GENOTYPING OF SOUTH AFRICAN *ACANTHAMOEBA* KERATITIS ISOLATES

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Background

Acanthamoebic keratitis (AK) is a rare but often severe, sight-threatening infection caused by certain free-living amoeba species (Figure 1).

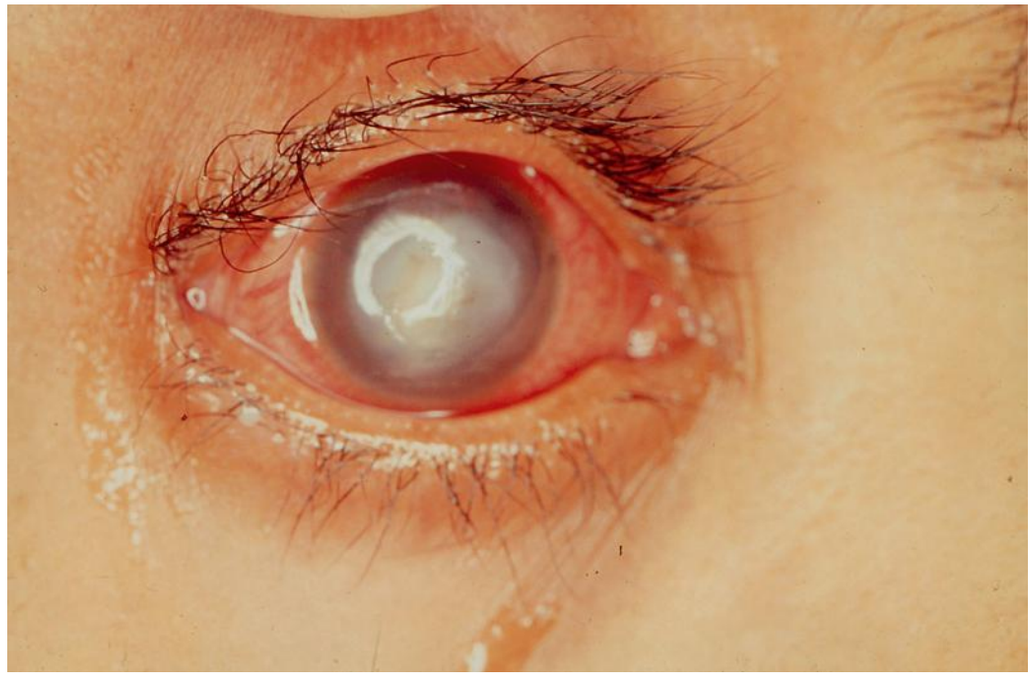

Figure 1: *Acanthamoebic* keratitis. Note the corneal infiltrate and the intense conjunctival reaction (image credit: Prof. T Carmichael)

Most free-living amoebae are non-pathogenic, but the genera *Acanthamoeba*, *Naegleria* and *Balamuthia* contain species that are potentially invasive. Free-living amoebae are ubiquitous in the environment. *Acanthamoeba* spp. can therefore be found in natural surface water bodies, both saline and fresh, and artificial environments like swimming pools, domestic and industrial water pipes, water tanks, air conditioners, cooling towers, drains, shower heads and taps. They occur in soil, compost, sewage, sediments, and in humans, on skin, and in the upper respiratory tract following inhalation of cysts.[1] Under unfavourable conditions the amoebae form resistant cysts that can survive in dry environments like dust and air, which can therefore be sources of infection. The cornea (particularly in those who wear contact lenses), and central nervous system (in the form of granulomatous amoebic encephalitis in immunocompromised persons) are the main target organs for invasive *Acanthamoeba* strains. Skin infections may also occur, and in immune-compromised patients, provide a route for haematogenous spread to the brain. People who handle contact lenses with unclean hands, who do not take proper care of contact lenses, or who wear them in inappropriate conditions or for excessive periods, are at risk for acanthamoebic keratitis. The infection is very

painful and progressive, and once deep corneal penetration and ulceration is established, vision is threatened. Aggressive, complicated and prolonged treatment is required, and sometimes only corneal transplant will save the eye. Prognosis is therefore dependent on the speed of accurate diagnosis and initiation of proper treatment. The traditional methods used for diagnosis are direct microscopic examination and culture of corneal material on non-nutrient agar seeded with *Escherichia coli*, which the amoebae phagocytose (Figure 2).

