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Detection of amphizoic amoebae *Acanthamoeba spp.* from the ocular discharge of buffalo

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Abstract

Acanthamoeba spp. are free-living, amphizoic amoebae widely distributed in soil, water and air, with the ability to cause opportunistic infections in both humans and animals. In livestock, ocular acanthamoebiasis can lead to conjunctivitis, keratitis and potential vision loss, resulting in economic losses due to decreased productivity and animal welfare concerns. This study reports the occurrence of Acanthamoeba spp. from ocular discharge samples of buffaloes showing signs of ocular discomfort, such as lacrimation. The water bathing practices were found to be the main cause of eye infections in the present study. Water bodies are heavily loaded with a variety of contaminants, including microbes, bacteria, fungi, pathogenic protozoa and organic substances. High levels of different nutrients in water bodies are responsible for enhancing bacterial growth, such as E. coli. This, in turn, favours the growth of amphizoic amoeba, occasionally present in water bodies. The presence of amphizoic amoebae in water bodies poses a risk for buffalo to contract amoebic infections.

Keywords*: Free living amoeba, buffalo, ocular infection, Kukrail Nadi*

Acanthamoeba are free-living pathogenic amoeba, mainly found in the water bodies. Earlier it was believed that *E. histolytica* was the only pathogenic amoeba that causing diseases in humans but this notion was broken in 1957 with the discovery of small free-living amoeba of genus *Naegleria* and *Acanthamoeba* which were found to cause infection in nervous system and eyes of human being and animals (Khan, 2006, Kinde *et al.,* 2007, Qvarnstrom *et al.,* 2013). These species of free-living amoebae are capable of invading mammalian tissue, causing severe pathogenic conditions and frequently leading to death (Somayeh Bahrami *et al.,* 2025). These amoebae are primarily free-living, but they also opportunistically infect the brain and eye; thus, they can survive as both free-living and endoparasites which is known as an amphizoic amoeba.

Schuster and Visvesvara (2004) reported *Acanthamoeba* spp. in a variety of mammals, *viz*., Indian buffalo, dogs and kangaroo. The amphizoic amoebae were isolated from water bodies, nasal swabs, CSF and corneal scrape or tears of human beings and animals (Somayeh Bahrami *et al.,* 2025). The incidence of amphizoic amoebae infection is likely to be higher in warmer regions than in colder parts of the world and in less well-sanitized areas. These amoebae are commonly found in water used for bathing, swimming, and water sports, which can lead to the inadvertent infection of humans and domestic animals.

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Disease Primary amoebic meningoencephalitis (PAM) is caused by Naegleria spp. whereas diseases Granulomatous amoebic encephalitis (GAE) and Amoebic Keratitis (associated with eyes) is caused by different species of *Acanthamoeba*. Both pathogenic and non-pathogenic species of *Naegleria* and *Acanthamoeba* are found worldwide in soil, water, air, and dust (Coşkun *et al.,* 2013). Granulomatous amoebic encephalitis (GAE) is a chronic, progressive disease of the central nervous system that typically occurs in animals with compromised immunity or other debilitating health conditions. During contact with contaminated water, amoebae occasionally invade the corneal stroma via the epithelium or through intact epithelium (Betanzos *et al.,* 2019). This disease is marked by intense eye pain, inflammation, reduced vision, and a stromal infiltrate that is often ring-shaped and primarily made up of neutrophils. If not managed properly, the condition can lead to vision loss and even blindness (Khan, 2005; Lorenzo-Morales *et al.,* 2015; Somayeh Bahrami *et al.,* 2025).

Materials and methods

The study was conducted in buffaloes that were repeatedly bathed in a shallow, muddy pond during the peak summer season at Kukrail Nadi in Lucknow city (Figure 1). A total of 15 buffaloes that showed lachrymation and abnormal head movement were screened during the summer months (March to May, 2025). All the buffalo were around one year old, of which six of them were dairy buffalo. The method of Singh and Hanumaiah (1979) was used for the identification of amoeba. The conjunctival secretion was collected from the buffaloes in sterile, screw-capped small tubes and brought to the lab for culture on non-nutrient agar plates that were pre-seeded with *Escherichia coli*. Non-pathogenic *E. coli* cultured was maintained in the lab for amoebae growth. Amoebae were isolated from the samples. Isolated amoebae were sub-cultured 3-4 times by cutting a small square piece of agar on which plenty of amoebae were present and placed facing downward on the fresh bacterial circle (*E. coli*) in a non-nutrient agar plate. These plates were incubated in a BOD incubator at 37^0C for 5-10 days. The cyst suspension was diluted 3-4 times so that the few cysts remained in the cavity slide. Cysts were observed under a microscope.

