Acanthamoeba species keratitis in a soft contact lens wearer molecularly linked to well water

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Acanthamoeba species keratitis has been associated with soft contact lens wear. In the present report, an epidemiological link was established between the patient's isolate and well water from the home using molecular methods. To the authors' knowledge, this is the first case in Canada where such a link has been established. Primary care practitioners and specialists, including ophthalmologists and infectious diseases specialists, must maintain a high degree of clinical suspicion in soft contact lens wearers with keratitis unresponsive to conventional topical and systemic treatment.

Kératite à *Acanthamœba* moléculairement liée l'eau d'un puits, chez une porteuse de lentilles cornéennes souples

La kératite à *Acanthamœba* a été associée au port de lentilles cornéennes souples. Dans le présent rapport, un lien épidémiologique a été établi à l'aide de méthodes moléculaires entre l'isolat prélevé chez la patiente et l'eau du puits qui alimentait son lieu de résidence. À la connaissance des auteurs, il s'agit du premier cas où un tel lien est établi au Canada. Les médecins de premier recours et les spécialistes doivent maintenir un fort degré de suspicion face à une kératite qui ne répond pas aux traitements topiques et systémiques d'usage courant chez les porteurs de lentilles cornéennes.

Key Words: Acanthamoeba *species; Keratitis; Soft contact lens*

CASE PRESENTATION

An otherwise-healthy 14-year-old female soft contact lens wearer, who lived in a rural area where the tap water source for the home was a well, presented to a community hospital with a four-week history of progressive redness, swelling and blurred vision of her left eye despite treatment with topical neomycin and corticosteroids. A dendritiform corneal epithelial lesion, corneal edema and inflammation in the anterior chamber were noted on initial examination, and treatment for herpes simplex keratouveitis with oral valacyclovir and topical trifluridine 1% was initiated. There was no clinical improvement, and six weeks after the onset of symptoms she was referred to a corneal specialist for evaluation and further management. The patient's visual acuity was 20/80 and her corneal sensation was intact; slit-lamp examination revealed diffuse corneal edema, areas of punctate epithelial staining, poorly defined subepithelial and anterior stromal infiltrates, a partial ring infiltrate and 2+ cell and flare in the anterior chamber (Figure 1). The patient underwent corneal debridement, and scrapings were sent for viral, bacterial and *Acanthameoba* culture. She was admitted to the hospital, and polyhexamethalene biguanide 0.02% and diamidine propamidine isothianate eye drops were added to antiviral therapy and topical neomycin. Over the next few days, her visual acuity decreased to hand movement only, the corneal edema intensified, and midstromal peripheral vascularization developed. Her corneal epithelium healed promptly following debridement. Treatment with oral itraconazole, topical chlorhexidine 0.02% and topical ciprofloxacin were added after *Acanthameoba* species were isolated from corneal scrapings. Over the next few months, the patient improved markedly (her left visual acuity was 20/50) despite significant corneal vascularization and persisting central stromal opacity.

Clinical samples, including corneal scrapings, contact lenses, contact lens solution, tap water and well water, were inoculated directly onto non-nutrient agar with *Escherichia coli* overlay (1), as well as sheep's blood agar, MacConkey agar and chocolate agar. The non-nutrient agar with *E coli* overlay was incubated at room temperature. The agar surface of the plates was examined daily with an inverted microscope at 10× magnification. On day 5 after planting, the plates inoculated by corneal scrapings were positive for both the cyst (Figure 2) and trophozoite forms of *Acanthamoeba* species. In addition, *Serratia marcescens* and *Stenotrophomonas maltophilia* were isolated from corneal scrapings that were inoculated onto sheep's blood agar, MacConkey agar and chocolate agar. Environmental samples consisting of well water and tap water from the patient's home, in addition to samples from the contact lens case, were also positive for *Acanthamoeba* species. *Acanthamoeba* species isolates were forwarded to the Ohio State University Department of Molecular Genetics (Columbus, Ohio, USA), where molecular epidemiological analysis was undertaken. This consisted of amplification and sequencing of 700 base pairs of the mitochondrial 16S *rRNA* gene and the full nuclear 18S *rRNA* gene. Sequencing of the mitochondrial 16S *rRNA* gene revealed that both clinical and environmental isolates had the T4 genotype.

