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The molecular identification, Isolation, morphologic, serologic of *Acanthamoeba sp*. throw the brain Of Sprague-Dawley Male Rats-(Case Record)

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Normally, free-living amoebae, individuals from the family *Acanthamoeba* can be found in a scope of organic specialties, like soil, new and saline water, airborne dust, channels for warming, ventilation, and cooling, as well as swimming pools and hot tubs. Now and again, they have likewise been connected to infections of the focal sensory system in the two individuals and creatures. Right now, amoebic disease related passing in Sprague-Dawley rodents prompted the segregation of amoeba into culture from their minds. In light of culture characteristics, sore shape, and immunofluorescence tests, the tainting one-celled critter was recognized in the ongoing study as *Acanthamoeba sp*. Furthermore, by sequencing an indicative part of the atomic little subunit ribosomal RNA quality, the one-celled critter was recognized as *Acanthamoeba sp*.

Keywords: Rat brain , *Acanthamoeba*, Isolation, Molecular identification, Ribosomal RNA gene

INTRODUCTION

The sort *Acanthamoeba* is notable for being a crafty reason for illnesses in the two individuals and creatures (Carrasco-Sánchez et al. 2021; Marciano-Cabral and Cabral 2003; Schuster and Visvesvara 2004a).*Acanthamoeba* can infect humans' lungs, skin, nasal sinuses, eyes, and produce severe lesions via the zzcentral nervous system (CNS). Moreover, they have contaminated lower primates, canines, ponies, bovines, birds, reptiles, fish, and spineless creatures, bringing about difficult ailment or passing (Álvarez et al. 2021)(Carrasco-Sánchez et al. 2021; Marciano-Cabral and Cabral 2003; Schuster and Visvesvara 2004a),(Visvesvara, Moura, and Schuster 2007a). In this report, we discuss the disclosure of a one-celled critter that killed a 5-month-old male Sprague-Dawley rodent. We identified it as *Acanthamoeba* in view of pimple morphology, immunofluorescence and sub-atomic assays.

MATERIALS AND METHODS

Experimental rat model

The Medical Research Center Animal House at the Faculty of Medicine at Umm Al-Qura University provided a 5-month-old male Sprague-Dawley rat, which was handled in compliance with the moral standards specified by the ethics council of Umm Al-Qura University.

Rats were quarantined for the customary 30 days. Actual assessment, full blood count, and biochemical profile were all inside normal ranges preceding the review except for an expanded creatine phosphokinase. No parasites or ova were found when the faeces were directly, and flotation examined. Up until 8 days after their arrival, when 50% of the rats were discovered dead without showing any preceding clinical indications, the rats seemed to be doing well.

2 hours after death, a post-mortem examination was performed. Brain cultures both aerobic and anaerobic were obtained. We gathered examples of cerebrum tissue, fixed them in 10% phosphate-cushioned formalin with a pH of 7.4, dried them in ethanol of expanding strength, washed them in toluene, and mounted them in liquid paraplast at 58-62°C. Hematoxylin and eosin was used to stain each of the following histological sections (H&E). The modifications in the brain were studied using light microscopy. Representative samples of the intestines, lungs, kidneys, liver, and spleen were frozen.

***In vitro* culture**

Before being thawed in a water bath at 37 °C, the frozen brain was separated into three sections, minced, and tiny fragments. Both halves were incubated at 37°C, into a human lung fibroblast (HLF) cell which have a one hundred g/ml gentamicin in addition to the other into a non-nutrient agar plate with a layer of *Escherichia coli*. (Schuster and Visvesvara 2004a). No bacteria were found despite the addition of gentamicin, 5% foetal bovine serum, and amoebae that had grown on agar plates to liquid culture medium (PYG). (Visvesvara, Moura, and Schuster 2007a).