Results and discussion

Three buffaloes were found to be positive for amoebic infection. *Acanthamoeba* in active locomotion exhibits a broad anterior lobopodium, from which more than three spike-like projections know as acanthopodia are formed. The ectoplasm and endoplasm are distinctly defined, and there is one contractile vacuole present (Fig.2). The cyst consists of two walls, with the outer cyst wall being highly wrinkled. Cyst morphology is the key to identify different species of *Acanthamoeba*. Jongwntimes *et al.* (2000) described the *Acanthamoeba castellanii* cyst as having a wrinkled ectocyst and stellate endocyst, featuring 6-8 ray. Moreover, the cyst was double-layered and the largest in size, with a thick, circular, and irregularly folded ectocyst and a stellated endocyst (Fig. 3). The species of *Acanthamoeba* were identified on the basis of typical cyst morphology (Page, 1967a, 1976b, Visvesvara, 1991 and Khan, 2001). Jongwntimes *et al.,* (2000) described *A. castellanii* cyst as mammillated or wrinkled ectocyst and stellate endocyst with 6-8 ray.

Fig. 1. Sampling site, Kukrail Nadi, Lucknow

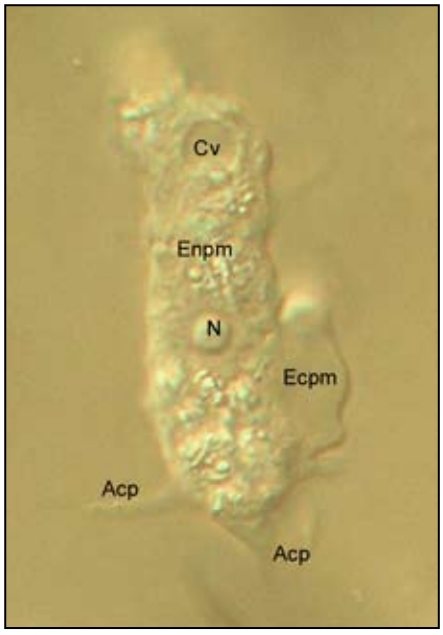

Fig. 2: Trophozoite of *Acanthamoeba castellanii* (magnification x200) Cv, Contractile vacuole; N, Nucleus; Acp, Acanthopodia; Enpm, Endoplasm; Ecpm, Ectoplasm

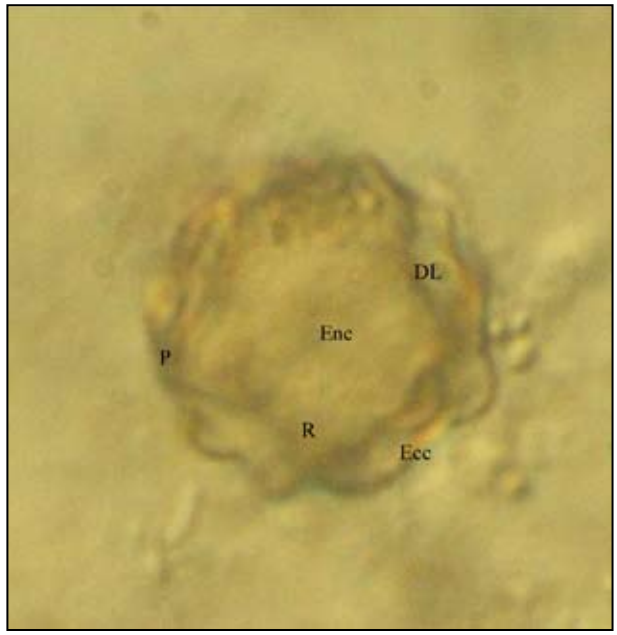

Fig. 3: Cyst of *Acanthamoeba castellanii* (magnification x200) DL, Double layer; P, Pore; Enc, Endocyst; Ecc, Ectocyst; R, Rays

Samples were collected during summer, when buffalos regularly bathed to balance their body temperature according to the surrounding temperature. An opportunistically infected buffalo developed an eye infection, resulting in redness and lachrymation within a few days. Buffaloes commonly encounter trauma in the eyes due to grass blades, straw, sticks, or rough feed, sand or dust particles and scratches during fighting or rubbing eyes on rough surfaces. Pre-existing eye diseases, caused by viral, bacterial, or fungal infection, can also weaken corneal surfaces. In such conditions, *Acanthamoeba* has a chance to infect the eyes and invade insight via the cornea. Finally, amoeba adheres to the corneal surface, particularly in areas where the epithelial layer has been damaged (Walochnik, 2018). It secretes proteolytic enzymes that break down tissues and allow deeper penetration. It can form resistant cysts within the cornea, making the infection chronic and hard to treat (Wang *et al.,* 2023).

