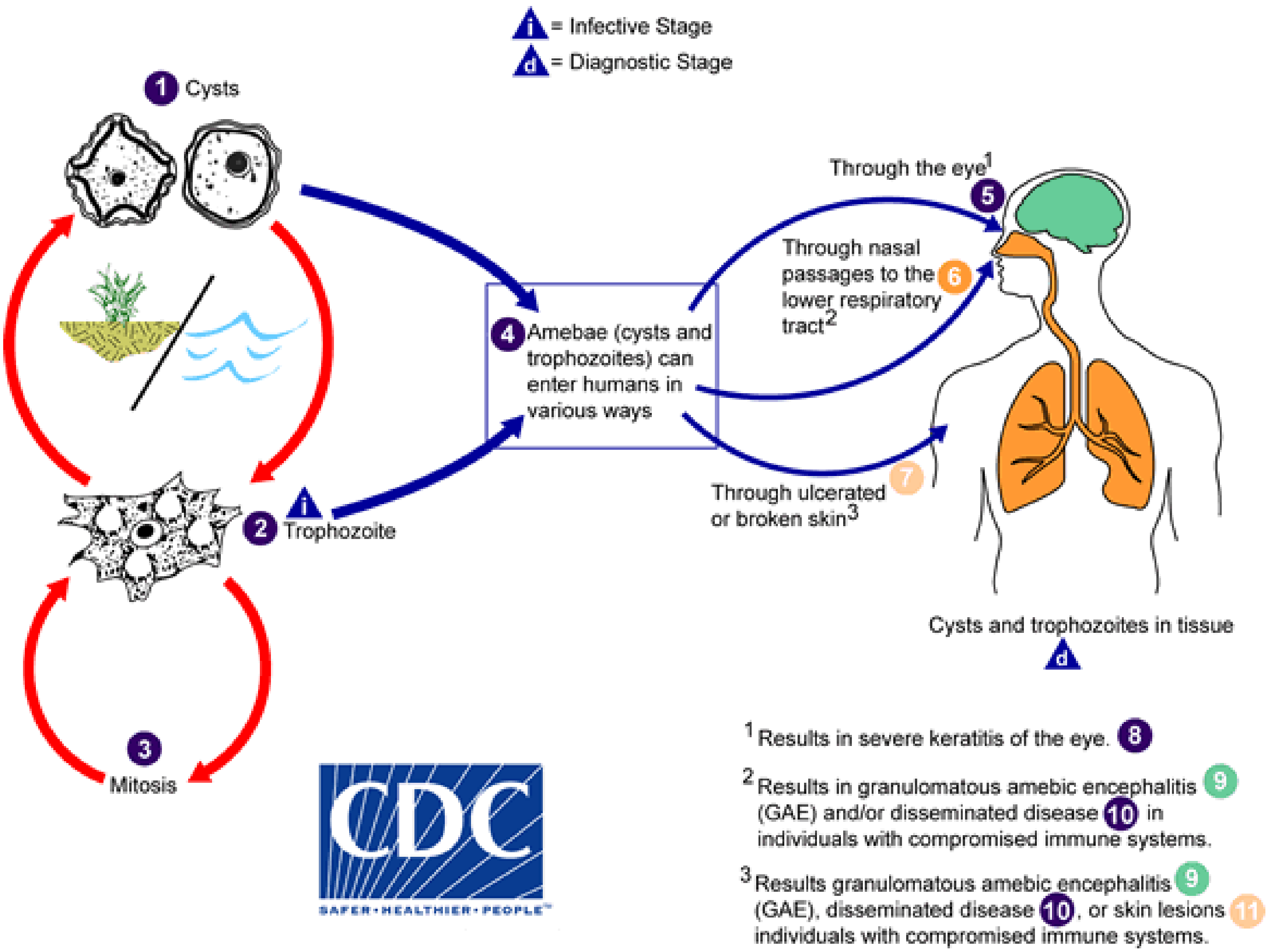

Figure 2.1. ***Acanthamoeba*** **Life Cycle. Source: CDC (2019)**

Under certain circumstances, including an abundance of food, a neutral pH, the proper temperature, and an osmolarity of between 50 and 80 mOmol, amoeba can persist in the trophozoite phase for a considerable amount of time. On the other hand, unfavourable circumstances, like inadequate nourishment, sharp fluctuations in osmolarity, severe fluctuations in temperature, or sharp variations in pH, cause trophozoites to develop into cysts (Khan, 2006). In short, the trophozoite forms a protective shell around itself and becomes metabolically sluggish. Proteins and polysaccharides make up the cyst's outer walls, whereas cellulose makes up the inner wall (Siddiqui and Khan, 2012; Marciano-Cabral and Cabral, 2003).

During the cyst formation stage, excess food, water, and particles are expelled, leading the trophozoite to condense into a rounded structure termed a "precyst." This precyst undergoes further development into a double-walled cyst, with the outer wall serving primarily as a protective barrier, shielding the parasite from harsh environmental conditions. The cyst's diameter can range from 5 to 20 μm, influenced by the specific species or genotype. Remarkably, cysts possess the ability to become airborne, potentially facilitating the widespread dissemination of *Acanthamoeba* throughout the environment and the potential transmission of infections to susceptible hosts. Numerous studies indicate that cysts can retain their capacity to cause illness for several years, suggesting their potential role in the dissemination of *Acanthamoeba* infections (Mazur *et al.,* 1995).

The "ostioles" that cysts have on their bodies allow them to sense changes in their surroundings. The life cycle is completed when trophozoites leave the cysts and reproduce actively, leaving behind their outer shell when the environment is right (Khan, 2006). Cycloheximide, a drug that prevents protein synthesis, can impair the active synthesis of macromolecules involved in both the encystment and excystment processes.

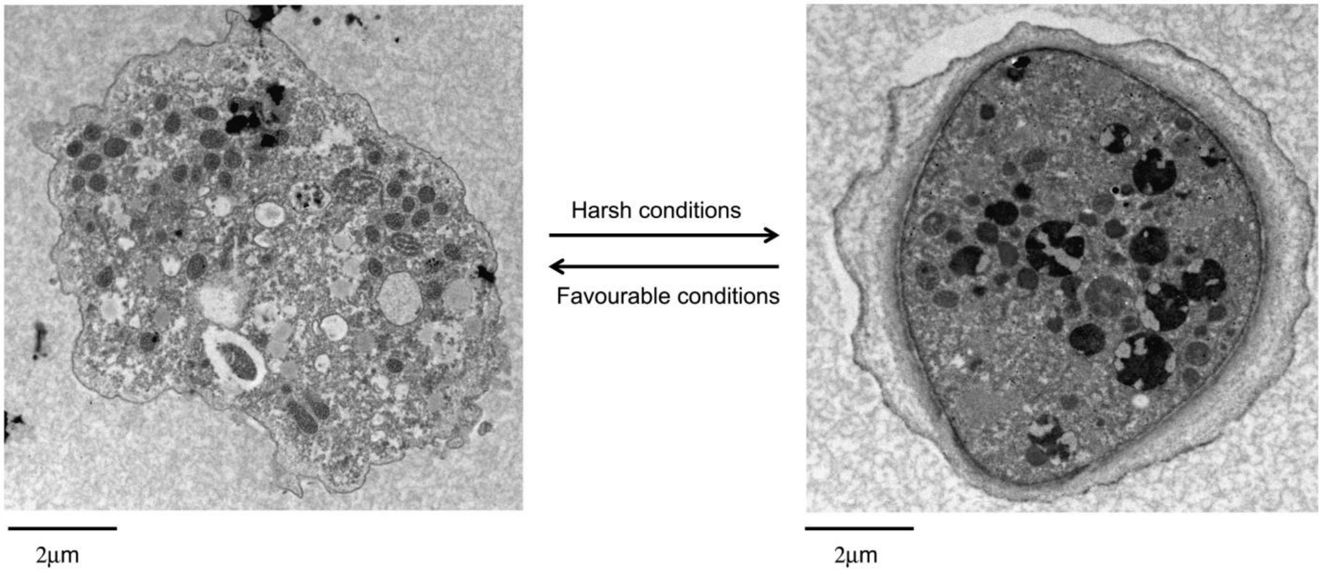

Figure 2.2. Developmental process of *Acanthamoeba spp.* Under favourable conditions, *Acanthamoeba* remains in the trophozoite form and divides mitotically (1st image) and produces infection, while under harsh conditions amoeba transforms into a dormant cyst form (2nd image) that is highly resistant to harsh conditions. Siddiqui and Khan, 2012)

When *Acanthamoeba* trophozoites are exposed to non-nutritive environments, they can be stimulated to change into cysts, and when the environment becomes more favourable, they undergo excystation. Researchers have studied processes related to eukaryotic RNA transcription, RNA polymerase functions, and cellular differentiation in both trophozoites and cysts using *Acanthamoeba* as a model system (Marciano-Cabral and Cabral, 2003).

2.2 Biology

Over the years, numerous electron microscopy studies have revealed *Acanthamoeba's* cellular organisation (Marciano-Cabral and Cabral, 2003). Regarding its organelles, *Acanthamoeba* is similar to higher eukaryotic cells. It is noteworthy because it has contractile vacuoles, which help with osmotic regulation by allowing water to be expelled. Lysosomes, digestive vacuoles, and multiple vacuoles containing glycogen are among the other vacuoles found in the cytoplasm. According to Siddiqui and Khan (2012), the components of the plasma membrane are proteins (33%), phospholipids (25%), sterols (13%), and lipophosphoglycan (29%).

Phosphatidylcholine (45%), phosphatidylethanolamine (33%), phosphatidylserine (10%), phosphoinositide (6%), and diphosphatidylglycerol (4%), are the main phospholipids found in *Acanthamoeba*. Longer polyunsaturated (20–30%) and oleic (40–50%) fatty acid chains make up the main fatty acid chains. Glycolipid concentrations are low; in whole cells and in plasma membranes, glucose makes up about 60% of the sugars in glycolipids. Within the total lipids extracted from pathogenic *Acanthamoeba* trophozoites, the non-saponifiable fraction contains ergosterol and 7-dehydrostigmasterol, two types of sterols.

Furthermore, prostaglandins are produced by *Acanthamoeba* (Siddiqui and Khan, 2012). In its trophozoite stage, the organism also has free ribosomes, mitochondria, a Golgi complex, smooth and rough endoplasmic reticula, and microtubules (Marciano-Cabral and Cabral, 2003). The cytoplasm may contain bacteria or other symbiotic microorganisms. Trophozoites have a diameter of roughly 15–45 µm and a round, oval, or irregular shape, according to scanning electron microscopy (Sun and Wang, 2017). Their surface has a rough appearance and is covered in many spiny, cone-shaped processes. The cellular features that set *Acanthamoeba* species apart from *Naegleria* species are unique. On solid substrates or at the water-air interface, amoeba moves similarly. Amoebae are able to move passively without separating from the water's surface due to adhesion forces that are stronger than gravity between *Acanthamoeba* and the water-air interface (Siddiqui and Khan, 2012). Generally, the side of the cell that the trophozoite travels along is where a fan-shaped pseudopodium, also called a lobopodium, forms (Sun and Wang, 2017).