Indirect immunoflourescence

Indirect immunoflourescence (IIF) was carried out on formalin-fixed, paraffin-embedded slices of brain by deparaffinizing and coating each section with a 1:200 dilution of one of three distinct rabbit antisera produced against *Acanthamoeba castellanii*, *Balamuthia mandrillaris*, and *Naegleria fowleri*

Indirect immunoflourescence (IIF) was done on formalin-fixed, paraffin-embedded cuts of cerebrum via deparaffinizing in addition to covering each part with a 1:200 weakening of one of three unmistakable bunny antisera created against *Acanthamoeba castellanii*, *Balamuthia mandrillaris*, in addition to *Naegleria fowleri* (Schuster and Visvesvara 2004b).

DNA extraction, PCR, and sequencing

DNA was extracted using an *Acanthamoeba sp*. culture (CDC: V601) produced from the brain. This sample was designated OSU: 08-016. Total DNA was isolated from the culture sample OSU: 08-016 using a DNeasy kit from Qiagen, Inc., Valencia, CA. Utilizing genus-specific primers for the *Acanthamoeba* 16S r-RNA after DNA extraction, PCR was utilize to amplify the *Acanthamoeba* nuclear SSU rDNA sequences (F 5- TTATATTGACTTGTACAGGTGCT-3) and *Acanthamoeba* 16S rRNA 2 (R 5-CATAATGATTTGACTTCTTCTCCT-3), This makes a portion of the SSU r louder (Wang et al. 2009). The newly found sequence is compared to a big data-base of previously acquired sequences from both our lab in addition to other labs in order to determine the Acanthamoba genotype.

The first approach for differentiating between different rDNA genotypes was developed by (Stothard et al. 1998).This method categorised an isolate into a certain genotype if its entire rDNA sequences changed by five percent or more in comparisons of sequence alignment. This caused the emergence of three unique *Acanthamoeba* genotypes at the time. (Stothard et al. 1998). Since then, other genotypes have been discovered using this methodology (Debette et al. 2011; El-Sayyad et al. 2015).

By contrasting another successions got from this genotypically instructive rDNA district to existing genotype reference groupings, *Acanthamoeba* researchers may rapidly decide genotype. A denaturing stage at 94 °C for 10 minutes, trailed by 45-55 patterns of 11 °C at 95 °C, 11 °C at 57 °C, and 21 °C at 72 °C, were the PCR conditions used in this experiment.(Dyková et al. 1999; Lédée et al. 2017; Stothard et al. 1998). The PCR result was filtered utilizing the QIAquick PCR cleaning pack utilizing a microcentrifuge (QIAGEN Ltd., Crawley, UK) in accordance with the manufacturer's instructions in order to sequence and characterise the strains. In essence, 5 ml of the PCR output and 25 ml of buffer PB were mixed. The mixture was put to a QIAquick column after it became yellow, and it was centrifuged at 17900 x g for 60s. A additional 10 l of 3M sodium acetate (pH 5.0) was added to the mixture. After discarding the flow-through, the product was washed with 750 l of buffer PE and centrifuged at 17900 x g for 60s.The QIAquick column was centrifuged for an additional 60 seconds at 17900 x g after discarding the flow-through once again. After that, the QIAquick column was placed in a clean 1.5 ml microcentrifuge tube and 30 l of elution buffer was added to the centre of the QIAquick membrane to elute the DNA. The column was kept at room temperature for one minute before being centrifuged at 17900 x g for 60s.(Dyková et al. 1999; Lédée et al. 2017; Schroeder, Kwak, and Allen 2001; Stothard et al. 1998).

Sanger Sequencing Services (Eurofins Scientific, Wolverhampton, UK) were used to sequence purified PCR products for strain definition. The sequences were obtained using the forward *Acanthamoeba* 16S rRNA (R 5-CATAATGATTTGACTTCTTCTCCT-3) primer. From the *Acanthamoeba sp*. culture, OSU: 08-016, the *Acanthamoeba sp*. sequencing from this experiment was submitted to Gen-Bank with the accession number GQ889265. (CDC: V601)

RESULTS

Upon post-mortem examination, it was discovered that the rats were underweight and had lost around 20% of their body weight. A thorough examination of the hepatic parenchyma revealed splenomegaly and many 1-2 mm white lesions. While the brain's anaerobic culture was unsuccessful, the aerobic culture resulted in a significant development of hardy, non-hemolytic *E. coli*.

