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Viability of *Acanthamoeba* Cysts in Ophthalmic Solutions

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***Acanthamoeba* keratitis is a chronic infection of the human cornea. Many people who have this infection wear soft contact lenses. Usually lens wearers clean and maintain their lenses with various ophthalmic solutions including homemade saline. Recently it has been shown that homemade saline solutions play a role in lens contamination and thus in *Acanthamoeba* keratitis. We therefore evaluated the viability of cysts of three species of *Acanthamoeba* by exposing them for various time periods to saline, cleaning, and disinfectant solutions generally used to care for these lenses. We found that the viability of the cysts in saline solutions ranged from a minimum of 14 days to 90 days of exposure. In cleaning solutions, the survival times ranged from a minimum of 1 day to 90 days of exposure. Disinfectants, as expected, were the most effective of all tested solutions in killing *Acanthamoeba* cysts. The survival times ranged from 6 h to 14 days. None of these products were effective in destroying *Acanthamoeba* cysts in less than 6 h of exposure, which exceeds the suggested time that any given solution should be used for lens care.**

Small free-living amebae of the genus *Acanthamoeba* cause a chronic infection of the human cornea (1). The first case of *Acanthamoeba* keratitis in the United States was reported in 1973 in a south Texas rancher with a history of eye trauma (3). Since then, more than 200 cases have been recorded, mostly in soft-contact-lens wearers with no history of eye trauma (Visvesvara, unpublished data). A case-control study of patients with *Acanthamoeba* keratitis in 1987 suggested that contamination of homemade saline solutions used by the lens wearers contributed to this condition (8). A statistically significant association between keratitis and the use of homemade saline was noted. It was noted also that homemade saline solutions were more likely to be contaminated with bacteria and fungi than commercially prepared saline solutions. However, in another study (2), it was shown that 3% of disinfectant solutions, 15% of cleaners, and 13% of preserved saline solutions were contaminated with bacteria. Ophthalmic solutions contaminated with fungus and bacteria might support the growth of the ameba (8). Since *Acanthamoeba* species are ubiquitous in nature, lens care solutions could become contaminated with *Acanthamoeba* cysts in the air (4) or from lenses during regular use. If the organisms survive or multiply in these solutions, the eye could be exposed to a continuing source of *Acanthamoeba* cysts. Because ophthalmic solutions may play a role in lens contamination and thus in *Acanthamoeba* keratitis, we evaluated the viability of cysts of *Acanthamoeba* spp. in various soaking, cleaning, and disinfecting ophthalmic solutions.

MATERIALS AND METHODS

Three species of *Acanthamoeba* frequently implicated in cases of keratitis, *A. castellanii*, *A. polyphaga*, and *A. culbertsoni*, were chosen for the study. All three species, isolated from clinical cases, were grown on nonnutrient agar plates (9) seeded with *Escherichia coli* at 30°C for 4 to 6 weeks to ensure adequate growth and cyst formation. The surfaces of the agar plates were flooded with 5 ml of saline and gently scraped with an inoculating loop, and the cysts were harvested by centrifugation at 560 × *g* for 10 min in a CRU-5000 centrifuge (International Equipment Co., Needham Heights, Mass.). The supernatant was aspirated, and the sediment was washed twice in saline in order to eliminate most of the bacteria. The cysts were then counted with a hemacytometer and resuspended in saline to produce a final concentration of 10^7 cysts per ml.

Six different brands of ophthalmic saline solutions (S), cleaners (C), and disinfectants (D) were purchased from local retail stores. These 18 solutions were selected primarily to include all possible combinations of the most commonly used ingredients in each category (Table 1). A 1-ml amount of each solution was dispensed aseptically into labeled glass screw-capped tubes (15 by 100 mm). Control tubes were prepared containing normal saline. A 10-μl amount of a cyst suspension containing 100,000 cysts was pipetted into each tube. The caps of the tubes were tightened and sealed with Parafilm (American Can Co.) to avoid evaporation, and the tubes were incubated at 22°C until ready to be processed.