Although multinucleated cells in suspension culture are identical, *Acanthamoeba* amoebae are generally uninucleated. Binary fission is the process of reproduction. Cyst formation is the process by which a double-walled wrinkled cyst, consisting of an ectocyst and an endocyst, develops. The sizes of these cysts vary from species to species and range from 13 to 20 µm (Marciano-Cabral and Cabral, 2003). Unfavourable environmental factors like food scarcity, desiccation, and changes in pH and temperature are common causes of cyst formation. Cysts are resistant to antibiotics, chlorination, and biocides. Additionally, they can endure temperatures as low as 0 to 2°C (55). Cysts can be destroyed by treatments like autoclaving, methylene oxide exposure, or Freon exposure. Excystment is the process by which trophozoites leave the cyst when the environment is conducive to their emergence (Marciano-Cabral and Cabral, 2003).

2.3 *Acanthamoeba spp.* as opportunistic pathogens

2.3.1 *Acanthamoeba Keratitis*

Acanthamoeba-related diseases mainly present as two long-term illnesses: encephalitis and *keratitis*. Human infections with pathogenic free-living amoebae, like *Acanthamoeba keratitis*, usually result from unintentional contact or seize-the-occasion situations (Ghosh, 2018; Sun and Wang, 2017).

When it comes to infections of the cornea, lipopolysaccharides or mannosylated glycoproteins on the membranes of corneal epithelial cells are the first place where *Acanthamoeba* protozoa attach themselves. Toxin production and phagocytosis are involved in this initial contact. Bowman's membrane is breached and corneal epithelial cells thin or die as a result of the release of enzymes such as neuraminidase (Sun and Wang, 2017). Furthermore, this process compromises the integrity of the epithelial barrier, which permits *Acanthamoeba* protozoa to deeply penetrate the corneal stroma. Corneal stromal collagens are broken down by *Acanthamoeba* proteases, and trophozoites can cause radial keratoneuritis (Sun and Wang, 2017).

It is well known that contact lenses can act as mechanical vectors, making it easier for contaminated sources to transmit *Acanthamoeba* to the cornea (Sowka *et al*., 2016). But contact lenses also induce microtrauma to the corneal epithelium, which facilitates the entry of these pathogens. Other possible routes of infection include direct corneal injury (such as corneal abrasions) and exposure to contaminated materials, either simultaneously or later (Sowka *et al*., 2016).

The main cause of *Acanthamoeba keratitis* is improper contact lens cleaning. Research shows that contact lens wearers account for over 85% of cases of *Acanthamoeba keratitis*, which is frequently linked to specific behavioural factors. According to Niederkorn *et al*. (1999), for example, *Acanthamoeba keratitis* is more common in young males. This may be because of poor personal hygiene, inappropriate handling and maintenance of contact lenses or lens storage cases, and disregard for disinfection protocols, such as using homemade saline solutions (Brennan, 2002).

It is best to discard contact lenses that have scratches or other damage. Swimming and rinsing eyes while wearing contact lenses are additional risk factors. Since *Acanthamoeba* has a strong resistance to chlorine, it is not advisable to use chlorine-based disinfectants on a regular basis for cleaning lenses (Khan, 2006). Moreover, compared to traditional hydrogel lenses, *Acanthamoeba* exhibits noticeably higher adherence to silicone hydrogel contact lenses.

Additionally, *Acanthamoeba* protozoa damage corneal epithelial layers by three mechanisms: (a) endocytosis, which is similar to phagocytic activity, in which the protozoa can directly ingest a portion of the corneal cell membrane; (b) spontaneous exocytosis, in which the protozoa can release lysins that further damage the epithelial cell membrane, without the need for an activation process; and (c) membrane-related activation of exocytosis, in which *Acanthamoeba* protozoa form a coalition of trophozoites that can bind with receptors or ligands on the surface of the epithelial cell, thereby activating proteases. The epithelial cells may sustain damage as a result of the release of these proteases (Sun and Wang, 2017).

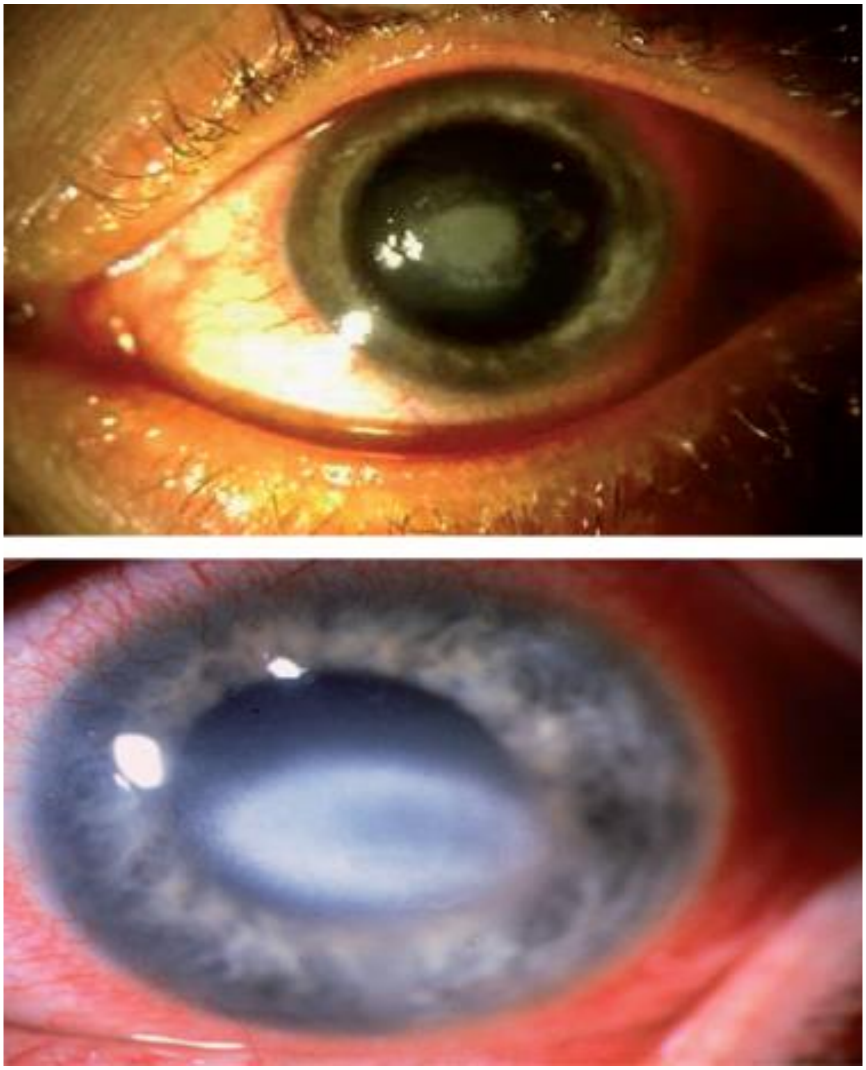

Plate 2.1 ***Acanthamoeba*****-infected eye exhibiting the severity of the disease. Note the ulcerated epithelium and stromal infiltration exhibiting corneal opacity in acute** ***Acanthamoeba keratitis*** **(published with permission from Elsevier) (Source: Khan, 2006)**

Taking care of *Acanthamoeba keratitis* is a difficult and complicated task. Numerous drugs, such as certain antibiotics, antiseptics, antifungals, antiprotozoals, antivirals, and antineoplastics, can be used to eradicate trophozoites (Sowka *et al.,* 2016). But *Acanthamoeba* cysts are sensitive to a very small range of agents, mostly biguanides and diamidines. When starting a treatment, doctors frequently start with a combination of two or more medications. A biguanide, such as polyhexamethylene biguanide (PHMB) 0.02% or chlorhexidine 0.02%, is typically used as part of an initial regimen in combination with a diamidine, such as propamidine isethionate (Brolene, Sanofi) 0.1% or hexamidine 0.1%. These drugs are given hourly, every hour, for the first 48 hours after corneal debridement. Following that, the frequency is lowered to once every hour during the day for a period of one or more weeks (Sowka *et al.,* 2016).

2.3.2 Granulomatous Amebic Encephalitis

With Granulomatous Amebic Encephalitis (GAE), the central nervous system (CNS), possibly including the lungs, is infected and the illness progresses slowly over time. It usually affects people whose immune systems are weakened (Ghosh, 2018). The illness arises from breathing in dried *Acanthamoeba* cysts. The illness has a protracted incubation period and advances slowly (Ghosh, 2018). Numerous *Acanthamoeba* species have been linked to GAE, according to serological laboratory testing (Marciano-Cabral and Cabral, 2003). Clinical symptoms of an *Acanthamoeba* infection may appear in a few weeks to months, though the precise incubation period is unknown. The respiratory tract, skin, sinuses, olfactory epithelium, and other regions are examples of portals of entry (Marciano-Cabral and Cabral, 2003).

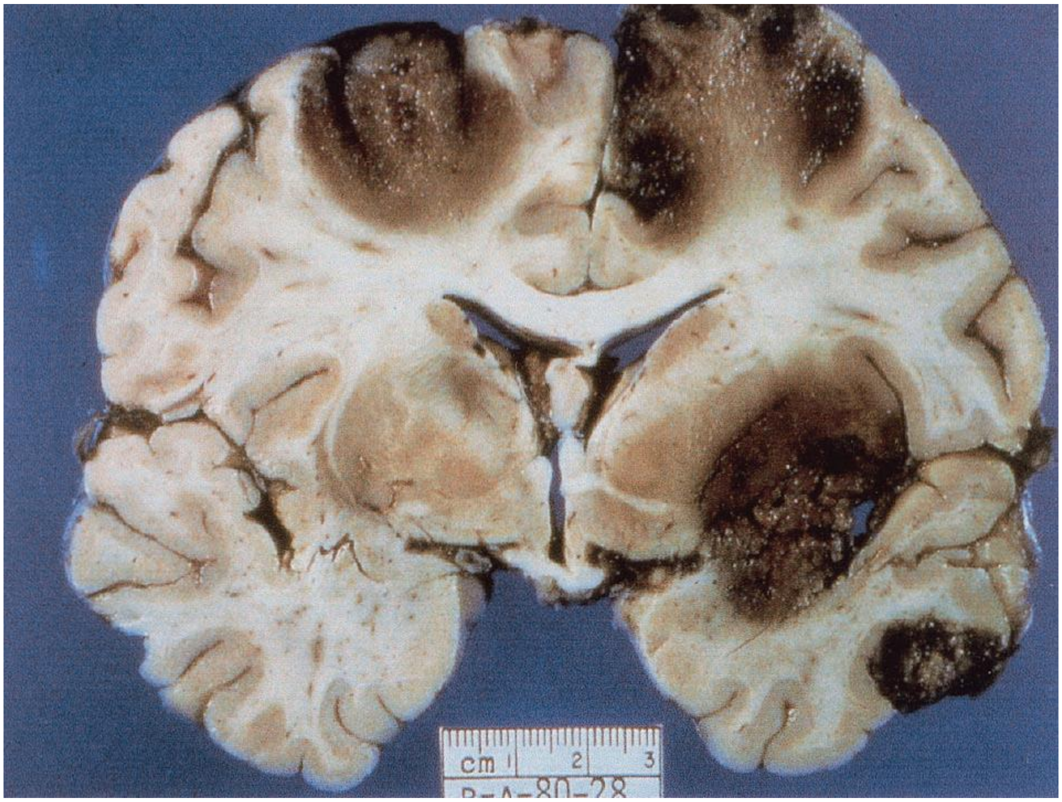

Plate 2.2. Coronal section of the cerebral hemispheres with cortical and subcortical necrosis from a fatal human case of GAE (Source: Marciano-Cabral and Cabral, 2003)

Granulomatous Amebic Encephalitis (GAE) typically affects people who already have underlying medical conditions, such as infection with the HIV virus, Hodgkin's disease, diabetes, renal failure, cirrhosis, tuberculosis, skin ulcers, or malignancies (Marciano-Cabral and Cabral, 2003).