3.1. Histopathology

The amoebae often caused significant necrosis and inflammation in the tissues of the brain. The nuclear chromatin of several neuroglial cells was clumped (pyknosis). Glial cell clumping shows that the brain tissue is inflamed.

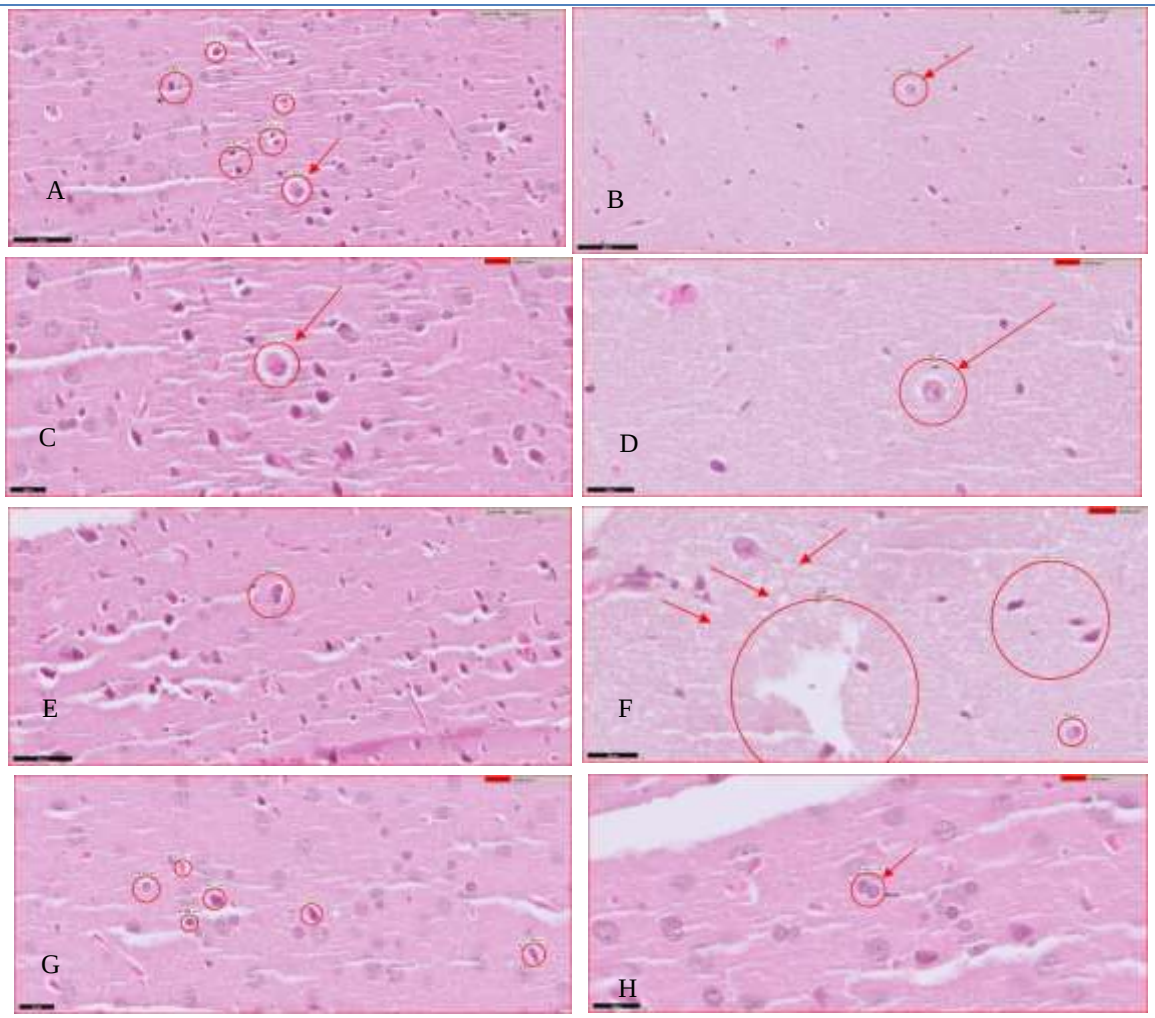

Figure 1: (A-H) . Photomicrograph shows the cerebellum's histological section stained with H&E. (A) Pyknotic cell count and arrowhead indicating Trophozoite; (B) Magnified version of (A); (C) Macrophage engulfing foreign body; (D) Pyknotic cells; (E&F) Trophozoite; (G) Severe degeneration of the brain tissue; and (H) Trophozoite going through mitosis.

