Acanthamoeba Keratitis: The Role of Domestic Tap Water Contamination in the United Kingdom

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PURPOSE. The incidence of acanthamoeba keratitis (AK) in the UK is some 15 times that in the United States and seven times that in Holland. To investigate reasons for this higher frequency, a study of the role of domestic tap water as a potential source of AK was undertaken.

METHODS. Tap outlets from the homes of 27 patients with culture-proven AK were sampled and cultured for free-living amoebae (FLA). For all Acanthamoeba isolates, mitochondrial DNA (mtDNA) restriction fragment length polymorphisms (RFLPs) and cytochrome oxidase (cox 1/2) sequence typing was performed to determine the similarity between corneal and tap water isolates.

RESULTS. FLA, including Acanthamoeba, were isolated from 24 (89%) of 27 homes, and the presence within the homes varied significantly with tap water temperature and location: 19 (76%) of 25 bathroom sink cold taps sampled compared with 6 (24%) of 25 hot and 9 (47%) of 19 kitchen cold taps compared with 3 (16%) of 19 of hot kitchen taps. Acanthamoeba were isolated from 8 (30%) of 27 homes (five bathroom sink cold taps, one cloakroom cold tap, one bath, and one bedroom sink mixer [hot/cold] taps). In six cases, identical Acanthamoeba mtDNA profiles were found for the clinical and home tap water isolates. In keeping with UK plumbing practice, 24 of 27 homes had internal roof water storage tanks to supply domestic taps, but the mains fed the kitchen cold tap.

CONCLUSIONS. Water storage tanks promote colonization of domestic water with FLA, including Acanthamoeba, and hence increase the risk of AK. This accounts for the significantly greater incidence of AK in the UK and supports advice to avoid using tap water in contact lens care routines. (Invest Ophtalmol Vis Sci. 2004;45:165-169) DOI:10.1167/iovs.03-0559

The Acanthamoeba is a genus of free-living amoebae, members of which can cause a potentially blinding keratitis in humans.1 The organism is characterized by a life cycle of feeding and replicating trophozoite and dormant cyst stages.2 The resistance of Acanthamoeba cysts to most antimicrobial agents makes acanthamoeba keratitis one of the most difficult ocular infections to treat with a mean treatment period of more than 5 months, surgical interventions in 50% of cases, loss of vision (6/18 or less) in more than 30% of patients and, ultimately, enucleation in recalcitrant cases.3-4 Contact lens wearers are most at risk of contracting acanthamoeba keratitis, accounting for some 90% of reported cases.4-5 Poor hygiene practices, such as the failure to comply with recommended lens cleaning and disinfection procedures and the rinsing and storing of lenses in nonsterile saline or tap water are recognized risk factors for infection.6-9

Acanthamoeba is one of the most common free-living amoebae found in the environment.2 Acanthamoeba cysts can withstand extremes of temperature, desiccation, and disinfection,10 which accounts for the reported isolation of the organism from soil, mud, rivers, ponds, lakes, chlorinated bathing pools, water cooling towers, tap water, and the atmosphere.11-12 This almost ubiquitous presence of Acanthamoeba in such environments is a constant challenge to the contact lens wearer as potential sources of infection. This also presents a difficulty in determining the precise source of a patient’s infection and is further complicated by the morphologic similarity between acanthamoeba keratitis strains.10-13 However, the admission of many patients to rinsing or storing lenses in tap water or tap-water–prepared saline solutions has strongly implicated such practices to be a significant risk factor in acquiring the infection.4,6-14

Since acanthamoeba keratitis was recognized in 1973, several hundred cases have occurred in the UK, with 180 culture-positive cases treated at Moorfields Eye Hospital between January 1990 and December 2000; details of 183 cases are now in the literature.3,5,15 Acanthamoeba keratitis is not a notifiable disease and therefore the true incidence of infection in the UK or worldwide is difficult to ascertain. However, studies have estimated the incidence among contact lens wearers to be 1.36 per million in the United States14 (although studies using non–culture-based diagnostic methods have proposed an incidence one order of magnitude higher),16 3.06 per million in Holland17 and between 17.53 and 21.14 per million in the United Kingdom.18,19,20 However, it has been suggested that the incidence in England and Wales may be 33% higher than reported.16