Alcoholism, drug addiction, steroid therapy, radiation, chemotherapy for cancer, and organ transplantation are among the risk factors for GAE. Although immunocompetent children and adults are more vulnerable to infection due to compromised immune systems and incapacitating conditions, cases of GAE caused by *Acanthamoeba* have been reported (Marciano-Cabral and Cabral, 2003).

The most likely ways for an infection to occur are either through skin lesions or inhalation through the lungs and nasal passages of amoebae. The amoebae can then enter the central nervous system (CNS) directly through the olfactory neuroepithelium or hematogenously from a primary site in the skin or lungs (Marciano-Cabral and Cabral, 2003).

Headaches, disorientation, nausea, vomiting, fever, lethargy, stiff neck, focal neurologic deficits, or indications of elevated intracranial pressure are among the symptoms of a central nervous system infection.

Inflammation, fibrin thrombi, and severe hemorrhagic necrosis are typically seen in pathological findings. Moderate to severe oedema is frequently observed in the cerebral hemispheres. The midbrain, brain stem, corpus callosum, and cerebellum can all be localised by multiple lesions (Marciano-Cabral and Cabral, 2003). Usually seen over the cortex, inflammatory exudate is primarily made up of mononuclear cells and polymorphonuclear leukocytes. In certain instances, severe angiitis is evident, marked by perivascular cuffing with lymphocytes. In addition, the tissue is dotted with many trophozoites. Parenteral pentamidine, oral itraconazole, and topical chlorhexidine gluconate have all been effective treatments for skin abscesses resulting from this illness (Martinez and Visvesvara, 2001).

2.3.3 Infections in Patients with AIDS

In individuals with compromised immune systems, such as those with acquired immunodeficiency syndrome (AIDS), widespread infections can affect the skin, lungs, sinuses, and various organs either individually or in combination (Ghosh, 2018). According to certain authorities, compromised host defense mechanisms in immunocompromised individuals can lead to infections spreading from the primary site to other organs and tissues, potentially exacerbating the condition. Immunocompromised individuals may not mount a robust granulomatous response (Marciano-Cabral and Cabral, 2003). Typically, those who contract *Acanthamoeba* in this population have advanced HIV disease and a low CD4 T-cell count (less than 200/mm3). The clinical course of *Acanthamoeba* infection progresses rapidly, with most patients succumbing within a month of the onset of neurological symptoms (Marciano-Cabral and Cabral, 2003). Non-AIDS *Acanthamoeba*-infected individuals may exhibit cerebrospinal fluid (CSF) lymphocytosis, but HIV-positive patients may not show any cells in their CSF.

2.4 Methods of Isolation

When found in environments that are favourable to *Acanthamoeba*, these organisms usually eat yeasts, other protozoa, bacteria, microscopic organisms, and organic particles. It is interesting to note that these things can act as growth substrates for *Acanthamoeba* in lab settings, though there are some technical drawbacks to this (Khan, 2006). Specifically, the complex preparations required for yeast and protozoa make them difficult to use as growth substrates (Khan, 2006). Particularly yeasts grow quickly, making it difficult to remove them in order to obtain pure axenic *Acanthamoeba* cultures. In the meantime, organic materials such as glucose and proteose peptone provide an abundance of nutrients, which unintentionally promote the growth of undesirable organisms like bacteria, yeasts, fungi, and other protozoa. Specific protocols, like using simple plating assays, are now used in response to these technical challenges and to increase the likelihood of successfully isolating *Acanthamoeba* from environmental and clinical samples. These are described below.

2.4.1 Isolation of *Acanthamoeba* Using Non-Nutrient Agar Plates Inoculated with Gram-Negative Bacteria

This is the most widely used technique for separating *Acanthamoeba* from a wide range of sources, including clinical and environmental samples. The basic idea behind this technique is that *Acanthamoeba* primarily feeds on Gram-negative bacteria, usually *Escherichia coli* or *Enterobacter aerogenes* (formerly known as *Klebsiella aerogenes*). These non-nutrient agar plates—which are specifically designed to contain the least amount of nutrients possible—are seeded with these Gram-negative bacteria in order to effectively stop the growth of undesirable microorganisms (Khan and Paget, 2002).

The process can be summed up like this: Plates devoid of nutrients that contain 1% (w/v) Oxoid no. 1 agar in Page's Amoeba Saline (PAS). Additionally, there is 4% (w/v) malt extract and 4% (w/v) yeast extract, and KOH has been used to carefully adjust the pH to 6.9. These non-nutrient agar plates are evenly coated with about 5 mL of late log-phase cultures of Gram-negative bacteria, usually *Escherichia coli* or *Enterobacter aerogenes*. Following a brief incubation period of five minutes, any surplus culture fluid is eliminated, and the petri dishes are allowed to air dry before being infused with either clinical specimens or environmental samples (Khan, 2006; Marciano-Cabral and Cabral, 2003).

After the inoculation, the plates are carefully observed every day for the presence of *Acanthamoeba* trophozoites while being incubated at 30°C. Trophozoites usually become visible after a few hours, sometimes as soon as 12 hours, depending on the initial amoeba count in the sample (Khan, 2006). If amoebae are available, keep an eye on the plates for a maximum of seven days. When the bacterial food supply runs out, *Acanthamoeba* differentiate and eventually form recognisable cysts. Future research may focus on these issues as well as possible differences in bacterial preferences among *Acanthamoeba* isolates belonging to different species/genotypes. While some questions remain unanswered, such as why *Escherichia coli* or *Enterobacter aerogenes* are the preferred food substrates, or the specific bacterial preferences of *Acanthamoeba*—Gram-negative versus Gram-positive bacteria—may be addressed (Khan, 2006).

2.5 *Acanthamoeba* species in Tap Water and Other Water Systems

Waterborne routes are one way that *Acanthamoeba* species can spread. They typically enter the human body through forceful surface water inhalation, especially from poorly maintained swimming pools and other aquatic sources. A discernible rise in the number of waterborne disease outbreaks reported in the past few decades has coincided with the discovery of hitherto undiscovered waterborne parasites. Tap water, runoff from heavy rains, and agricultural residues are some of the factors that spread these diseases by transferring parasites from the soil into surface waters (Baqer *et al*., 2018).

Given these worries, a study was conducted to determine whether parasites were present in household water tanks throughout different parts of Baghdad, as well as in drinking water from the Al-Wahdaa and Al-Rasheed Drinking Project and rivers. Forty samples from the Drinking Project's river and drinking water as well as fifty-four samples from residential water tanks in various Baghdad regions were collected for the study. The free-living amoeba *Acanthamoeba* and *Naegleria* cysts were found using the wet mount slide method. Interestingly, compared to other months, cyst counts were higher in July and August in both household water tanks and the Drinking Project in all regions (Baqer *et al*., 2018).

As a result, the research made clear how important it is to screen water for parasites. One factor that has been linked to the infective stage of parasites contaminating water is the existence of cracks in drinking water pipes that connect stations and homes, especially in areas close to agricultural fields that store cow dung.

In addition, a study by Ghaderifar *et al*. (218), concentrated on the identification and isolation of *Acanthamoeba* from park pond water in a tropical and subtropical area of the Middle East. Ninety samples of pond water were collected from August to December in thirteen different parts of Mashhad City for the study. Following on-site determination of the physicochemical parameters, the samples were filtered and cultivated on Bacto-agar supplemented with *Escherichia coli*. Samples that tested positive in the culture were then subjected to PCR analysis, and the PCR products were sequenced. Nineteen (21.1%) of the samples tested positive for *Acanthamoeba*, and sequencing confirmed that the isolates of *Acanthamoeba* were of the T4 genotype. It is interesting to note that there was no discernible relationship found in the study between physicochemical parameters and *Acanthamoeba* presence. In summary, despite the relatively high frequency of *Acanthamoeba* in park pond water, no meaningful correlation was found between the physicochemical parameters and *Acanthamoeba* presence.

Kao *et al*. (2013), looked into the seasonal fluctuations and diversity of *Acanthamoeba* species in a subtropical river-shed in a different study. The goal of the study was to make a link between the seasonal and geographic distribution of *Acanthamoeba* species in Taiwan's Puzih River watershed, which is situated slightly above the Tropic of Cancer and has a typical subtropical monsoon climate. Using an amoebal enrichment culture method to identify *Acanthamoeba* species in water samples; PCR confirmation was required. Sixteen (11.7%) of the 136 water samples that were used in the investigation contained *Acanthamoeba* species. Summertime saw the highest percentage of *Acanthamoeba*-positive samples (32.4%), which were primarily from upstream regions. Results showed that seasonal variations existed in the detection rates of *Acanthamoeba* species, with percentages of 2.9% in the spring, 32.4% in the summer, 2.9% in the autumn, and 8.8% in the winter.

Summertime was the season when *Acanthamoeba* species were most common. These findings are consistent with earlier research indicating that the late summer months are when the presence of *Acanthamoeba* species in aquatic environments peaks.

In a similar vein, the study conducted by Al-Herrawy *et al*. (2017) which involved 144 tap water samples, showed that the summer months had the highest frequency of *Acanthamoeba* species in water samples (38.9%). In the spring, this percentage dropped to 30.6%; in the autumn and winter, it dropped to 25%. The *Acanthamoeba* isolates were identified at the species level using morphological criteria. Different species of *Acanthamoeba* were identified thanks to the unique characteristics of the cysts, such as their size, shape, number, and arrangement of cyst pores.

In a study conducted by Koyun *et al*. (2020) that investigated the presence of free-living amoebae in water sources within the Samsun province, 98 out of 192 water samples were found to contain *Acanthamoeba spp.* The study focused on various water sources, including rivers and tap water in Samsun along the Black Sea. Notably, despite the prevalence of *Acanthamoeba* species in the overall water samples, tap water samples, surprisingly, did not show any presence of *Acanthamoeba* species.

In order to separate and identify *Acanthamoeba* species from a variety of water sources, such as drinking water, tap water, swimming pools, and other recreational waters, Vijayakumar (2018), carried out an investigation. Fifty-seven water samples from various sources, including drinking water, swimming pools, and recreational water bodies, were used in the study. In order to isolate the *Acanthamoeba* species, all of the collected samples were processed and cultured on non-nutrient agar medium with an overlay of *Escherichia coli*. Microscopic observations of both the cyst and trophozoite forms were necessary for the identification of the organisms. Thermotolerance and osmotolerance assays were used to determine *Acanthamoeba's* pathogenicity. The findings revealed that 10 (17.5%) out of the 57 water samples that were analysed had *Acanthamoeba* in them. Notably, bore-well water kept in tanks (37.5%) and recreational water samples (26.7%) had the highest percentage of positive samples. Every processed sample of drinking water tested negative for *Acanthamoeba*. Three (30%) of the isolated strains were categorised as non-pathogenic, and four (40%) showed pathogenic characteristics.

