

Experimental Keratitis by *Acanthamoeba polyphaga*

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Abstract: The current study reported the presence of a species of amoeba belonging to the genus *Acanthamoeba* is *A. polyphaga* recording for the first time in Iraq, this species classified depending on morphological features of cyst phase. 33 samples were isolated from environmental source, including rivers and soils in Basra province, 18 of these samples were positive for genus *Acanthamoeba* and 2 were positive to *Acanthamoeba polyphaga*. We used non nutrient agar to growth the amoeba and incubation in 37C. This study showed the ability of *A. polyphaga* to infected rats with keratitis through 14 days by applying 10⁵ trophozoites for each rat. *A. Polyphaga* causing inflammation and severe necrosis in cornea stroma, spread amoeba has been noted in tissue section of rats eyes infected.

Introduction

Acanthamoeba spp. is organism that consist from one cell, living as free living , the life cycle of this amoeba contain two form, that is vegetable phase known as trophozoite and dormat phase known cyst, the cyst can be found in environmental for many years and when the environmental condition become suitable the cyst transfer to trophozoite and lead its vital function , *Acanthamoeba* is opportunistic amoeba that's mean its survive as free living in environmental but can be cause disease when enter the human and animals body and live as parasite. the genus of *Acanthamoeba* consist of 20 species some of them very important because causing infection such as *A. castellanii*, *A. polyphaga*, *A. triangularis*, *A. culbertsoni* and other .These species can be causing infection in skin, lung , eye and center nerve system (Gardner *et al.*, 1991).

Acanthamaba possesses the ability to live in different environments where isolated from different sources of water piped swimming pools , bottled water ,sea water ponds , canals for ventilation , refrigeration units, sewage, sediments, soil and beaches, vegetables and surgical instruments , contact lenses and air samples. The amoeba with absolute presence (Niyiyati *et al.*, 2009).

Moreover, *Acanthamaba* isolated from hospitals and units of dialysis stations eye wash samples pharynx, sinuses nasal humans and cuts the skin and biopsies of the cornea means the spinal cord, as well as the healthy people showed possessing antibodies to

Acanthamaba This shows the public exposure of them to these pathogens(Cursons *et al.*, 2005).

through life parasite cycle there are two Phase been fed vegetation and ranging dimensions of this phase between 35-16 microns and vary these measurements are different depending on isolation and has been fed complex looks like thorns on its surface called acanthapodia on the surface of cell, used to movement and catch prey and contain single nucleus at the rate of 1 /6 of the cell size.

Fed on bacteria, algae, yeasts or small organic molecules and thus we see many of the nutritional vacuoles in the cytoplasm of the amoeba, the amoeba multiplies by binary fission in split parental cell into two cells (Khan, 2006).

Material and methods

Samples collection

Thirty three samples were collection from river (Shatt-al Arab, Shatt-al Turk and Shatt-al Basra) and soil (dry soil and moist soil) by sterile bottles, the samples was transferred to laboratory, the rivers samples has been suspended with 100 ml distill water while the soil samples have been collection in sterile containers and after transferred to laboratory, culture 0.5 g from each sample on NNA medium and added 3 ml distal water, all samples incubation in 37C and examined after 4 days (Elder *et al.*, 1994).

Rats' infection

Twelve rats have been used in this study old ranged 3-4 weeks, the animals were breeding in specially

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cages in animal's house of the faculty of sciences. The rats infected by contamination eyes with 30µl solution contain 10000 trophozoites, The culture were washed with distill water , collection in sterile tubes and underwent a centrifuged at 4000cycl for 5 min. , The sediment was washed with sterile water and recentrifuge process then the sediment collected and suspended with two ml of sterile water, after that calculated the number of amoeba in stuck by hematocytometer. scarification the cornea right eye of each rat were done by a sterile syringe needle three times vertically, After that each eye contaminated with 10 µl from the solution containing the 10,000 trophozoites amoeba, It was suture the eyelid right eye of each rat for 24 hours so as to ensure full contact between cornea and *Acanthamoeba polyphaga* after that, removed sutures and follow-up of infected animals on a daily basis for a period of 14 days (Ran and Wu, 2010).

Rats were killed by ether and autopsied after 14 days of infection by medical scissors and forceps were sterile, eyes were extraction and cultured part of it on non nutrient agar medium with added sterile water, after that incubation in 37C.

The remaining parts of the rats eyes were saved in 10% formalin for histological study.