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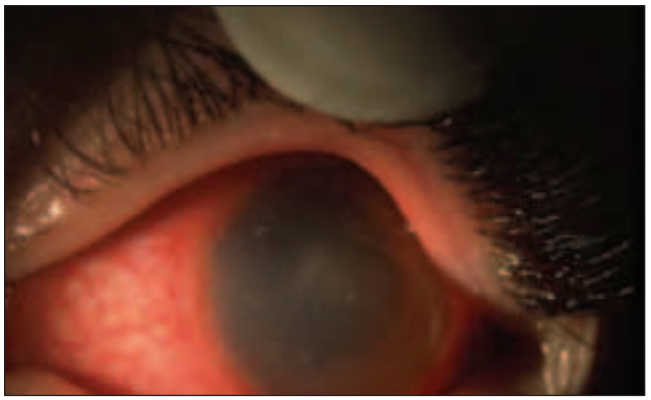

Figure 1) *Slit-lamp examination of patient's left eye demonstrating edema and poorly defined infiltrates*

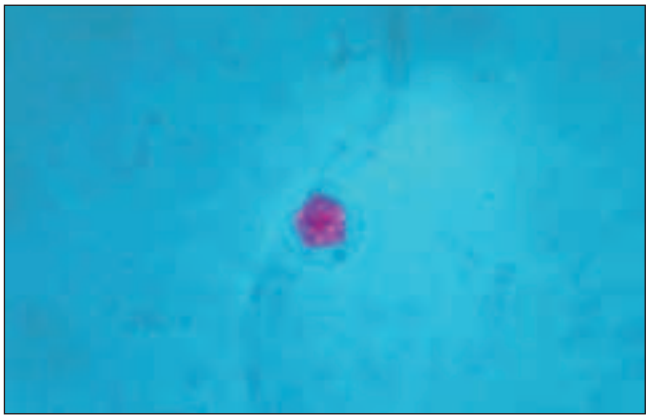

Figure 2) *Trichrome stain of* Acanthamoeba *species cyst seen on non-nutrient agar with* Escherichia coli *overlay after inoculation of corneal scrapings*

Figure 3) Acanthamoeba *species 18S* rRNA *gene tree. The distance scale is the number of base pair substitutions calculated per base pair*

In addition, sequencing of the more variable 18S *rRNA* gene yielded an identical sequence homology for all of the isolates, indicating that both clinical and environmental isolates were the same strain. The phylogenetic gene tree presented is based on an alignment of 2690 sites of the 18S *rRNA* gene (Figure 3). The alignment was performed using the sequence alignment program Eyeball Sequence Editor (ESEE version 3.0, University of Rochester, USA) (2). A total of 2860 sites were used to calculate distances using the Kimura 2-parameter model in the phylogenetic analysis program Molecular Evolutionary Genetics Analysis version 2 (MEGA2, Molecular Evolutionary Genetics Analysis, USA) (3). The tree is rooted with *Acanthamoeba* species V006, which is a genotype T1 isolate. The isolates from the current study included in the phylogenetic tree are *Acanthamoeba* species: Canada 03-002, GenBank accession number AY552093 (lens case B); 03-003, GenBank accession number AY552094 (tap water); 03-004, GenBank accession number AY552095 (well water); and 03-005, GenBank accession number AY552096 (left corneal scrape). These isolates are presented in bold-faced type on the gene tree (Figure 3).

Acanthamoeba species are free-living amoebas with a global distribution, and have been isolated from soil as well as a wide variety of water sources, including tap water (4). The *Acanthamoeba* species' life cycle consists of trophozoite and cyst stages, the latter promoting persistence in both the environment and an infected host. Resistance to chlorination permits survival in contact lens paraphernalia after contamination (1). The annual incidence of microbial keratitis associated with daily wear soft contact lenses is estimated to be 1:2500 (5). Risk factors for *Acanthamoeba* species keratitis (AK) include inappropriate soft contact lens care (6), and exposure to swimming pool water. A British survey (7) spanning two years, studying 106 reported cases of AK, estimated an annual incidence of 17.53 to 21.14 per 1,000,000 contact lens wearers. Of note, the incidence has been rising since the first descriptions of AK in the 1970s (8). This may be due to a combination of increased soft contact lens wear as well as improved microbiological detection.

