Acanthamoeba keratitis: Experience in a non-specialist microbiology laboratory

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Abstract

Acanthamoeba keratitis is an unusual complication of wearing soft contact lenses: a case was diagnosed by culturing corneal scrapings. Acanthamoeba are tolerant of variations in osmolarity in the culture media. Page's saline, recommended for culture agar plates, can easily be substituted with salines which are suitable for cell culture media, thereby avoiding the cumbersome manufacture of special saline. Early diagnosis as a result of successful isolation may avoid the need for keratoplasty.

We recently received an unusual emergency request for *Acanthamoeba* spp culture. We had no previous experience of this uncommon or perhaps underdiagnosed pathogen.¹

Case report

A 32 year old man had been wearing soft contact lenses for four years. He was referred to the eye clinic with a punctate ulcer of his right cornea. Bacterial and viral cultures were negative. He showed no improvement after courses of local prednisolone, acyclovir, tetracycline and chloramphenicol. Two months later his visual acuity deteriorated and he was seen as an emergency at the ophthalmology clinic. Acanthamoeba keratitis was suspected and deep corneal scrapings were taken. The patient was sent home with propanidine isothionate (Brolene) eye drops. He returned with further deterioration of vision the next day and further biopsy specimens were taken. There was no further deterioration on subsequent reviews.

The accepted method of monoxenic culture on 1.5% non-nutrient agar (oxoid L28) in Page's saline,¹ inoculated with a suitable bacterial species, is found in many microbiology or parasitology textbooks. Our laboratory, like most general laboratories, does not stock Page's saline, nor the full complement of salts for its manufacture. Having to use an immediate substitute, we compared Page's saline with Dulbecco A saline (phosphate buffered saline, PBS, Oxoid BR14a) used in the virology department for cell culture media. From our own experience (personal communication, M Sillis) and earlier reports of contamination of cell cultures with *Acanthamoeba*,² we felt that the higher osmolarity of PBS would be tolerated.

The first specimen was very small, aspirated on to the tip of a hypodermic needle. To avoid

desiccation media were prepared immediately, consisting of thin, non-nutrient agar plates seeded with *Escherichia coli* (NCTC 10148). The material for culture was washed gently from the needle on to culture plates, which were sealed and incubated at 30°C. The plates were scanned daily with an inverted microscope. Cysts and trophozoites were detected on day 5. Subsequent specimens were negative.

Discussion

Unfortunately, Acanthamoeba keratitis is too often a last resort diagnosis after lengthy exclusion of other pathologies.^{3,4} This may be attributed to a lack of awareness of the prevalence and pathogenicity of this organism. The rapid and extensive damage from the disease due to late diagnosis almost always necessitates keratoplasty.^{3,4} Laboratory diagnosis is usually limited to postoperative histology of the corneal button. Microbiological diagnosis from corneal scraping is rare, cases reported in the United Kingdom are few,^{4,5} and therefore information on medical treatment of this disease is scanty. Propanidine is the drug of choice based on sensitivity testing.⁵

There is also a scarcity of information outside specialist centres of culture methods for this hardy organism on alternative media. Unlike *Naegleria*, *Acanthamoebae* are relatively tolerant of variation in osmolarity (personal communication, Dr D Warhurst). By replacing Page's saline with a commercial alternative stocked by most laboratories, we successfully cultured *Acanthamoeba* from corneal scrapings and showed the importance of early diagnosis. We feel that most general laboratories should be able to offer this service to ophthalmologists and avoid delays from interlaboratory referral or shortage of suitable media. It is satisfying to learn that our patient's eye remained stable with a course of propanidine eye drops.

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