Results

The current study showed distribution of *A. polyphaga* in river samples, the number of positive samples 2, from total samples was collected from the waters of some rivers and soils dry and wet in Basra. *Acanthamoeba polyphaga* has two form in life cycle, the trophozoite and the cyst, the trophozoites irregular in shape, characterized with slowly move, has pseudopodia like thorn with tapered end known as acanthopodia, the cytoplasm contain a central nucleus with contractile vacuoles and several food vacuoles.

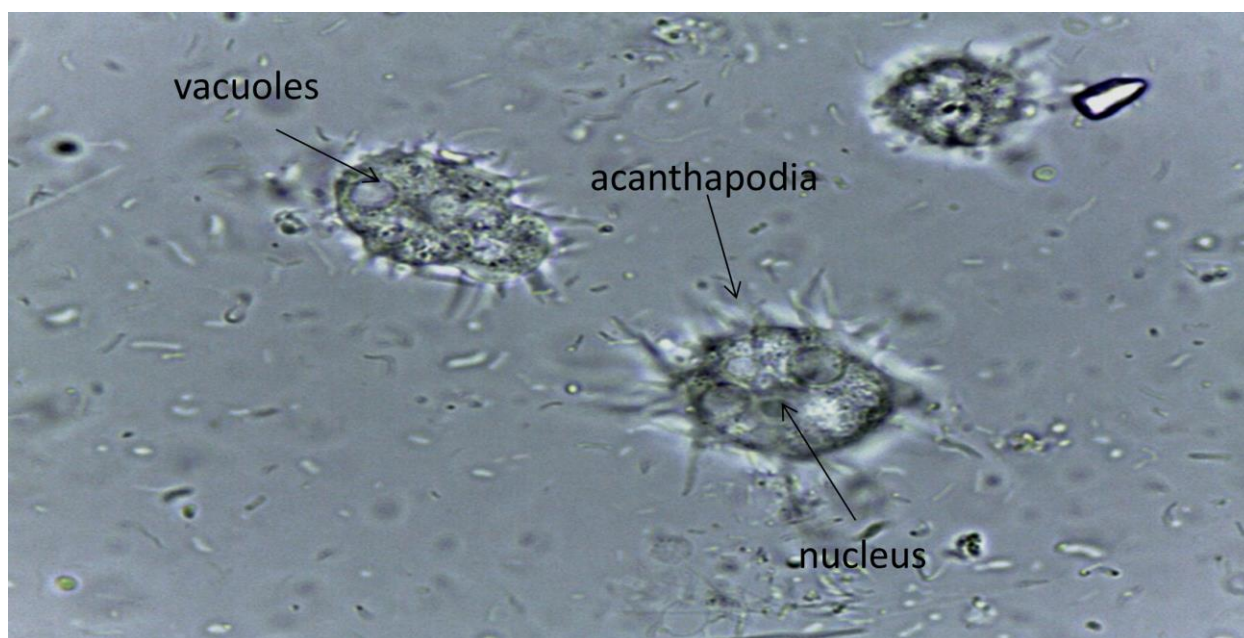


figure (1) *Acanthamoeba polyphaga*. Shows acanthopodia, vacuoles and nucleus.

A. polyphaga cyst has outer membrane (ectocyst) wrinkled and inner membrane (endocyst) polygonal with 4-5 ribs and central nucleus, the diameter of cyst is 16.1-18.4µm with range 17.25 µm.