At 6 h, 24 h, 3 days, 7 days, 14 days, 30 days, and 90 days, one tube of each ophthalmic solution and a control tube were gently shaken, placed in an ice-water bath for 5 min, rolled between the palms of the hand a few times to loosen organisms that might be sticking to the glass, and centrifuged at 560 × *g* for 10 min. The supernatant was discarded, and the pellet was washed once in normal saline. After centrifugation, the supernatant was removed, leaving 0.5 ml in the bottom of each tube. This material was then spread onto a plate (15 by 60 mm) of nonnutrient agar seeded with *E. coli* and incubated at 30°C for 10 days. The edges of the plates were sealed with Parafilm after the first day of incubation to prevent the agar from drying out. Plates were exmained daily for *Acanthamoeba* trophozoites with an inverted microscope. If no trophozoites were observed at the end of 10 days, the cysts were considered nonviable. All tests were run on three separate occasions.

RESULTS

The exposure times and viability of cysts of *A. castellanii*, *A. polyphaga*, and *A. culbertsoni* in the different saline (Fig. 1), cleaning (Fig. 2), and disinfectant (Fig. 3) solutions are

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TABLE 1. Ophthalmic solutions tested

Code	Brand name	Manufacturer	Additive(s)
Saline solutions			
S1	Lens Plus	Allergan	None
S2	Sensitive Eyes Saline	Bausch & Lomb	Sorbic acid, 0.1%; EDTA
S3	Sterile Preserved Saline	Bausch & Lomb	Thimerosal, 0.001%; EDTA
S4	Sterile Saline	Bausch & Lomb	EDTA
S5	Softmate ps Saline	Barnes & Hinde	Potassium sorbate, 0.13%; EDTA, 0.025%
S6	Softmate Rinsing Solution	Barnes & Hinde	Thimerosal, 0.001%; chlorhexidine, 0.005%; EDTA, 0.1%
Cleaning solutions			
C1	Pliagel	Coopervision	EDTA, 0.5%; sorbic acid, 0.25%
C2	Sensitive Eyes Daily Cleaner	Bausch & Lomb	EDTA, 0.5%; sorbic acid, 0.25%
C3	Sterile Daily Cleaner	Bausch & Lomb	EDTA, 0.2%; thimerosal, 0.004%
C4	Preflex	Alcon	EDTA, 0.1%; sorbic acid, 0.1%
C5	Softmate ps Daily Cleaner	Barnes & Hinde	EDTA, 0.2%; potassium sorbate, 0.013%
C6	LC-65	Allergan	EDTA, 0.01%; thimerosal, 0.001%
Disinfectant solutions			
D1	Disinfection Solution	Bausch & Lomb	Thimerosal, 0.001%; chlorhexidine, 0.005%; EDTA, 0.1%
D2	Flex-Care	Alcon	Thimerosal, 0.001%; chlorhexidine, 0.005%; EDTA, 0.1%
D3	Hydrocare	Allergan	Thimerosal, 0.002%
D4	Softmate Disinfection	Barnes & Hinde	Chlorhexidine, 0.005%; EDTA, 0.1%
D5	Softmate Disinfection/Storage	Barnes & Hinde	Thimerosal, 0.001%; chlorhexidine, 0.005%; EDTA, 0.1%
D6	Lensept	American Optical	H_2O_2, 3%

shown. The survival times shown for these solutions refer to the maximum length of time that the cysts survived.

Some variation was noted between the three species in their survival in the four saline solutions that contained no preservatives (S1, S2, S4, and S5). Cysts survived at least 3 months in these saline solutions. In the other two solutions (S3 and S6), one containing thimerosal and the other thimerosal and chlorhexidine, cysts survived 14 but not 30 days.

Some variation was also noted between the survival of the three species of *Acanthamoeba* in different cleaning solutions. Among the three species, *A. castellanii* survived the longest, for example, 3 days but not 7 days in C6, 7 days but not 14 days in C3, and at least 90 days in C2, whereas *A. culbertsoni* and *A. polyphaga* survived 1 day but not 3 in C6, 3 days but not 7 in C3, and 30 days but not 90 in C2. All three species survived 14 but not 30 days in C1 and at least 90 days in C5.