Valladares *et al.* (2014) reported that domestic pets such as dogs get *Acanthamoeba* infection through the cornea by contact with contaminated water sources. The immunocompromised animals also get infected if exposed to contaminated water (Ayers *et al.,* 1972). Chavatte *et al.* (2016) and Cardoso *et al.* (2024) reported that *Acanthamoeba* could migrate to the gastrointestinal tract, but more investigation is required to establish this infection route.

Acanthamoeba spp. infection can cause more serious tissue damage in aquatic animals, resulting in high mortality rather than terrestrial animals, but route of infection is same via contaminated water (Laoprasert *et al.,* 2009). McFadin and Kauffman (1974) first recognised *Acanthamoeba* as a veterinary pathogen in cattle. Wijesekara *et al.* (2007) reported *Acanthamoeba* spp. occurrence in the eyes of captive elephant in Srilanka. Gomaa *et al.* (2016) reported infection in a Friesian bull suffering from nervous signs. *Acanthamoeba spp.* infection was reported in a 1-year-old horse. The immunohistochemistry (IHC) result was positive for *Acanthamoeba spp.* located in different internal organs (Gonzalez *et al.,* 2020).

In southern India, genotype T4 was the most common *Acanthamoeba* strain found in AK patients. The study also reported genotype T12 for only the second time in India, a type that is rare worldwide. Most clinical isolates carried intracellular microbes, which could influence the severity and features of *Acanthamoeba* keratitis (Rayamajhee *et al.,* 2022). Environmental water surveillance for free-living amoebae (FLA) in North India focuses on detecting pathogenic genera such as *Acanthamoeba*, *Naegleria*, and *Balamuthia* in sources like rivers, lakes, ponds, canals, and tap water. *Acanthamoeba* spp. and *Naegleria* spp. were identified in the samples collected from different locations (Krishnamoorthi *et al.,* 2022). The environmental samples and corneal swabs from naturally infected animals tested for *Acanthamoeba castellanii*, the free-living amoebae was detected more frequently in environmental sources than in infected animal corneas based on microscopic examination (Basher *et al.,* 2018). Although *Acanthamoeba* infections have been reported in various animal species, they are often overlooked in veterinary practice (Bahrami *et al.,* 2025).

The present study, based on cyst morphology, confirmed the presence of *Acanthamoeba castellanii* in conjunctival secretion of buffalo. Observed symptoms in buffalo were discomfort, excessive lachrymation, redness and swelling of infected eyes. Buffaloes may rub their eyes after bathing, which can worsen minor injuries to the eye, leading to the further spread of infection (Wijesekara *et al.,* 2007). During hot weather, buffaloes are bathed in Kukrail Nadi in Lucknow to cool down. However, these water sources can be heavily contaminated with a variety of bacteria, especially *E. coil, which* serves as an excellent food source for *Acanthamoeba*. The presence of faeces and organic matter in the water can trigger growth of bacteria, leading to an increase the number of *Acanthamoeba* in these water bodies. *Acanthamoeba* flourish in warm and moist environments with high nutrients levels, making summer water ponds ideal breeding grounds for them.

Conclusion

The detection of amphizoic amoebae *Acanthamoeba* spp. from the ocular discharge of buffalo underscores the potential role of these free-living protozoa as emerging ocular pathogens in livestock. Although most Indian reports focus on human keratitis, the ecological conditions in which buffalo are reared—frequent exposure to stagnant or contaminated water, ocular trauma from vegetation, and close contact with shared environments—create a plausible risk for infection. Eye problems caused by *Acanthamoeba* can lead to poor vision or blindness, reducing the animal's ability to work and affecting milk production, resulting in financial losses. Timely veterinary check-ups, clean water sources, and early laboratory testing can help detect and treat infections before they cause permanent damage.

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Conflicts of Interest

The authors declare no conflict of interest.

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