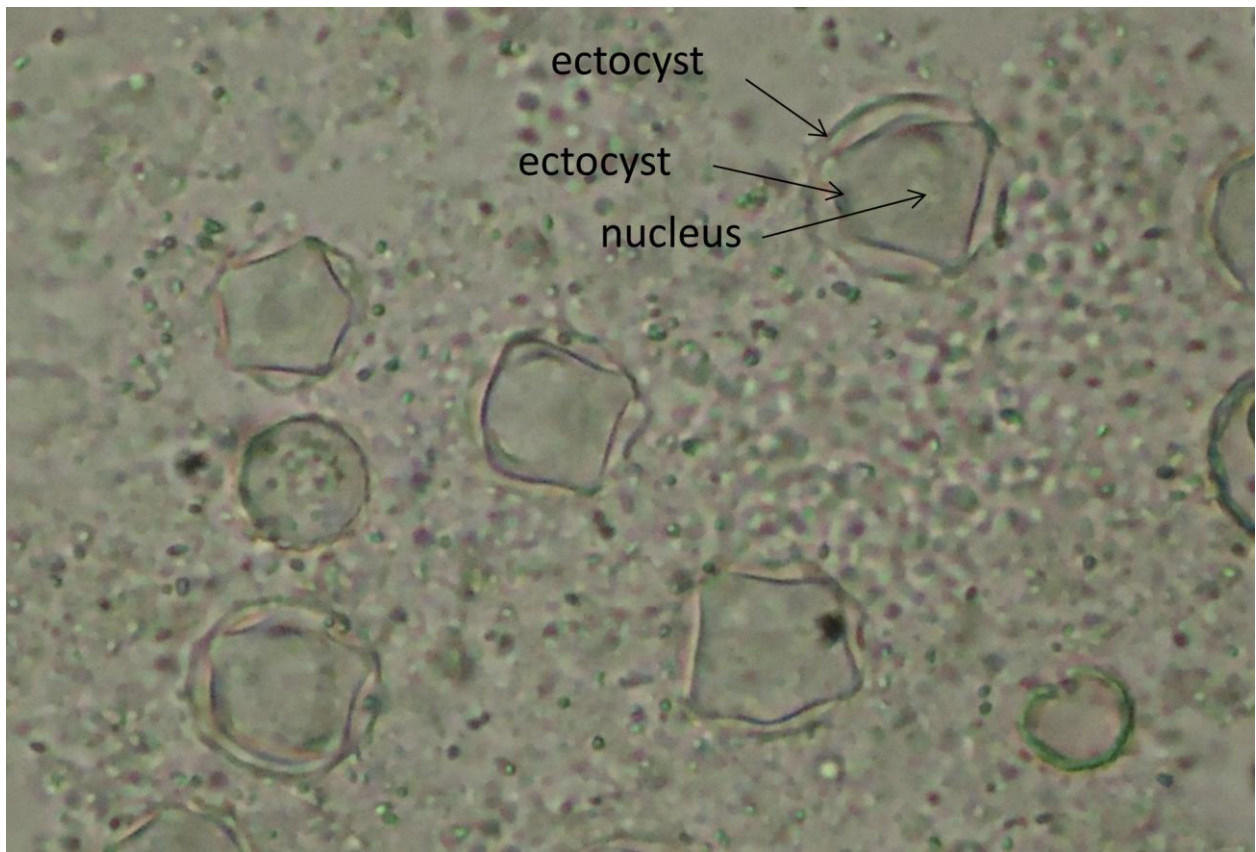


Figure (2) Cysts of *Acanthamoeba polyphaga*.

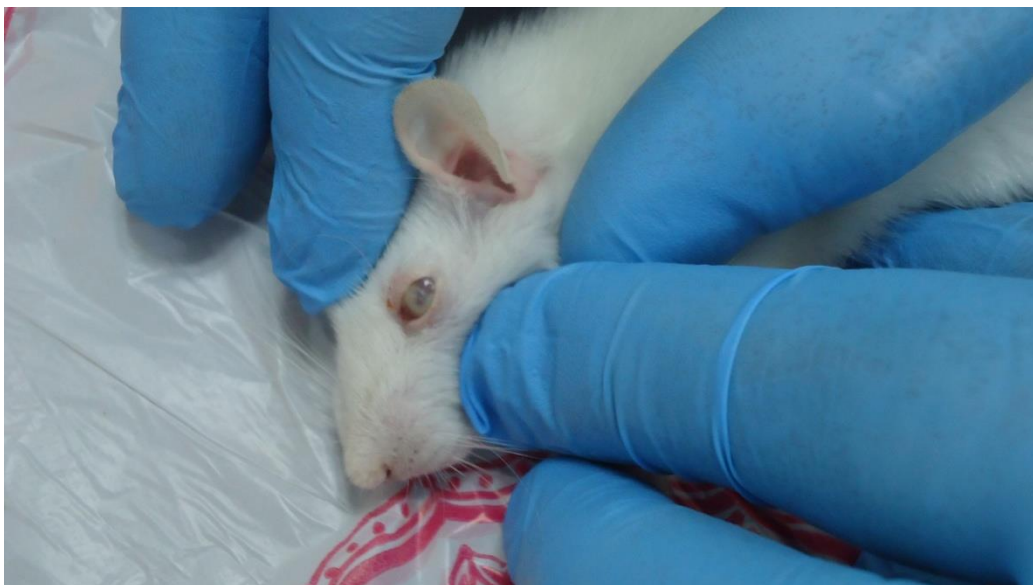


Figure (3) Construction of *Acanthamoeba* keratitis in rat experimental infected with *A. polyphaga*.