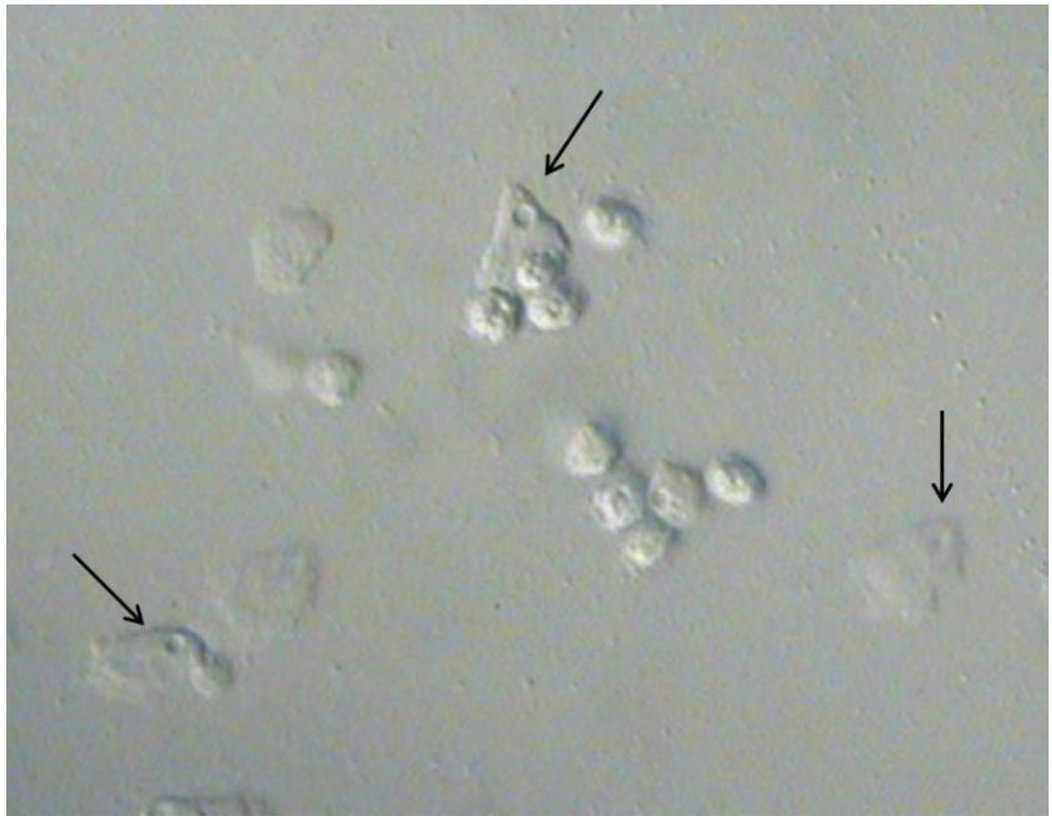

Figure 2: Agar plate culture showing *Acanthamoeba* trophozoites and their contractile vacuoles (arrows) and immature cysts (interference contrast, 400x).

Isolation of acanthamoebae from contact lenses and their containers sometimes provides indirect but highly suggestive corroborative evidence of the cause of keratitis. Morphological distinction between species is difficult and often unsatisfactory, so classification based on 18S ribosomal RNA gene sequences has allowed genotypic characterisation of strains of *Acanthamoeba* species. Seventeen genotypes have been distinguished, with most isolates associated with AK belonging to the T4 genotype, but T2a, T3, T5, T6, and T11 are also linked to AK. Certain genotypes (T1, T10, T12) are associated with granulomatous encephalitis, and no disease association has yet been found with the others.[1-4]

The aim of this study was to confirm the presence or absence of *Acanthamoeba* species in samples previously tested by culture and microscopy, using a molecular-based approach to determine the *Acanthamoeba* species genotypes of the positive samples.

Materials and methods

Forty-six archived patient samples, comprising 28 culture-

confirmed positive and 18 negative samples, collected between 1996 and 2014, were assayed. Among the positives, nine samples previously confirmed to be *Acanthamoeba* T4 genotype[5] were included as controls. A reference *A. castellanii* strain (ATCC 30010D) was also used. Stored samples were re-cultured and the genomic DNA was extracted manually using QIAamp DNA Mini kit (Qiagen, USA). Amplification of an approximately 500 bp 18S ribosomal gene sequence was done as previously described.[5] PCR products were electrophoresed on 2% agarose gel stained with SYBR safe DNA dye. Results were recorded on a UV gel documentation system. Amplicons of all positive isolates were sequenced (Inqaba Biotech, Pretoria) and compared to existing nucleotide sequences on the GenBank databases to determine the genotypes.

Results

DNA from 25 *Acanthamoeba* subcultures was amplified and sequenced, of which 24 were phylogenetically similar to genotypes previously deposited in GenBank. One sample did not align with any GenBank database sequence. Twenty-one isolates were identified as genotype T4 (of these, 13 were unnamed *Acanthamoeba* species and eight were *Acanthamoeba castellanii)* and three as genotypeT3 (unnamed species). Control samples were all T4 genotype, as expected.

Discussion and conclusion

A previous study showed that South African cases of AK were caused by the *Acanthamoeba* T4 genotype.[5] The results presented here support this finding. Accurate and rapid diagnostic test results will contribute to potentially sight-saving clinical outcomes. The much reduced turnaround time for PCR, compared to up to 10 days for culture, is clearly beneficial for clinical management. Additionally, PCR is highly sensitive and specific, and can be applied to other relevant clinical samples from cases of suspected acanthamoebic infections, such as skin and brain biopsies. To date, the only case of *Acanthamoeba* encephalitis reported in South Africa was a farm animal,[6] but it is possible that undiagnosed or misdiagnosed cases of this devastating condition occur amongst the country's large burden of HIV-related immune-deficient persons.

References

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