2.6 Studies on Boreholes and other Water systems with other Microbes

Oyedum *et al*. (2016), collected 20 aseptic water samples, 10 from each of the pipe-borne and public borehole sources, as part of a comparative research project to evaluate coliform contamination in Bosso town's water supplies. With the exception of samples from Rafin-Yashi, Maikunkele, Federal University of Technology (F.U.T.) Minna, and Tudun Fulani, the majority (60.0%) of the borehole water samples showed coliform counts within 10 cfu/100ml.

In contrast, coliform counts greater than 10 cfu/100 ml were found in all (100.0%) pipe-borne water samples. The organisms that were identified comprised a range of species, such as *Salmonella, Shigella, Clostridium, Bacillus, Yersinia, Serratia, Pseudomonas, Streptococcus,* and *Staphylococcus*. The most common species found was *E. coli* (20%). This was followed by *Yersinia spp.* (1.7%), *Proteus vulgaris* (3.3%), *Pseudomonas aeruginosa* (6.7%), *Bacillus subtilis* (6.7%), *Shigella spp.* (11.7%), *Clostridium spp.* (8.3), *Streptococcus faecalis* (8.3%), and *Klebsiella spp.* (3.3%), *Klebsiella spp.* (3.3%), *Proteus vulgaris* (3.3%), *Yersinia spp.* (1.7%), and *Serratia spp.* (1.7%). This investigation revealed that Bosso's borehole and pipe-borne water samples were both contaminated, with the pipe-borne water showing a greater level of contamination.

Additionally, in the Benin metropolis, Edo State, Idibie *et al.* (2018) conducted a comparative microbial analysis of borehole water and various other water sources. The aim of the study was to use standard microbiological techniques to identify differences in water quality between different sources. To guarantee fair representation, water samples were carefully collected in sterile containers. In order to avoid silt inclusion, samples taken from rivers and wells were taken below the water's surface. After letting the tap run for about five minutes, samples of tap water were taken. The eight water sources that were sampled were well water from Ologbo and Owa, river water from Ikpoba and Ogbekpan, rainwater from Isihor and Uwelu, and water from boreholes.

The samples from the Ologbo well and Ikpoba river had the highest mean counts of bacteria, which varied from 2 × 104 to 32 × 104. Remarkably, the mean bacteria count of the water from the Isihor borehole was the lowest. Notably, for all of the different water samples included in this study, the counts of bacteria obtained exceeded the accepted limit set by the WHO. The water from the Ogbekpan River had the highest total coliform count (6 MPN/ml) while the water from the Isihor borehole had the lowest total coliform count (6 MPN/ml).

2.7 *Acanthamoeba* Infection and Contact Lens Wearers

The tendency of *Acanthamoeba* to stick to surfaces is a critical first step in the pathogenesis of *Acanthamoeba keratitis*, a condition that is especially common in contact lens wearers. Two different ways that contact lenses impact the integrity of the corneal epithelium are directly through abrasions related to lens fitting and indirectly through interference with normal cellular metabolism and physiological processes (Ibrahim *et al*., 2009). These changes cause the epithelial cells to become hypoxic, which ultimately compromises the integrity of the cells. When contact lens wear is prolonged beyond the recommended times, corneal oxygenation is significantly decreased, particularly in individuals who sleep with their lenses overnight.

In 2007, Bharathi *et al.* examined the sensitivity and specificity of smears in identifying *Acanthamoeba* and looked into the epidemiological and clinical features of *Acanthamoeba keratitis*. All cases of *Acanthamoeba keratitis* confirmed by culture between October 1999 and August 2002 were retrospectively reviewed for this study. Using established protocols, corneal scrapes were subjected to microscopy and culture.

The findings showed that 33 patients (1.04%) out of 3183 consecutive patients with corneal ulcers were diagnosed with *Acanthamoeba*. Of these cases, people under the age of 51 accounted for 24 (72.72%) out of 33 ($P < 0.001$). A total of 26 patients (78.79%) were agricultural workers ($P = 0.031$), and all patients ($P < 0.001$) were from rural areas. All 33 patients had previously sustained a corneal injury ($P < 0.001$), of which 28 (84.85%) had been caused by mud exposure ($P < 0.001$). Prior medical treatment was received by all 33 patients (100%) ($P = 0.009$), and 10 patients (30.3%) had used conventional eye remedies ($P = 0.183$).

In 15 patients (45.45%), a ring infiltrate was indicative of a clinical pattern. In 27 (81.82%) of the eyes, the corneal ulcer had a diameter larger than 6 mm ($P < 0.001$). At the time of initial presentation, 26 patients (78.79%) had visual acuity limited to light perception ($P < 0.001$), and 24 patients (72.73%) were able to maintain this level as their ultimate visual outcome. In terms of identifying *Acanthamoeba* cysts, the 10% potassium hydroxide (KOH) preparation was found to have a higher sensitivity ($P < 0.001$).

Concurrently, Gatti *et al*. (2019), evaluated the sensitivity and specificity of a PCR assay based on sequence analysis of the 18S rRNA gene in comparison to conventional parasitological techniques in their investigation on the isolation and genotyping of *Acanthamoeba* strains from corneal infections in Italy. Using genus-specific primers JDP1 and JDP2, a specific 405 bp region of the 18S rRNA gene (ASA.S1), including diagnostic fragment 3 (DF3), was amplified. The nuclear small-subunit rRNA gene sequence's ASA.S1 segment was subjected to phenetic analysis for the purpose of genotype classification; the highly variable DF3 region was left out. Using phylogenetic analysis, sequences were obtained.

In every reported instance, patients indicated the presence of infection in a single eye, with 11 individuals (68.75%) acknowledging that they had neglected to clean their contact lenses (CL). Among the 16 individuals assessed, 14 (87.5%) had corneal scrapings that were stained with calcofluor white and hematoxylin and eosin. Microscopy revealed the presence of *Acanthamoeba* cysts in 10 cases (62.5%). In vitro culture on 3% non-nutrient agar plates yielded positive results in all cases (100%), and axenic growth and cloning confirmed 14 amoebic stocks (87.5%). PCR analysis demonstrated 100% sensitivity and specificity, with positive amplification from 15 isolates when compared to in vitro axenic culture. Notably, all identified *Acanthamoeba* strains belonged to the T4 genotype, which is the most prevalent genotype globally in cases of *Acanthamoeba keratitis* (AK). These findings underscore the significance of a comprehensive diagnostic protocol for biological specimens and AK diagnosis, incorporating a PCR assay.

Unlike *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which are other common causes of *keratitis*, Gomes *et al.* (2016) looked into *Acanthamoeba* in discarded contact lenses. According to the study, 175 healthy Madrid residents submitted their old contact lenses and answered questions regarding their personal hygiene routines. DNA was extracted from the contact lens solutions and subjected to real-time PCR analysis to detect the presence of *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Acanthamoeba*. To isolate *Acanthamoeba*, contact lenses and solutions were cultivated on non-nutrient agar.

The findings showed that 87 cases (49.2%) of the 177 samples had DNA from *Acanthamoeba*, 14 cases (7.9%) had DNA from *P. aeruginosa*, and 19 cases (10.7%) had DNA from *S. aureus*. However, only one sample (0.6%) that contained amoebae was obtained. This isolate was identified as T4 by genotyping. The contact lens wearers disclosed certain behaviors that were known to be risk factors for AK. The presence of *Acanthamoeba* DNA was found to be statistically significantly correlated with "not cleaning the contact lens case" in this study. Although the presence of *P. aeruginosa* suggested that *Acanthamoeba* may have been inhibited in these samples, the investigated bacterial DNA did not show a statistically significant association with the practices under study.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted in Egor Local Government Area, in Benin City, Edo State, Nigeria. Egor LGA lies between latitude 6.3737°N and 6°21'26.93"N and longitude 5.57547°E and 5°34'31.69"E (Fig. 3.1). The town of Uselu is the headquarters. It is also one of the LGAs that make up Benin City's greater metropolitan area. According to the 2006 census, it has a 93 km^2 area and a population of 339,899 (Idibie *et al.*, 2018). Egor Local Government Area is divided into ten wards: Uselu I, Uselu II, Uwelu, Okhoro, Oliha, Otubu, Ugbowo, Ogida/Use, Egor and Evbareke.

Benin City, is positioned at 6° 20' 0" North, 5° 38' 0" East. It is strategically located along the main highways linking Lagos to Asaba and as well as the eastern states. Situated on a branch of the Benin River, the city is well-connected by air, with access to the ports of Koko and Sapele in the Niger River Delta, as well as road links to Sapele, Siluko, Okene, and Ubiaja (Idibie *et al.*, 2018).

3.2 Research Design

This study is a prospective, observational cross-sectional investigation. Water samples from tap water and boreholes, were collected from various locations in Egor LGA. Samples were collected separately into sterile, dry, and clean polypropylene containers. The samples were then taken to the laboratory for processing. The study was conducted during the rainy season, and the samples' temperature range was 20–37 °C.