In an attempt to explain the significantly higher incidence of acanthamoeba keratitis in the United Kingdom, we investigated the prevalence of Acanthamoeba in domestic water sources of 27 patients with culture-proven acanthamoeba keratitis. Mitochondrial DNA (mtDNA) typing was then used to compare Acanthamoeba corneal and domestic tap water isolates and provide a definite epidemiologic link for the source of infection.
METHODS

Subjects

Twenty-seven patients with culture-proven acanthamoeba keratitis were investigated, of whom 23 were contact lens (CL) wearers. The study was retrospective, with a 3- to 10-month interval between corneal culture and obtaining domestic tap water samples. In addition, a previously unreported case from Northern Ireland was included. The study complied with the provisions of the Declaration of Helsinki.

Domestic Water Sampling

Each patient was sent a sampling pack comprising a questionnaire and sterile cotton-tipped swabs individually contained in sterile plastic transport tubes (Bibby Sterilin, Staffordshire, UK). Written instructions indicated that before first daily use, each household tap should be sampled by rubbing the swab inside the spout of the tap and a little water from the tap run into the transport tube. The swab was then replaced into the tube sealed with adhesive tape and labeled according to tap location (e.g., bathroom sink, kitchen, bath) and temperature (hot, cold, or mixed hot/cold). The samples and completed questionnaire were then returned for analysis.

Questionnaire

Patients answered questions relating to possible risk factors for the development of acanthamoeba keratitis. These included CL wear, CL type, disinfection and storage methods, presence of a roof water storage cistern, CL contact with tap water, and CL wearing while swimming or when showering.

Microbiology

In the laboratory, the tap samples were vortexed, and 0.5 mL of water inoculated over the surface of a nonnutrient agar plate seeded with a dense suspension of Escherichia coli (NNA-E. coli). After the water had absorbed the plates were incubated at 32°C in sealed polythene bags and examined daily for up to 7 days using an inverted light microscope for the presence of FLA. Isolates of FLA were identified by morphologic examination of the trophozoite and cyst forms. Acanthamoeba isolates were cloned by microcapillary manipulation of a single cyst on to a fresh NNA-E. coli plate. Excysted trophozoites from these clones were adapted to axenic (bacteria free) culture at 32°C. Mitochondrial DNA RFLP and Sequence Typing

Acanthamoeba isolates from the patients and domestic taps were compared for their mtDNA restriction fragment length polymorphisms (RFLPs). The mtDNA was isolated from axenic trophozoites by the alkaline lysis method used for the isolation of bacterial plasmid DNA, as previously applied to Acanthamoeba. Approximately 2 to 5 μg of mtDNA was digested with the restriction endonuclease HindIII (Roche Diagnostics, Sussex, UK) and separated by electrophoresis in a 0.7% agarose gel at 2 V/cm for 18 hours in 0.5× TBE buffer. DNA standards of A-hindIII/FOX-174 Rf-HaeIII digests (Amersham Pharmacia Biotech, Milton Keynes, UK) were included as size markers. The gels were stained with 1.0 μg/mL ethidium bromide in distilled water and photographed under UV transillumination (665 nm; Polaroid, Cambridge, MA; and a Wattern no. 9 orange filter; Eastman Kodak, Rochester, NY).

PCR was used to amplify part of the cytochrome oxidase subunit-1 and -2 (cox1/2) from purified mtDNA (Cos-PCR). Primers were forward (gaattagctgctccgggttc) and reverse (tcaggataatcggggatccttc) designed to amplify a 1.2-kbp fragment. PCR was performed in a 50-μL volume consisting of: 1× Taq DNA polymerase buffer (20 mM (NH₄)₂SO₄, 75 mM Tris-HCl [pH 8.8] at 25°C, 0.01% vol/vol Tween 20), 1.5 mM MgCl₂, and 0.2 mM each dNTP, 0.5 μM of each primer, 1 U of Taq DNA polymerase (Red Hot; Advanced Biotechnologies Ltd., Epsom, UK) and approximately 100 ng of template DNA. Thermal cycling conditions were: 4 minutes 96°C, followed by 35 cycles of 1 minute 95°C, 1 minute 52°C, and 1.5 minutes 72°C. After a final 10 minutes at 72°C, samples were held at 4°C. Amplified products were analyzed by agarose gel electrophoresis and sent for sequencing (MWG-Biotech, AG, Ebersberg, Germany). DNA sequences were aligned (ClustalW) and a phylogenetic tree constructed (TreeView Win32 1.6.6; University of Glasgow, UK).