Greater variability was noted between the three *Acanthamoeba* species in their survival in disinfectant solutions. Cysts of *A. castellanii* and *A. polyphaga* survived in D2 and D5 with thimerosal-chlorhexidine for 6 h but not 24 h, while cysts of *A. culbertsoni* survived for 3 days but not 7 days. Cysts of all three species survived for 6 h but not 24 h in D6 and for 24 h but not 3 days in D4. Cysts of *A. castellanii* and *A. culbertsoni* survived at least 24 h and cysts of *A. polyphaga* for 6 h but not 24 h in D1. Cysts of *A. culbertsoni* and *A. polyphaga* survived 7 days but not 14 days, while *A. castellanii* cysts survived 14 but not 30 days in D3, which contained only thimerosal.

DISCUSSION

The procedures and solutions used for contact lens care vary depending on the type of lens and the recommendations

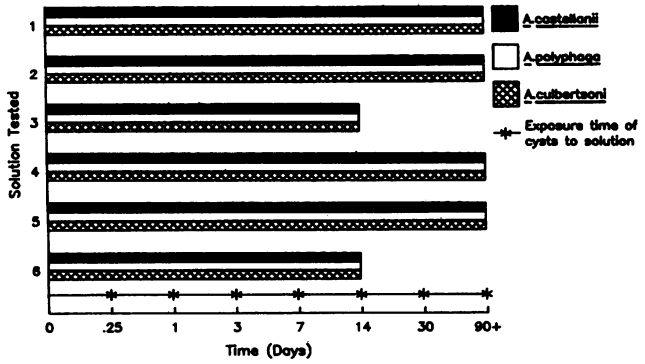

FIG. 1. Viability of cysts of *A. castellanii*, *A. polyphaga*, and *A. culbertsoni* in saline solutions at different exposure times. Bars: 1, Lens Plus; 2, Sensitive Eyes Saline; 3, Sterile Preserved Saline; 4, Sterile Saline; 5, Softmate ps Saline; 6, Softmate Rinsing Solution. See Table 1 for details.

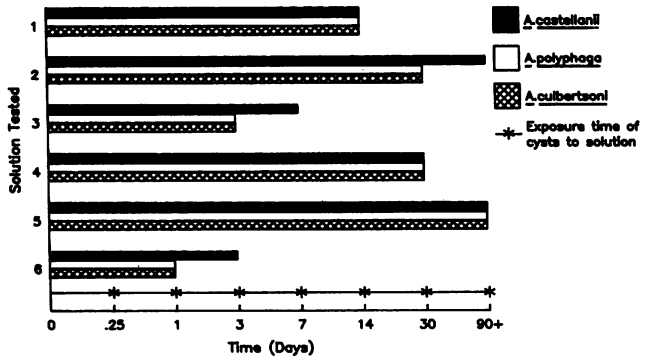

FIG. 2. Viability of cysts of *A. castellanii*, *A. polyphaga*, and *A. culbertsoni* in cleaning solutions at different exposure times. Bars: 1, Pliagel; 2, Sensitive Eyes Daily Cleaner; 3, Sterile Daily Cleaner; 4, Preflex; 5, Softmate ps Daily Cleaner; 6, LC-65. See Table 1 for details.

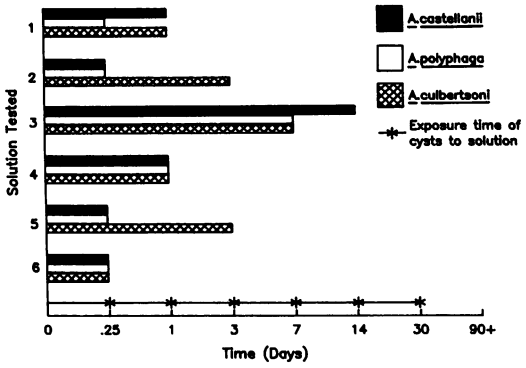

FIG. 3. Viability of cysts of *A. castellanii*, *A. polyphaga*, and *A. culbertsoni* in disinfectant solutions at different exposure times. Bars: 1, Disinfection Solution; 2, Flex-Care; 3, Hydrocare; 4, Softmate Disinfection; 5, Softmate Disinfection/Storage; 6, Lensept. See Table 1 for details.

of the patient's eye care specialist. Soft-contact-lens wearers generally follow similar procedures in caring for their lenses. After removal, the lenses are first cleaned with one of the cleaning solutions and then rinsed with either saline or disinfectant. The lenses are then disinfected either with a chemical disinfectant or in a thermal disinfecting chamber and rinsed again before being stored overnight or worn. Daily-wear soft lenses should be cleaned enzymatically once a week to eliminate protein deposits. Extended-wear soft lenses should be cleaned enzymatically at least once every 2 weeks. Shortcuts in any of these steps lessen the chance of effective results from use of these solutions (8).