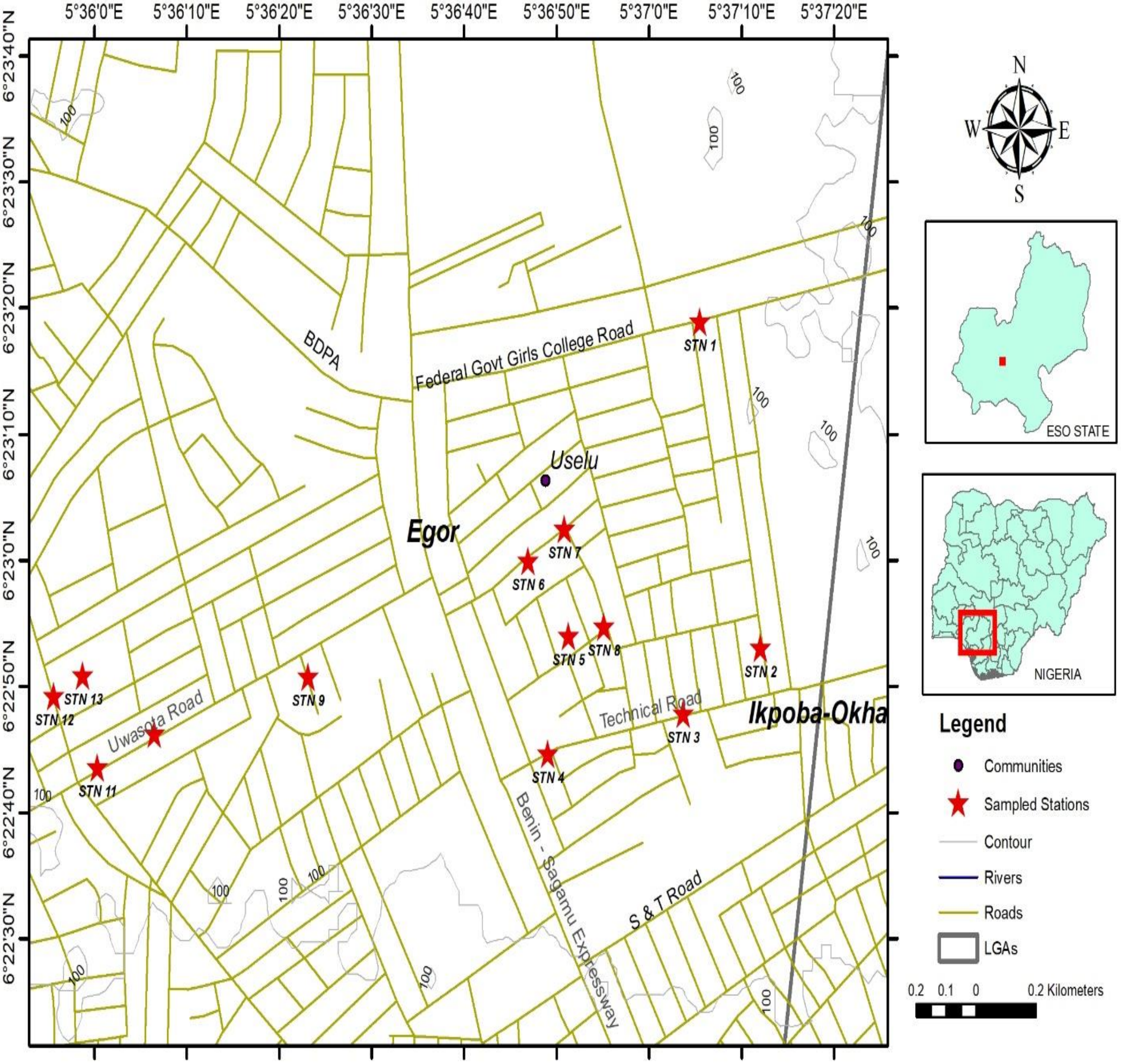

Fig. 3.1. Map of study area showing the sampled stations within Egor Local Government Area, Edo State, Nigeria

3.3 Materials

Sterile polypropylene containers

Nitrocellulose membrane filter (0.45 µm pore size and 47 mm diameter).

Stainless steel filter

Suction pump

Non-nutrient Agar

Heat-killed *Escherichia coli*.

3.4 Procedure for Detection of *Acanthamoeba* species

3.4.1 Detection of the Parasites

In the process of identification of *Acanthamoeba* species, 500 mL of water samples underwent filtration through a 0.45 µm-pore-sized cellulose nitrate membrane. Subsequently, the membrane was covered with parafilm and placed in an incubator at 30°C to facilitate the growth of free-living amoebae. After a three-day incubation period, the plates were examined under the microscope to detect the presence of *Acanthamoeba* trophozoites. Throughout this phase, the plates were maintained at 26°C. If, after the initial incubation, no parasite was observed, the plates were continuously monitored for up to 14 days under the same conditions. Identification of *Acanthamoeba* was achieved by using microscopy to determine the morphological characteristics of trophozoites and cysts, with sample plates serving as a reference guide (Al-Herrawy *et al*., 2017).

3.4.2 Centrifugation Technique

This method required the use of 10 mL centrifugation containers. Nine containers filled with sample water were centrifuged at 5000 rpm. Subsequently, the supernatant water was discarded, leaving the centrifuged sediments. Freshly prepared sediment specimens were viewed microscopically via a slide stained with iodine.

3.4.3 Identification of *Acanthamoeba*

Identification of *Acanthamoeba* relied on observing the morphological characteristics of trophozoites and cysts through microscopy and comparing same from sample plates as a guide (Al-Herrawy *et al*., 2017; Al-Herrawy *et al*., 2014). The distinctive features of the cysts, including their shape, size, the number and arrangement of the cyst pores, were instrumental in identifying different species within the genus *Acanthamoeba*.

3.5 Limitation of Study

The rising cost of laboratory procedures incurred during this study constituted a limitation. The reluctance of borehole owners to allow the collection of water samples became a constant challenge.

3.6 Data Analysis

Basic statistical measurement of percentage distribution was utilized to express the relative size of each component within the dataset of *Acanthamoeba spp.* found in water samples from pipe and tap. This made easier the comparison and analysis of different variables. Statistical analysis, including paired samples t-tests was performed to compare *Acanthamoeba* presence between bore-hole source water and tap water and also between the two methods (culture and centrifugation). Ultimately, data obtained from this study were analyzed with SPSS version 22.0.

CHAPTER FOUR

RESULTS

4.0 RESULTS

4.1 Overall prevalence of *Acanthamoeba* species in borehole source water and tap water from Egor LGA, Edo State, Nigeria

A total of 52 water samples (26 each from borehole source water and tap water) were examined for *Acanthamoeba* species in this study and 27 were infected with an overall prevalence of 51.92%. *Acanthamoeba* species were detected in 15 (57.69%) of the 26 samples from borehole source water while 12 (46.15%) of the borehole tap water had *Acanthamoeba* species In most cases, the trophozoite and cystic forms became visible after a week; in other samples, this happened after a month. Ten *Acanthamoeba* species including *A. triangularis, A. royreba, A. astronyxis, A. comandoni, A. mauritaniensis, A. culbertsoni, A. quina, A. lenticulata, A. polyphaga*, and *A. castellanii* were detected in the borehole source water and tap water examined in this study.

4.2 Prevalence and mean intensity of *Acanthamoeba* species infection in borehole source water from Egor LGA, Edo State, Nigeria after culture

Nine species of *Acanthoemoeba* namely *A. polyphaga*, *A. triangularis*, *A. royreba*, *A. quina*, *A. comandoni*, *A. castellani*, *A. culbertsoni*, *A. astronyxis* and *A. mauritaniensis* were detected with using the culture method of identification (Plate 4.1). *Acanthamoeba polyphaga* had the highest prevalence and mean intensity of 50.0% and 1.33±0.13, respectively. This was followed by *A. triangularis* (34.62%), *A. royreba* (30.77%), *A. quina* (26.92%), *A. mauritaniensis* (23.08%) and *A. castellanii* (19.23%). *Acanthamoeba comandoni* and *A. astronyxis* had a prevalence and intensity of 11.54% and 0.33±0.19 parasite per infected borehole water each. The lowest prevalence (7.69%) of parasitic infection was recorded for *A. culberstsoni*. *Acanthamoeba mauritaniensis* and *A. castellanii* had mean intensity of 0.53±0.19 each. The prevalence and mean intensity of *Acanthamoeba* species in borehole source water after culture are presented in Table 4.1.

Plate. 4.1. Cyst stages of detected *Acanthamoeba* species from Bore-hole water source after culture. Key: 1= *A. polyphaga*, 2= *A. triangularis,* 3= *A. royreba*, 4= *A. quina*, 5= *A. comandoni*, 6= *A. castellani*, 8= *A. culbertsoni*

Table 4.1 Prevalence and mean intensity of *Acanthamoeba* species in borehole source water after culture

***Acanthamoeba* species**	**Number examined**	**Number infected**	**Prevalence (%)**	**Number of parasites**	**Mean intensity ± SD**
Acanthamoeba triangularis	26	09	34.62	19	1.27±0.12
A. polyphaga	26	13	50.0	20	1.33±0.13
A. culberstsoni	26	02	7.69	04	0.27±0.18
A. comandoni	26	03	11.54	05	0.33±0.19
A. mauritaniensis	26	06	23.08	08	0.53±0.19
A. castellanii	26	05	19.23	08	0.53±0.22
A. royreba	26	08	30.77	13	0.87±0.24
A. astronyxis	26	03	11.54	05	0.33±0.19
A. quina	26	07	26.92	10	0.67±0.21

SD – Standard deviation

4.3 Prevalence and mean intensity of *Acanthamoeba* species infection in borehole source water from Egor LGA, Edo State, Nigeria after centrifugation

Table 4.2 shows the prevalence and mean intensity of borehole water source from Egor LGA, Edo State, Nigeria after centrifugation. *Acanthamoeba triangularis* and *A. culberstsoni* showed the highest prevalence, both at 30.77%, followed closely by *A. mauritaniensis* at 26.92%. These species appear to be relatively common in the borehole source water samples after centrifugation. The study also showed that *A. culberstsoni* had the highest mean intensity at 1.13 ± 0.31, followed by *A. mauritaniensis* at 0.93 ± 0.27 parasites per positive sample, indicating a relatively higher concentration compared to other species. The mean intensity of other species include *A. polyphaga*, 0.87 ± 0.27; *A. Triangularis*, 0.47 ± 0.22; and *A. lenticulata*, 0.33 ± 0.19. *Acanthamoeba royreba* and *A. comandoni* showed intensity of 0.53 ± 0.24 each. *Acanthamoeba astronyxis* has the lowest prevalence and mean intensity of 7.69% and 0.13 ± 0.09 parasites per positive sample, respectively.

Table 4.2. Prevalence and mean intensity of *Acanthamoeba* species in borehole source water from Egor LGA, Edo State, Nigeria after centrifugation

***Acanthamoeba* species**	**Number examined**	**Number infected**	**Prevalence (%)**	**Number of parasites**	**Mean intensity ± SD**
A. culberstsoni	26	08	30.77	17	1.13 ± 0.31
A. qina	26	03	11.54	06	0.40 ± 0.21
A. royreba	26	05	19.23	08	0.53 ± 0.24
A. mauritaniensis	26	08	30.77	14	0.93 ± 0.27
A. polyphaga	26	07	26.92	13	0.87 ± 0.27
A. Triangularis	26	04	15.38	07	0.47 ± 0.22
A. comandoni	26	04	15.38	08	0.53 ± 0.26
A. lenticulata	26	03	11.54	05	0.33 ± 0.19
A. castellanii	26	03	11.54	04	0.27 ± 0.15
A. astronyxis	26	02	7.69	02	0.13 ± 0.09

4.4 The prevalence and mean intensity of *Acanthamoeba* species in borehole tap water from Egor LGA, Edo State, Nigeria after culture

Table 4.3 shows the prevalence and mean intensity of borehole water source from Egor LGA, Edo State, Nigeria after culture. *Acanthamoeba triangularis* exhibits the highest prevalence at 26.92%, and mean intensity 0.92 ± 0.29; followed by *A. royreba* at 19.23% and mean intensity of 0.75 ± 0.33. Other species that follow include; *A. polyphaga* and *A. quina* which exhibited similar prevalence at 15.38% and intensity 0.58 ± 0.26. *Acanthamoeba mauritaniensis* showed an identical mean intensity but not same prevalence. *Acanthamoeba castellanii* and *A. comandoni*, also showed similar prevalence and intensity at 7.69% and 0.25 ± 0.18, respectively.