RESULTS

Free-Living Amoebae

FLA were cultured from one or more taps of 24 (89%) of 27 households of acanthamoeba keratitis patients. FLA presence varied significantly with the tap water temperature and location. Water-main-supplied kitchen cold taps yielded 9 (47%) of 19 FLA compared with 19 (76%) of 25 bathroom sink cold taps (ANOVA; P < 0.05). Hot water taps were markedly less contaminated with only 3 (16%) of 19 kitchen and 6 (24%) of 25 bathroom sink taps positive (P < 0.05 compared with cold water equivalents). Bath taps, 5 (45%) of 11 cold and 2 (18%) of 11 hot taps, were also positive. Mixed taps were positive in two of seven kitchen, one of two bathroom sink, and two of four bath samples. Two of four shower samples were also positive.

The FLA isolated were identified as Acanthamoeba spp., Hartmannella spp., Naegleria gruberi, Vahlkampfia sp., and Vannella sp.. Contamination of domestic water sources by Acanthamoeba spp. was found in 8 (30%) of 27 separate homes. Within these homes, five came from bathroom sink cold taps, one from a cloakroom cold tap, one from a bath hot-and-cold mixer tap and 1 from a bedroom sink mixer tap. In one patient’s home, all hot and cold taps in the kitchen, bathroom sink, and bath cold tap were positive for Acanthamoeba.

mtDNA Typing

In six of eight keratitis cases, identical HindIII mtDNA RFLPs were found for the Acanthamoeba isolate from the patient cornea and domestic tap water (Fig. 1). Cox-PCR sequence comparison also confirmed the DNA relatedness of these clinical and tap-water-related strains (Fig. 2). A reference strain of A. castellanii (CCAP 1501/1a) was included in the analysis and phylogenetic tree construction.

In the acanthamoeba keratitis case from Belfast, Northern Ireland, Acanthamoeba spp. were isolated from the patient, the bedroom cold water tap of the patient’s accommodation,
and the bathroom cold tap water of the parental home in the Irish Republic. Identical mtRFLPs and Cox-PCR sequences were found with the patient isolate and that from the Belfast tap water, but not that from the parental home (Pt-27) as shown in Figures 1 and 2.

**CL Habits**

All 27 patients in the study returned questionnaires. Of these, 23 (85%) reported that they were CL wearers: 17 (77%) used soft lens types, and 6 (26%) gas-permeable lenses. None of the patients admitted to storing his or her lenses in tap water. However, all but three reported that their lenses or storage case occasionally came into contact with tap water. Twenty-four of 27 patients were aware of having water storage tanks on their roofs, including 20 of 23 of the CL–wearing patients.

Of the eight homes from which Acanthamoeba were isolated, seven were occupied by CL wearers and all had roof storage tanks. Of these, four patients wore daily wear soft lenses, two disposable daily wear lenses, and one rigid gas-permeable lenses. Lens disinfection systems used were chlorine tablets (n = 1) one-step hydrogen peroxide (n = 3), and multipurpose solutions (n = 1). Two patients using daily wear disposable CLs used saline solution only for storage of the lenses. FLA were isolated from the tap water outlets of all the four non-lens wearers who had acanthamoeba keratitis. However, Acanthamoeba was isolated only from a bath mixer tap of one of the homes, the patient having had mud splashed into his eye at a motocross rally 3 days before evidence of infection. The strain from the tap was a different strain from that grown from his eye (Pt-22).

**DISCUSSION**

Although acanthamoeba keratitis is now well recognized as an infection associated with CL wear and there have been significant improvements in CL care systems, the incidence within the UK remains significantly higher than in the remainder of Europe and the United States.4,14,15,17,18 In this study, the hypothesis that domestic tap water is a reservoir for Acanthamoeba causing keratitis was investigated. The isolation of amoebae from the domestic tap water outlets of almost 24 (89%) of 27 of patient homes, of which 8 contained Acanthamoeba, demonstrates that this is a significant source of these organisms. Furthermore, the use of mtDNA typing methods for Acanthamoeba spp. strain differentiation confirmed that in six cases the patients’ domestic tap water was the source of infection.