Numerous necrotic, edematous, and spongiform foci of deterioration were seen, and some histiocytes contained hemosiderin or phagocytized cell debris. There were a few spots of necrosis and haemorrhage that were linked to a few amoebae. There were a few free and phagocytized amoebae that had severely degraded brain tissue FIGURE 1 (A-H).

3.2. Immunofluorescence analysis

Intense apple green fluorescence was only produced by amoebic organisms in tissue samples that had been exposed to anti- *A. castellanii* serum. Because they did not react to sera, the amoebae were of the genus *Acanthamoeba* (anti-B. man- drillaris and anti-N. fowleri).

3.3. *In vitro* culture

On agar incubated at 37 and 42 degrees Celsius , amoebae developed and within 48 hours had fully covered the plate. After the majority of the bacteria had been devoured, the amoebae started to encyst. The trophozoites had a big nucleus with a huge, spherical nucleolus positioned in the centre and were around 8–14 m in size. Acanthopodia, which resemble thorn-like filamentous projections that protrude from the surface of the trophozoites, were another characteristic of these organisms. The cysts were polygonal, ranging in size from 7 to 12 m, and had two walls, the inner of which was either round or oval and the outside of which was wrinkled. *Acanthamoeba* was used to identify the amoebae, and CDC: V600 was used to identify the isolate. In the cell culture flasks, amoebae also proliferated, albeit it took a few days before they were visible. (Figure 2)

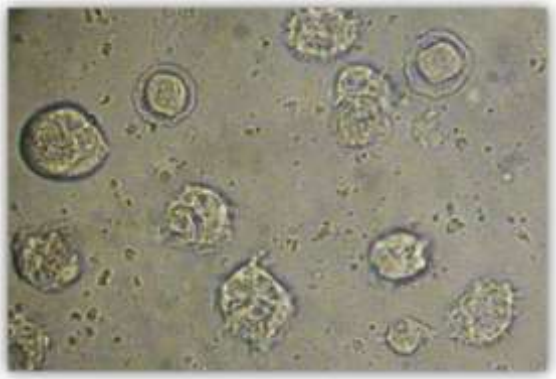

Figure 2: Presence *of Acanthamoeba spp.*inthebrain tissue samples. shows *Acanthamoeba* trophozoites and cysts on PYG.X40 objective(bars = 10µm).

3.4. PCR and sequencing

For the partial sequencing of the 16s rRNA gene (mitochondrial subunit), DNA was successfully amplified (Figure 3). Sequences were purchased from Eurofins Scientific, Wolverhampton, UK. With MUSCLE (v3.8.31), the sequence alignment was carried out using the default configuration and set to the greatest precision. MEGA and the phylogeny.fr platform were used to reconstruct the phylogenetic tree (Figure 4). Through the use of TreeDyn, the tree was graphically depicted and altered (v198.3).

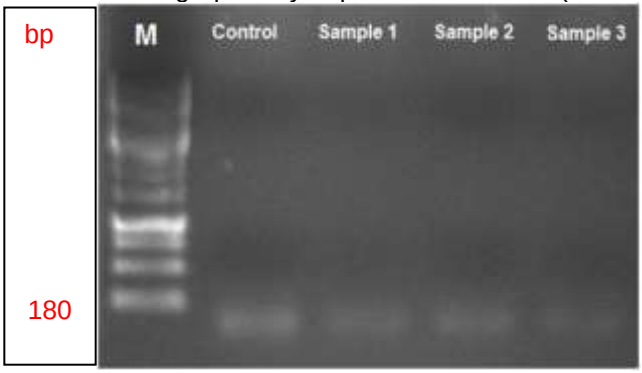

Figure 3 : 16s rRNA primer-amped PCR products. All lanes showed bands that were rather close to the predicted 180 bp.
M– kbp DNA ladder, C– *Acanthamoeba* (T7 - control), 1 to 3 – positive brain tissue.