Table 4.3. Prevalence and mean intensity of *Acanthamoeba* species in borehole tap water after culture

***Acanthamoeba* species**	**Number examined**	**Number infected**	**Prevalence (%)**	**Number of parasites**	**Mean intensity ± SD**
A. castellanii	26	02	7.69	03	0.25 ± 0.18
A. comandoni	26	02	7.69	03	0.25 ± 0.18
A. Triangularis	26	07	26.92	11	0.92 ± 0.29
A. polyphaga	26	04	15.38	07	0.58 ± 0.26
A. mauritaniensis	26	03	11.54	07	0.58 ± 0.31
A. quina	26	04	15.38	07	0.58 ± 0.26
A.royreba	26	05	19.23	09	0.75 ± 0.33
A. culberstsoni	26	03	11.54	03	0.25 ± 0.13

SD – Standard deviation

4.5 The prevalence and mean intensity of *Acanthamoeba* species in borehole tap water from Egor LGA, Edo State, Nigeria after centrifugation

Nine species of *Acanthoemoeba* namely *A. polyphaga*, *A. triangularis*, *A. royreba*, *A. quina*, *A. comandoni*, *A. castellani*, *A. culbertsoni*, *A. astronyxis* and *A. mauritaniensis* were detected with the centrifugation method of identification. Plate 4.2 showed *A. lenticulata* which was not visible in the previous plate obtained after culture (Plate 4,1). *Acanthamoeba culberstsoni* showed the highest prevalence of 30.77% with a mean intensity of 0.92 ± 0.23, followed closely by *A. triangularis* at 26.92% with the same mean intensity as *A. culberstsoni*. *Acanthamoeba comandoni* showed a prevalence at 23.08% while *A. castellanii* exhibited a prevalence of 19.23%. *Acanthamoeba astronyxis* had the lowest prevalence of 7.69% (mean intensity, 0.42 ± 0.29). *Acanthamoeba triangularis* and *A. culberstsoni* have the highest mean intensity, both at 0.92 ± 0.26 and 0.92 ± 0.23 parasites per positive sample, respectively. *Acanthamoeba qina, A. royreba,* and *A. astronyxis* had the lowest mean intensity, all at 0.42 parasites per positive sample.

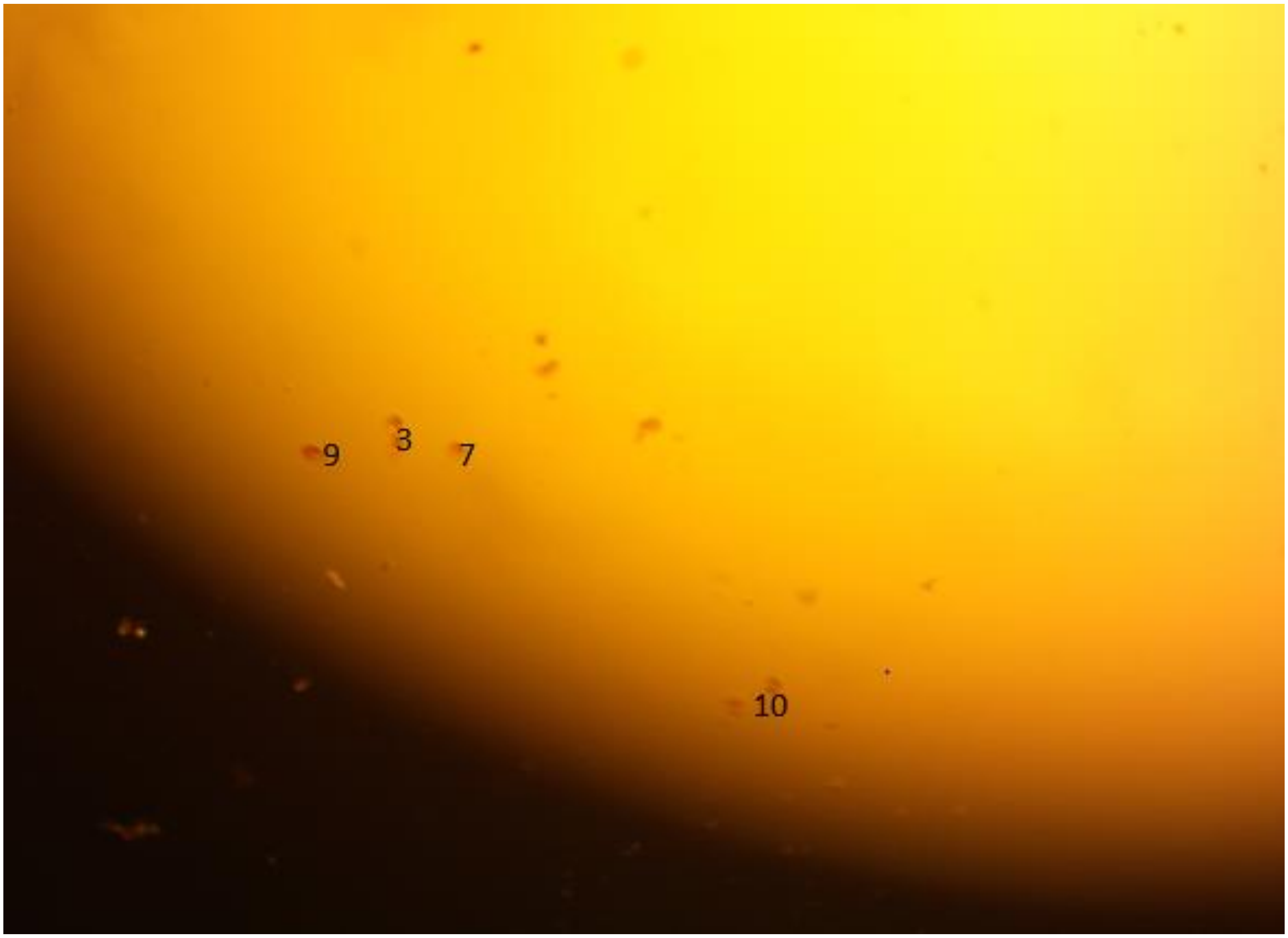

Plate. 4.2. Cyst stages of detected *Acanthamoeba* species obtained from Bore-hole tap water after centrifugation. Key: 7= *A. lenticulata*, 9= *A. mauritanienses*, 10= *A. astronyxis*

Table 4.4 Prevalence and mean intensity of *Acanthamoeba* species in borehole tap water after centrifugation

***Acanthamoeba* species**	**Number examined**	**Number infected**	**Prevalence (%)**	**Number of parasites**	**Mean intensity ± SD**
A. Triangularis	26	07	26.92	11	0.92 ± 0.26
A. culberstsoni	26	08	30.77	11	0.92 ± 0.23
A. mauritaniensis	26	05	19.23	08	0.67 ± 0.26
A. qina	26	03	11.54	05	0.42 ± 0.23
A. royreba	26	04	15.38	05	0.42 ± 0.19
A. comandoni	26	06	23.08	09	0.75 ± 0.25
A. astronyxis	26	02	7.69	05	0.42 ± 0.29
A. polyphaga	26	03	11.54	06	0.50 ± 0.26
A. castellanii	26	05	19.23	08	0.67 ± 0.28

SD – Standard deviation

4.6 Statistical tests

For cultured sample, the tests showed that a significant difference exist between the presence of *Acanthamoeba* species ($p = 0.007$) in borehole source and tap in Benin City, while there was no significant difference ($p = 0.277$) between the presence of *Acanthamoeba* species in centrifuge samples of borehole source and tap as shown in Tables 4.5 and 4.6 respectively.

Table 4.5: Paired samples *t*-test between water sources after culture

Source	N	mean±SD	Mean difference ±SD	*t*	*p*-value
Borehole source	9	10.22±5.95	4.67±3.84	3.645	0.007
Borehole tap	9	5.56±3.50			

SD – Standard deviation, N – Sample size, *p*-value – Probability value

Level of significance (α) = 0.05

Table 4.6: Paired sample *t*-test between water sources after centrifugation

Source	N	mean±SD	Mean difference ±SD	*t*	*p*-value
Borehole source	10	8.40±4.79	1.60±4.37	1.156	0.277
Borehole tap	10	6.8±3.33			

SD – Standard deviation, N – Sample size, *p*-value – Probability value

Level of significance (α) = 0.05

4.7 Comparison of both Methods

Although the paired t-test for significant difference in the mean count of *Acanthaoemba* species identified in borehole source samples using both methods was statistically insignificant at the 5% level of significance (t = 0.528, p = 0.610), it is worth noting that two more *Acanthamoeba* species (*A. lenticulata* (5) and *A. triangularis* (7)) were identified through the centrifuge method (Table 4.7).

The paired t-test for significant difference in the mean count of *Acanthaoemba* species identified in borehole tap samples using both methods was statistically insignificant at the 5% level of significance (t = 1.455, p = 0.184) (Table 4.8), the centrifuge method however, detected one more *Acanthamoeba* species (*A. astronyxis* (5)).

Table 4.7: Comparison between mean count of *Acanthamoeba* species in culture and centrifuge methods for borehole source samples

		Mean Count ± SD	Difference in Mean counts ± SD	t	*p* value
Pair	Culture	7.30 ± 6.06	1.10 ± 6.59	0.528	0.610
	Centrifuge	8.40 ± 4.79			

SD – Standard deviation, α = 0.05

Table 4.8: Comparison between mean count of *Acanthamoeba* species in culture and centrifuge methods for borehole tap samples

		Mean Count ± SD	Difference in Mean counts ± SD	t	*p* value
Pair	Culture	5.56 ± 3.50	2.00 ± 4.12	1.455	0.184
	Centrifuge	7.56 ± 2.46			

SD – Standard deviation, α = 0.05

CHAPTER FIVE

5.0 DISCUSSION

The morphological charateristics of various *Acanthamoeba* species are similar. In a culture, they appear to be pleomorphic and move on the substrate by producing endocytic structures and emitting slight cytoplasmic microprojections from the cell surface. These projections are formed by hyaline cytoplasm and they are related to motion structures such as acanthopodia and lamellipodia, in which actin provides a framework that allows rapid changes in morphology. In the cytoplasm abundant vacuoles of different size and content are seen (Gonzalez-Robles *et al.*, 2013). By means of electron microscopy, it is possible to observe the compact fibrogranular appearance of the cytoplasm, along with the main cellular organelles such as the Golgi complex, the endoplasmic reticulum, digestive vacuoles, mitochondria and contractile vacuoles.

For *A. triangularis*, endocyst could appear stellate, polygonal and triangular. Ectocyst is thick wrinkled and corrugated but is not spherical. Rays of endocyst are broad and slightly curved. The average number of pores was 3 or 4. The diameter of cysts was 13 µm (Al-Herrawy *et al*., 2017).

Like *A. triangularis, A. polyphaga* also belongs to group II in morphological classifications and has relatively smaller cysts with an average diameter of less than 18 µm. The outer cyst wall is folded or wavy, and the inner cyst wall is various in shape, which is wavy, tetragonal shape (Wang *et al*., 2023). Other species exhibit varying levels of prevalence and mean intensity, suggesting differences in their distribution and concentration within the borehole water.

This study examined the prevalence of *Acanthamoeba* in tap water and borehole sources in Egor Local Government Area of the metropolitan Benin City. Available literature showed that there is no prior research on *Acanthamoeba* in Nigerian borehole water.

According to this study, 57.69% of the 26 samples had *Acanthamoeba* in the borehole water sources, and 46.15% of the 26 samples had positive results for the borehole water tap. *Acanthamoeba triangularis*, *A. culbertsoni*, *A. mauriteniensis*, and *A. polyphaga* were the most frequently occurring species in this study. *Acanthamoeba* species were more prevalent in borehole source water compared to borehole tap water. This difference in prevalence between source and tap water suggests that the distribution process may affect the presence of *Acanthamoeba* species, potentially leading to a reduction in their occurrence in tap water.