Exposure to *Acanthamoeba* species is common due to its ubiquitous nature. Unlike granulomatous amoebic encephalitis with *Acanthamoeba* species, subacute and chronic corneal infection is seen in immunocompetent hosts (9). In some cases, the source has been proven to be tap water, which is colonized with *Acanthamoeba* species, subsequently contaminating contact lenses, which serve as vectors (4). Recent advances in molecular epidemiology of *Acanthamoeba* species have established clinical and environmental links (10,11). The pathogenesis of AK is likely secondary to a combination of exposure and trauma. Trauma induced by contact lens wear disrupts the integrity of the corneal epithelium, and preceding trophozoite or oocyst adherence to the contact lens is followed by attachment to the corneal epithelium via mannose-binding proteins, which have been found to be expressed on the surface of

Acanthamoeba castellanii. Invasion of the underlying stroma via cytolysis and corneal desquamation, and the elaboration of proteases follows at a later stage (12). Acanthapodia-dependent phagocytosis has been demonstrated in pathogenic strains of *Acanthamoeba* species (13). Radial keratoneuritis is secondary to cytolysis of corneal nerves, and is responsible for the pain generally associated with AK (14). In the current case, the pain was unusually attenuated. This may have been due to early topical corticosteroid application.

Classically, *Acanthamoeba* species have been identified based on morphological characteristics of cysts and trophozoites (12). Advances in molecular diagnostics have permitted characterization at the genotype level (15) and have established epidemiological links between isolates. Twelve, possibly more, different *Acanthamoeba* species genotypes have been identified (T1 to T12). One particular genotypic cluster containing T3, T4 and T11 genotypes has been shown to be pathogenic in vitro, demonstrated by growth at higher temperatures, cytotoxicity and elaboration of proteases (11). Genotype T6 has also been associated with AK (16). In the present case, 18S *rRNA* gene sequencing suggests that AK was secondary to contact lens case colonization with well water containing *Acanthamoeba* species genotype T4, which has been described in other cases of AK (10,17). Considering the fact that genotype T4 was identified in the present case, the most likely species of *Acanthamoeba* is *A castellanii*. *Acanthamoeba polyphaga* has also been demonstrated to have the same genotype, although this has not been demonstrated consistently and the organism may be considered polyphyletic. By identifying the same genotype in clinical and environmental isolates, we have linked the present case of AK with exposure to contaminated well water from the patient's home.

Topical therapy with cysticidal agents remains the mainstay of therapy (18,19). Polyhexamethylene biguanide has been demonstrated to be effective, especially if applied in conjunction with other agents. Chlorhexidine applied hourly is also effective, and kills both cysts and trophozoites (20). A successful outcome in patients treated medically is noted in up to 88%, the remainder requiring corneal debridement and possibly penetrating keratoplasty. In the latter case, failure has been associated with relapsing *Acanthamoeba* species infection (21).

Since the present case was described, two more cases of soft contact lens-associated AK have been diagnosed at Misericordia Health Centre, Winnipeg, Manitoba. *Acanthamoeba* species was isolated from corneal scrapings, and contact lens case washings were inoculated onto non-nutrient agar with *E coli* overlay. A 16-year-old male patient also had a history of exposure to well water. Well water samples were not available for analysis because the patient had moved to a new residence. In addition, *Acanthamoeba* species were also isolated from corneal scrapings of a male soft contact lens wearer working on a farm. These cases highlight the importance of appropriate specimen collection and direct plating to an appropriate media.

A high degree of clinical suspicion of AK is warranted in soft contact lens wearers with keratitis unresponsive to conventional topical and systemic treatment for herpetic, bacterial or fungal keratitis.

To our knowledge, this is the first case of AK to be molecularly linked to Canadian well water. This organism is common in the environment, and molecular typing is helpful in determining the source of the infection and establishing epidemiological links.

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