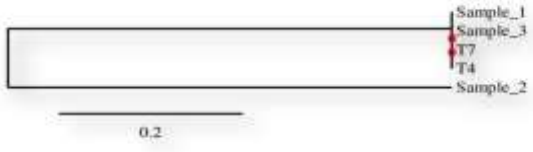

Figure 4: Phylogenetic trees for the 16s rRNA gene based on the three samples of brain tissue and two reference sequences. There were two reference sequences used: AF479550.1 for *Acanthamoeba castellanii* (T4) and AB795716.1 for *Acanthamoeba* spp. (T7). Which are contained in the blue box are *Acanthamoeba castellanii*, and which are contained in the green box are *Acanthamoeba spp*. The maximum likelihood approach was used by the PhyML tool (v3.1/3.0 aLRT) to reconstruct the phylogenetic tree. Using TreeDyn, the tree was graphically visualised and altered (v198.3).

DISCUSSION

This case's gross and histologic alterations are reminiscent of lethal amoebiasis, which is seen in several bird species and has just been documented in a toucan(Visvesvara, Moura, and Schuster 2007a). The distribution of the lesions points to an infection that started in the cecum before spreading hematogenously to adjacent tissues. Death was attributed to the severe necrosis and inflammation that was typically present when amoebae were present in these tissues.

It has been observed that tiny, free-living amoebae from the genera *Acanthamoeba* and *Naegleria* live in soil and fresh water and eat bacteria and debris(Carrasco-Sánchez et al. 2021; Schuster and Visvesvara 2004a). *B. mandrillaris* has very sometimes been isolated from soil (Schuster and Visvesvara 2004a). Balamuthia amoebae are thought to eat other microscopic amoebae that are prevalent in the environment rather than bacteria. There is some debate on this amoeba's dietary needs.

A few types of *Acanthamoeba* (*A. castellanii*, *A. culbertsoni*, *A. healyi*, *A. polyphaga*, and *A. rhysodes*), the main known types of Balamuthia B, have been connected to CNS contaminations in people, primates like gorillas, mandrills, and gibbons, as well as other mammals like canines, steers, bison, ponies, and kangaroos. mand (Yang et al. 2019; Schuster and Visvesvara 2004b; 2004a). Furthermore, *Acanthamoeba* is known to cause *Acanthamoeba keratitis*, a contamination of the human cornea that can be life-threatening. (Visvesvara, Moura, and Schuster 2007b).

The biological effects of neurotrophins rely on their concentrations and receptor affinities, which can play a range of functions by interacting with different receptors and ion channels. (Lin, Kuo, and Luh 2022) . The significance of neurotrophins in the control of neuroinflammation, apoptosis, blood-brain barrier permeability, memory function, and neurite regeneration has been shown in several research.(Zambusi and Ninkovic 2020; Tauber et al. 2005).Similar to neurodegenerative diseases, parasitic infections like amoebic encephalitis in the brain are characterized by complicated pathogenesis.

A few *Acanthamoeba* segregates from fish and those related with *Acanthamoeba keratitis* are individuals from a similar T4 phylogenetic group, as per a new report utilizing SSUrRNA quality sequencing, suggesting that the very characteristics that permit these amoebae to taint animals may likewise help these amoebae contaminate people. (Dyková et al. 1999).These amoebas are known as

amphizoic amoebas because they can live inside animal tissue and cohabit with other free-living species in the natural environment. (Lis et al. 202).

CONCLUSION

In the current work, we identified two new *Acanthamoeba* strains and demonstrated that an *Acanthamoeba* strain with a genotype sp. capable of infecting people could also be capable of infecting rats fatally.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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