According to Al-Herrawy *et al*. (2017), 44.4% of the 144 tap water samples obtained from drinking water that were analysed in Egypt had *Acanthamoeba*. The most common species analysed in their study included *A. comandoni* followed by *A. astronyxis* and *A. triangularis*. This was less than what our investigation revealed probably due to the inconsistent treatment of borehole water in Nigeria.

In the study aimed at identifying *Acanthamoeba* in park pond water within a tropical and subtropical region of the Middle East and exploring its relationship with physicochemical parameters, Ghaderifar *et al.* (2018) found that 19 samples (21.1%) tested positive for the parasite. The presence of *Acanthamoeba* did not exhibit a significant correlation with any of the studied physicochemical parameters.

The results from the paired samples t-test for cultured samples, as presented in Table 4.5, revealed a significant difference ($p = 0.007$) in the presence of *Acanthamoeba* species between borehole water sources and taps in the study area. This observation aligned with the results of Sente *et al*. (2016), who investigated the prevalence of *Acanthamoeba* in specific environmental settings in Uganda, including the banks of the Kazinga channel (60.7%), fish landing sites (50%), the River Kyambura (39.5%), and the Kazinga mid-channel (5.3%). Their study demonstrated a significant variation in *Acanthamoeba* prevalence among sampling sites ($p = 0.001$). The isolated *Acanthamoeba* species in that investigation included *Acanthamoeba hatchetti*, and *Acanthamoeba polyphaga*.

In addition, the prevalence of *Acanthamoeba* species varied between the culture and centrifugation methods and among different species. Some species, such as *A. polyphaga*, exhibited consistent prevalence across methods, while others showed differences.

Comparing the results with data from Tables 4.2 and 4.4 borehole tap water after culture and borehole source water after centrifugation, there seemed to be differences in both prevalence and mean intensity for some species. These differences could be due to variations in environmental factors or sampling methods. For example, *A. polyphaga* showed a decrease in both prevalence and mean intensity after centrifugation. Mean intensity also varied between the culture and centrifugation methods. Some species maintained similar mean intensities, while others showed differences. Overall, *A. triangularis* and *Acanthamoeba culberstsoni* consistently demonstrated high mean intensities across both borehole water sources and methods.

Furthermore, It is imperative, therefore, to posit that differences in prevalence and mean intensity between culture and centrifugation methods might be attributed to the efficiency of each method in concentrating and detecting *Acanthamoeba* species. Centrifugation may provide higher sensitivity in detecting low concentrations of parasites but could also result in differences due to potential loss of viability during the process.

A study by Carnt *et al*. (2020) examined the potential seasonal variation in the prevalence of free-living *Acanthamoeba* in household tap water in the greater Sydney area of Australia. The investigation found that 12 (27.9%) out of 43 samples classified as *Acanthamoeba* were collected during the winter, while 16 (29.6%) out of 54 samples were obtained during the summer. The statistical analysis indicated no significant difference (p = 0.85) in the prevalence of free-living *Acanthamoeba* between summer and winter. According to a study conducted by Vijayakumar (2018), contact lens wearers might face an increased risk due to the high percentage of pathogenic strains

(40%) found in recreational water. The study further outlined the distribution of *Acanthamoeba* in various water sources in the central region of Saudi Arabia.

Individuals relying on soil and water for household chores, agricultural activities, farming occupations, and recreation might be at risk, as the discovery of *Acanthamoeba* in soil, water, and air suggested ongoing environmental contamination (Bunsuwansakul *et al*., 2019). While the primary focus of this study may not be on pathogenicity, it is reasonable to suggest that inadequate environmental sanitation could contribute to *Acanthamoeba* contamination in the surrounding area. Although *Acanthamoeba spp.* is typically considered an uncommon potential pathogen, it has been associated with various health issues in humans. These include cutaneous lesions, sinusitis, *Acanthamoeba Keratitis* (AK), Granulomatous Amebic Encephalitis (GAE), and a disseminated form, particularly affecting individuals with underlying medical conditions or compromised immune systems. Notably, AK has been reported in immunocompetent patients, especially those who wear contact lenses (Bunsuwansakul *et al*., 2019).

Additionally, there is a correlation between low *Acanthamoeba* trophozoite counts and extremes in temperature and dissolved oxygen levels. *Acanthamoeba spp.* are more prevalent in water that is contaminated and rich in bacteria, irrespective of the specific physico-chemical parameters of the water.

This study posits that the prevalence of *Acanthamoeba* in environmental water and borehole water within the Benin Metropolis is primarily attributed to the nature of the water facilities. Consequently, the availability of safe drinking water for the public is compromised in both domestic and environmental contexts. Abanyie *et al*. (2016) and Adamou *et al*. (2020) emphasized that providing the population with reliable access to potable water remained a significant challenge for developing nations globally.

In Nigeria, few studies have explored the use of chlorine for treating borehole water. Akerele *et al*. (2023) discovered significant deviations in various parameters of raw water samples from the approved levels of the Nigeria Standards for Drinking Water Quality (NSDWQ) and the World Health Organisation (WHO), highlighting the need for enhanced water treatment techniques. Their study aimed to assess the quality of raw water samples and the efficacy of conventional treatment methods commonly employed by residents in the Lekki area of Lagos.

It is noteworthy that, to date, there have been no prior studies conducted in Nigeria on *Acanthamoeba* or any other amoeba, making it challenging to ascertain the current impact of *Acanthamoeba* on human health in the region.

5.1 SUMMARY

This research investigated the prevalence of *Acanthamoeba* species in borehole water in the Egor Local Government Area of Benin City, Edo State, Nigeria. In this study, two methods were utilized and compared for the detection of the parasites. The study utilized a prospective, observational cross-sectional design, collecting 52 water samples from various locations. The samples underwent filtration and examination for the presence of *Acanthamoeba* species using the light microscopy.

Results showed the detection of *Acanthamoeba* species in both borehole water source (57.69%) and borehole water tap (46.16%). Ten species of *Acanthamoeba* were detected with *A.triangularis* and *A. polyphaga* being the most prevalent. The study also compared the efficacy of the two methods, culture and centrifugation, in detecting *Acathamoeba* species, noting differences in mean intensity between the methods.

Discussion highlighted the significance of the findings, emphasizing the potential health risks associated with *Acanthamoeba* contamination in water. The study suggested that inadequate environmental sanitation and water treatment could contribute to the prevalence of *Acanthamoeba* in the region. However, the research also underscored the paucity of studies on *Acanthamoeba* in Nigeria, highlighting the need for further research to assess its impact on human health.

Overall, the research provided valuable insights into the presence of *Acanthamoeba* in borehole water in Benin City, Nigeria and calls for improved water treatment measures to ensure public health and safety.

5.2 CONTRIBUTION TO KNOWLEDGE

The study has contributed to knowledge in the following ways:

1. There is substantial prevalence of *Acanthamoeba* species in Borehole water sources within Benin City.

2. The high prevalence of *Acanthamoeba* in borehole water used in various homes within the Benin Metropolis could be traceable to the nature of the Borehole and other environmental implications.

5.3 CONCLUSION AND RECOMMENDATION

Using the culturing method, this study discovered a significant difference in the presence of *Acanthamoeba* species between taps and borehole water sources in Benin City. However, applying the centrifuge method produced no discernible difference. These findings imply that the conclusion about the existence of *Acanthamoeba* species in the water sources may be influenced by the technique used to analyse the samples. Therefore, the nature of the borehole facility is responsible for the high frequency of *Acanthamoeba* in borehole water used in different homes within the Benin Metropolis. Additionally, the results of this study suggest that there is a good chance that *Acanthamoeba spp.* will contaminate household and environmental tap water systems. It might be necessary to conduct additional research to find out why these techniques yielded different results and to think about the effects on Benin City's water quality and safety. It is also critical to take into account the study's limitations, which include the sample size and the particulars of the water sources that were examined.