Ludwig et al. (5) reported that not all commercially available solutions are equally effective in killing *Acanthamoeba* organisms and that heat disinfection of contact lenses is superior to cold chemical disinfection.

In the present study, the disinfectant containing H_2O_2 was slightly more effective in killing cysts of all three *Acanthamoeba* species than the other disinfecting solutions. Disinfectant D3, containing only thimerosal, was clearly less effective. The reason for the differential susceptibility of the three species to the various disinfectants is not clear. It is possible that the cyst wall of *A. culbertsoni* is much more resistant to the disinfectant than that of the other two species. Reasons for the differences in the killing ability of different brands of disinfectant, even though all contained the same concentration of the preservative (0.005%), are unclear. All commercial solutions examined in this study were tested before their listed expiration dates. Variations in the concentration of the preservatives in different lots and even bottles of the same lot cannot be ruled out (6, 7). As might be expected, our study showed that saline solutions and cleaners were not effective in killing *Acanthamoeba* cysts.

Ophthalmic solutions could play an important role in potentiating *Acanthamoeba* infections. Although these solutions are sterile when purchased, they could become contaminated with bacteria and fungi during use, especially when they are opened and used for long periods of time (2). The bacterial and fungal contaminants will provide a food source for *Acanthamoeba* to feed on, multiply, and encyst. Our study shows that once the solutions are contaminated, the organisms could survive in them. This provides a continuing source of organisms for infecting the eye.

Since most of the lens care solutions are routinely used for no more than a few minutes to overnight, it is clear from our results that none of the solutions tested can by itself destroy *Acanthamoeba* cysts. Of the three types of solutions routinely used in contact lens care, disinfectants provided the greatest killing of *Acanthamoeba* cysts. However, cysts could be detected for at least 6 h after exposure in all disinfectant solutions and at 14 days in one. Contact lens care solutions currently on the market are not effective in eliminating *Acanthamoeba* cysts within a reasonable time frame.

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LITERATURE CITED

1. **Centers for Disease Control.** 1986. *Acanthamoeba* keratitis associated with contact lenses—United States. Morbid. Mortal. Weekly Rep. **35**:405–409.
2. **Donzis, P. B., B. J. Mandino, B. A. Weissman, and D. A. Bruckner.** 1987. Microbial contamination of contact lens care systems. Am. J. Ophthalmol. **104**:325–333.
3. **Jones, D. B., G. S. Visvesvara, and N. M. Robinson.** 1975. *Acanthamoeba polyphaga* keratitis and *Acanthamoeba* uveitis associated with fatal meningoencephalitis. Trans. Ophthalmol. Soc. U.K. **95**:221–232.
4. **Kingston, D., and D. C. Warhurst.** 1969. Isolation of amoebae from the air. J. Med. Microbiol. **2**:27–36.
5. **Ludwig, I. H., D. M. Meisler, I. Rutherford, F. E. Bican, R. H. S. Langston, and G. S. Visvesvara.** 1986. Susceptibility of *Acanthamoeba* to soft contact lens disinfection systems. Invest. Ophthalmol. Visual Sci. **27**:626–628.
6. **Penley, C. A., R. L. Schlitzer, D. G. Ahearn, and L. A. Wilson.** 1981. Laboratory evaluation of chemical disinfection of soft contact lenses. Contact Intraocul. Lens Med. J. **1**:101–110.
7. **Richardson, N. E., D. J. G. Davies, B. J. Meakin, and D. A. Norton.** 1977. Loss of antibacterial preservatives from contact lens solutions during storage. J. Pharm. Pharmacol. **29**:717–722.
8. **Stehr-Green, J. K., T. M. Bailey, F. H. Brandt, J. H. Carr, W. W. Bond, and G. S. Visvesvara.** 1987. *Acanthamoeba* keratitis in soft contact lens wearers: a case-control study. J. Am. Med. Assoc. **258**:57–60.
9. **Vera, H. D., and D. A. Power.** 1980. Culture media, p. 965–999. *In* E. H. Lennette, A. Balows, and W. J. Hausler, Jr. (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.