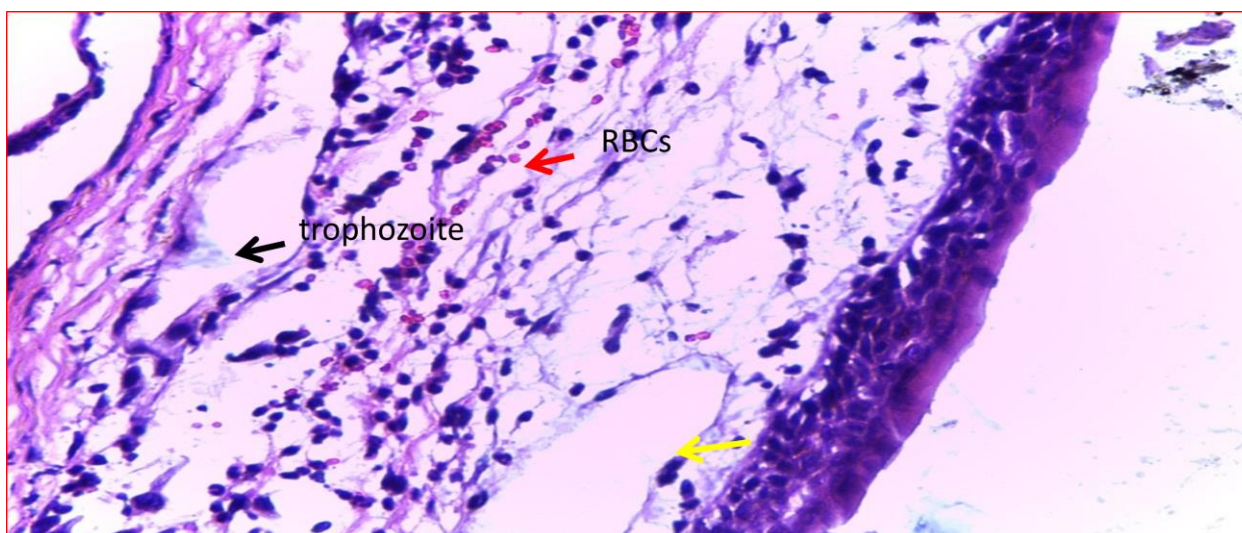


Figure (4) rat eye infected with *A. polyphaga* show sever bleeding in corneal stroma red arrow and necrosis of wide area of stromal region yellow arrow and presence of amoeba trophozoite black arrow at the bottom of stroma near posterior limiting lamina.



Figure (5) retina section of rat infected with *A. polyphaga* cause a little ulceration near amoeba yellow arrow .

Discussion

The genus *Acanthamoeba* consist from 20 species present as free living amoeba , some of these species opportunistic and pathogens to human and animals causing disease in center nerve system , eye and skin. *Acanthamoeba polyphaga* is an opportunistic amoeba, ubiquities (Page, 1967) .

We were isolated this amoeba from the water of the Shatt - al Arab that mean may be this amoeba favorite branch water and this agrees with Thomas (2011) who grow it in media contain sea water. In this study the diagnosis done depending on morphological feature to cyst such as the shape of ectocyst and endocyst; ectocyst is wrinkled and endocyst is polygonal consist from 4-5 ribs.

This species could be causing keratitis, Hong *et al.* (2014) recording infection in woman infected with *A. polyphaga*. The current study showed that infection of rats with trophozoites *A. polyphaga* by applied it and Sewing the eyelid for 24 hours causing infection in 10 laboratory rats from 12 using in study during 14 days and this is consistent with what Ren and Wu (2010) said that infected rats in this way causing keratitis.

The cyst and trophozoites have been showed in tissue sections of infected rats eyes, the method of infection may be lead to linked mannose binding protein (MBP) with receptor present on surface of cornea and penetrated epithelium layer. The studies showed MBP bind with glycoprotein present on the

corneal epithelium known as mannosylated (Garate, M. et al., 2004 ; Yang, Z. et al., 1997).

The trophozoites adhesion with the surface of the cornea is the basic conditions for the occurrence of the disease in humans and guinea pigs, and Chinese hamster (Niederhorn, J.Y. et al., 1992)

The main risk factors to occurrence infection in cornea are wearer contact lenses and lesion in cornea (Niederhorn, J.Y. et al., 1999) therefore the scrapping cornea or make injury in cornea in experimental rats is important step in *Acanthamoeba* keratitis studies.

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