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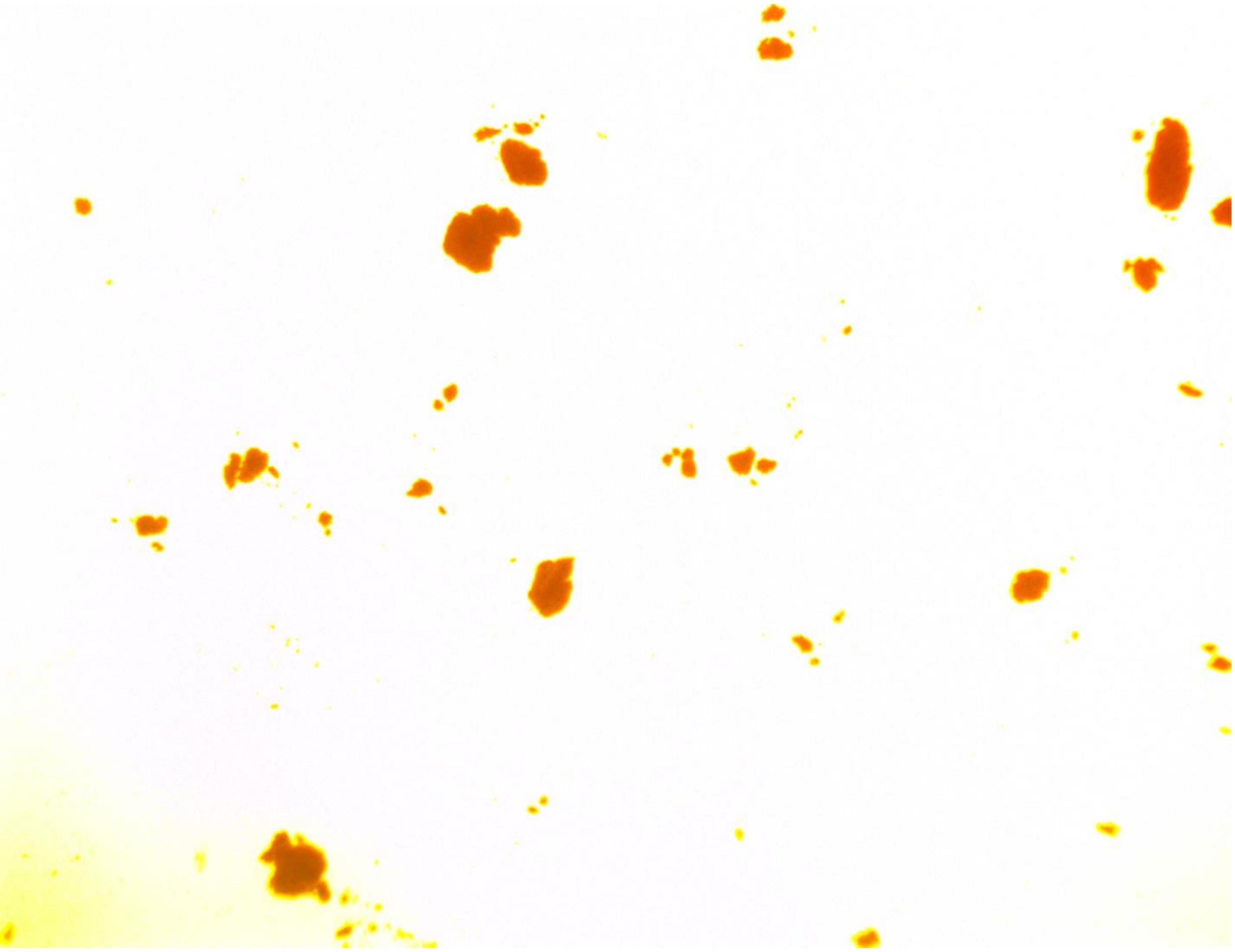
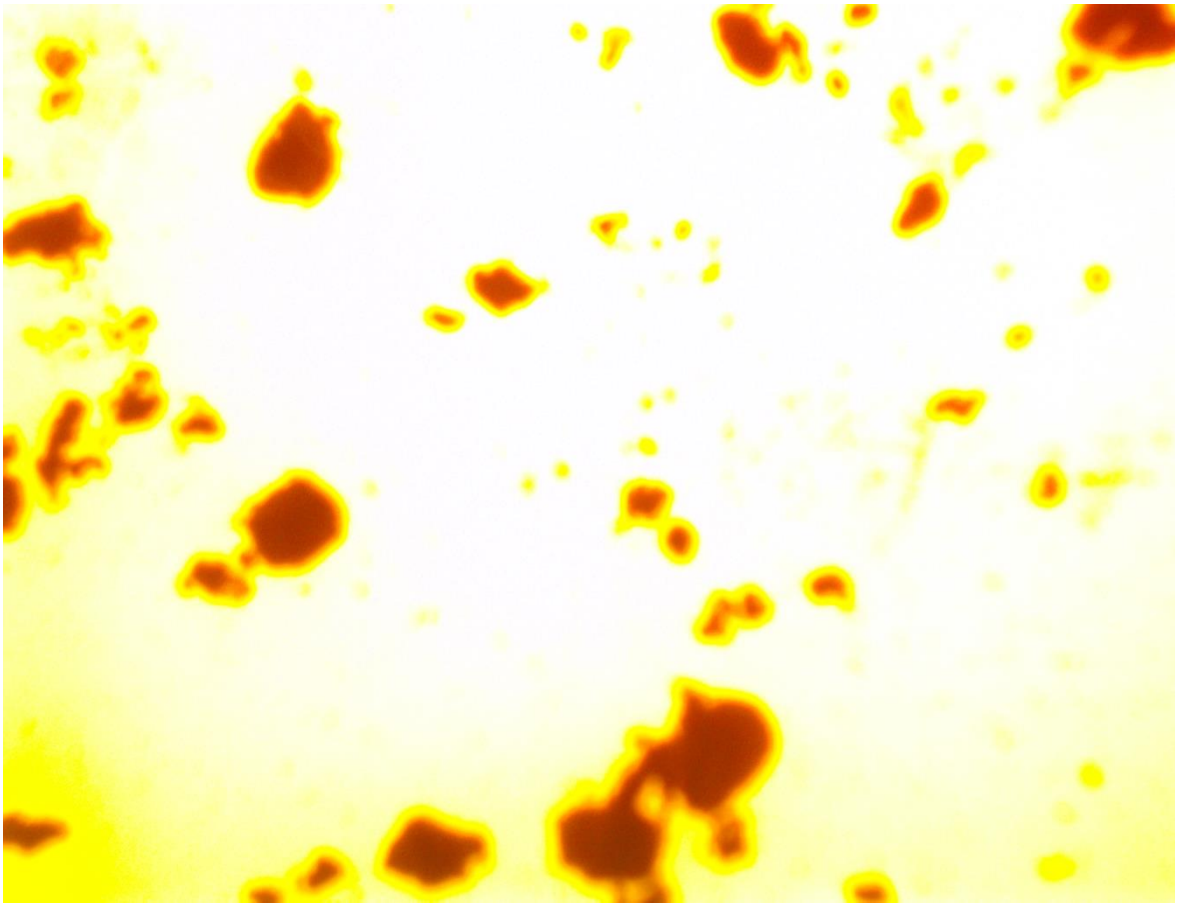
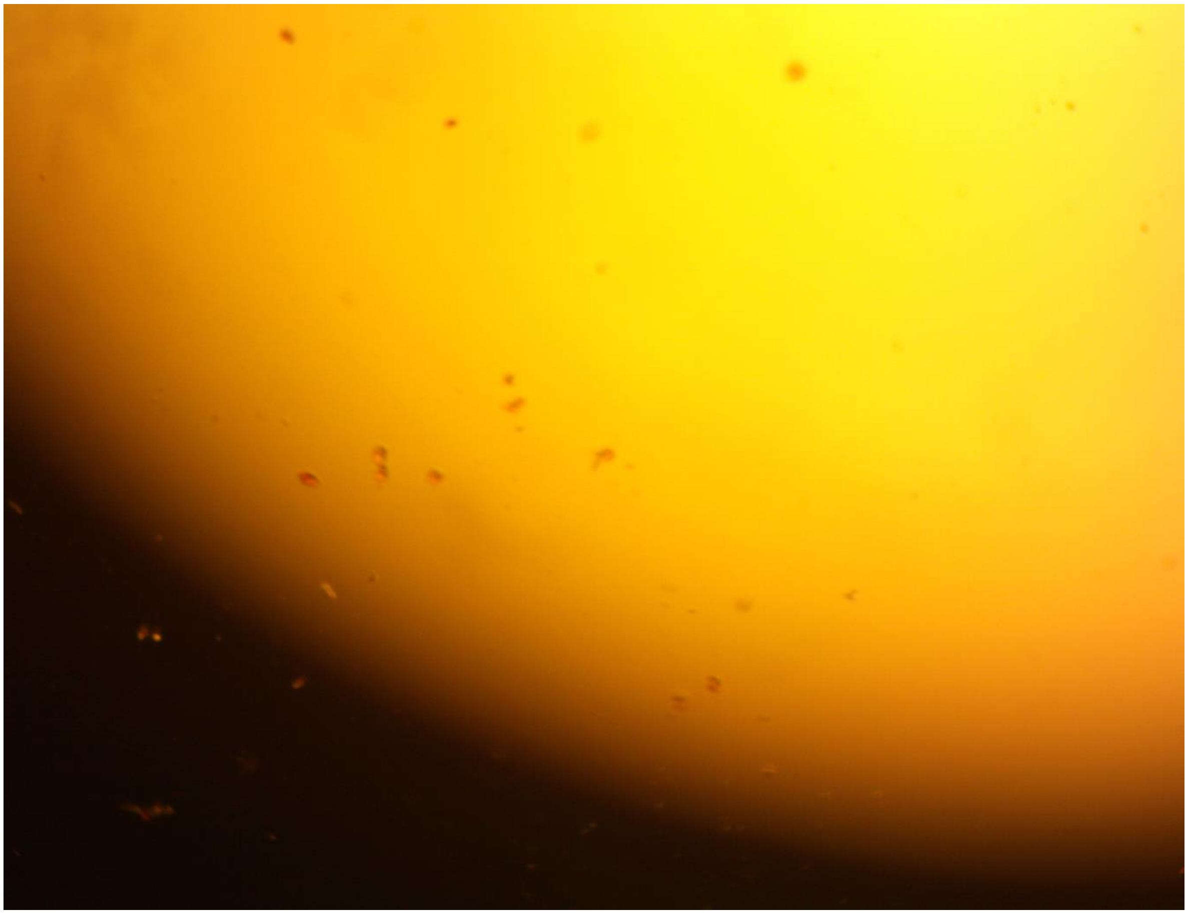
APPENDIX



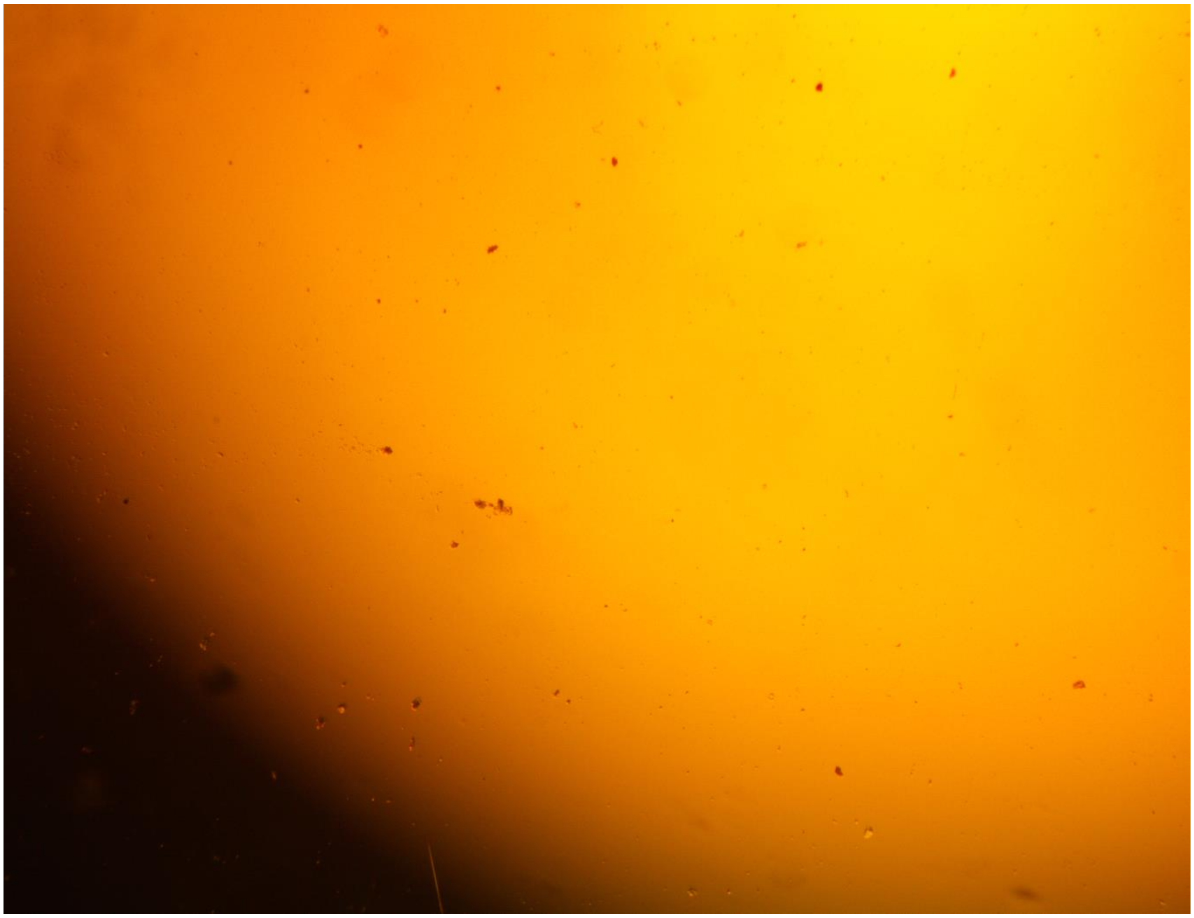
Distribution of *Acanthamoeba* species from Bore-hole water source after culture.



Distribution of *Acanthamoeba* species in Bore-hole tap water after culture.



Distribution of *Acanthamoeba* species in Bore-hole water source after centrifugation.



Distribution of *Acanthamoeba* species in Bore-hole tap water after centrifugation.