The taxonomic classification of the Acanthamoeba is based on morphologic observations of the trophozoite and cyst forms.2 Although this permits the identification of the genus and most species it is a subjective approach and does not permit strain differentiation.15 mtDNA RFLP typing has been shown to be a powerful technique for differentiating morphologically identical strains of Acanthamoeba and highlights the large degree of genetic diversity within species and strains classified by morphologic criteria.13,20 However, the technique requires that the isolates be adapted to axenic (bacteria-free) broth media, which is not always successful with some strains. The methods for purifying mtDNA and the RFLP analysis are also time consuming and laborious. Alternative typing schemes have been developed based on nucleotide sequence analysis of
It should be noted that this survey was conducted retrospectively with some 3 to 10 months elapsing between the diagnosis of infection and obtaining tap water samples. If tap outlet colonization was an intermittent feature or occurred transiently only around the time of the patients’ infection, then more taps may have contained Acanthamoeba if sampling had been undertaken nearer to the time of diagnosis. Acanthamoeba levels in ground water have also been shown to fluctuate with seasonal temperature changes. Although the acanthamoeba keratitis cases and tap water sampling occurred through all seasons, the possibility that tap water contamination is greater during the warmer months warrants investigation. It is also unlikely that the Acanthamoeba colonization is a feature only of the keratitis patient, as tap water organisms are found in February when no keratitis have been isolated from tap water outlets in England not associated with keratitis cases (Kilvington S, unpublished observations, 2003).

A common feature of the samples positive for FLA was the presence of biofilm on the swab specimens taken from inside the tap outlets. Although not investigated further, direct microscopic examination of the biofilm revealed numerous bacteria and fungal hyphae besides FLA trophozoites and cysts. Therefore, tap water, particularly from tank-fed supplies, may also be a potential source of other forms of microbial keratitis. FLA, including Acanthamoeba, have also been shown to support the intracellular growth and survival of pathogenic bacteria including Legionella pneumophila and Pseudomonas cepacia, and Mycobacterium avium. Accordingly, FLA in domestic water supplies may well serve as reservoirs for the presence and transmission of other human pathogens.

The findings of this study may be explained by the domestic plumbing practices used in the United Kingdom by which the water mains feed potable water to the kitchen cold tap of the home, augmented by a water storage cistern located in the roof. This is a historical feature originally intended to store water when supplies to households were intermittent, and the feature is retained today for the purpose of supplying other water outlets in the home, such as the toilet cistern and the bathroom cold taps. This arrangement is unique within the United Kingdom as in other European countries and the United States, all household taps are supplied directly by water mains. Only with the implementation of The Water Supply (Water Fittings) Regulations, 1999 has it been a requirement that the cistern should be covered with a rigid, close fitting and securely fixed cover which is not airtight but which excludes light and insects from the cistern. The regulations are not retrospective and do not have to be applied to storage cisterns that were installed before this legislation. A poorly maintained, infrequently flushed, and uninsulated cistern can allow microbes, including Acanthamoeba, to proliferate in the water and hence colonize tap outlets. FLA, including one isolate of Acanthamoeba, were also made from taps supplied from water mains. This source is obtained directly from the water purification plant where it is passed through filter beds and chlorinated to render it safe and potable. It is unclear whether the FLA and Acanthamoeba detected from the water mains-supplied taps originated directly from this supply or from within the home.

In conclusion, domestic tap water, notably that supplied from roof storage cisterns, is a source of Acanthamoeba causing keratitis in the United Kingdom and gives some explanation as to why the incidence of acanthamoeba keratitis in this country is at least 15 times that of the United States and 7 times the rest of Europe. None of the patients in this study admitted to rinsing or storing their lenses in tap water, suggesting that acanthamoeba keratitis can arise from indirect exposure to contaminated tap water. Even in compliant wearers, CL storage cases often contain bacteria and biofilm that provides a food source for Acanthamoeba. CL wearers in the United Kingdom and visitors to the country should be aware of the risks from Acanthamoeba in tap water. Acanthamoeba keratitis remains a rare but serious consequence of CL wear. It is recommended that wearers adhere strictly to the manufacturer’s recommended lens hygiene procedures and use only sterile, approved solutions. In addition, the findings of this study indicate that the manipulation and storage of CLs, both for cleaning–disinfection purposes and insertion, should take place away from sources of potential contamination, such as bathrooms and other sites that receive water from roof storage tanks.

References