An Epidemic of Presumed *Acanthamoeba* Keratitis That Followed Regional Flooding

Results of a Case-Control Investigation

Patricia A. Meier, MD, MSc; William D. Mathers, MD; John E. Sutphin, MD; Robert Folberg, MD; Taekyu Hwang, MSc; Richard P. Wenzel, MD, MSc

Objectives: To investigate an outbreak of presumed *Acanthamoeba* keratitis (AK), to identify risk factors associated with its development, and to characterize the changing epidemiology of AK.

Methods: We performed a pairwise-matched case-control study involving 31 patients who were diagnosed as having AK between July 1993 and December 1994. Risk factors were identified using conditional logistic regression analysis. To investigate the impact of regional flooding, we stratified counties within Iowa by whether their water facilities were affected and then calculated population-based estimates of the incidence of AK.

Results: During the study, 43 presumed incident cases of AK were diagnosed; 31 were included in the case-control study. Cases were diagnosed based on the clinical presentation of keratitis, positive tandem scanning confocal microscopy examination results, and confirmatory cytopathologic findings. There were no positive culture specimens. On average, cases had symptoms for 8 weeks before diagnosis, most notably photophobia (94%), red eyes (94%), and pain (80%). Contact lens use (odds ratio [OR] = 44.16; $P = .02$) and fishing (OR = 22.62; $P = .04$) were independent predictors of the development of AK. The presence of a humidifier in the home (OR = 0.08; $P = .03$) and having household water that originated from a private well instead of the municipal water supply (OR = 0.12; $P = .08$) were protective. Twenty-nine of 30 cases resided in counties in which the water supplies were affected by flooding as determined by the Department of Natural Resources, Des Moines, Iowa. The incidence of AK in these counties was more than 10 times higher than that in the unaffected counties (relative risk = 10.83, 95% confidence interval, 1.48-79.49; $P < .003$).

Conclusions: We describe an epidemic of keratitis that, based on clinicopathologic and epidemiological evidence, is consistent with AK. As in previous outbreaks of culture-proven AK, contact lens use was the major risk factor. Both the results of the case-control study and the population-based incidence estimates suggest that the recent outbreak may be caused, in part, by the effects of regional flooding. However, because the outbreak also coincided with a change in diagnostic techniques, we cannot eliminate recognition bias as the reason for the apparently changing epidemiology.

Arch Ophthalmol. 1998;116:1090-1094

From the Departments of Internal Medicine (Drs Meier and Wenzel), Ophthalmology (Drs Mathers and Sutphin), and Pathology (Dr Folberg), University of Iowa College of Medicine, and the University of Iowa Hospitals and Clinics, Iowa City; and the Department of Preventive Medicine and Environmental Health, University of Iowa Graduate School (Drs Meier and Wenzel and Mr Hwang). Dr Meier is now with Wilford Hall Medical Center, San Antonio, Tex, and Dr Wenzel is now with the Department of Internal Medicine, Medical College of Virginia, Virginia Commonwealth University, Richmond.

MICROBIAL keratitis is an important cause of corneal scarring and blindness.[1-3] *Acanthamoeba* keratitis (AK), first recognized in 1973,[4] is a rare protozoal infection with symptoms that mimic herpes simplex virus, thereby delaying diagnosis and treatment.[5-10] The early reported cases of AK were attributed to corneal injury or to exposure to contaminated water, but results of later reports suggested a link between contact lens use and AK.[10,11] In 1986, the Centers for Disease Control and Prevention issued a report of 24 cases of AK in which they noted that 20 cases (83%) were associated with contact lens use.[12] Between 1973 and 1988, there were 208 cases of AK reported in the United States, and 85% were among contact lens wearers.[13,14] In general, contact lens wearers are predisposed to ulcerative keratitis of varied causes, with an estimated 2.0 to 20.9 cases per 10 000 wearers per year.[3]

In 1994, ophthalmologists at the University of Iowa Hospitals and Clinics (UIHC), Iowa City, recognized that they were diagnosing more cases of AK than previously (**Figure 1**) and that fewer than half of the patients wore contact lenses.[15] We hypothesized that this increase could be related to exposure to contaminated water resulting from the flood of 1993, which had devastated the state of Iowa. Because the outbreak also coincided with the introduction of a new diagnostic tool used to examine patients

PARTICIPANTS AND METHODS

SETTING

The UIHC is the major referral hospital for the state, which has a population of 2.78 million. The UIHC ophthalmology clinic registers 60 000 outpatient visits annually.

STUDY DESIGN

A case-control study design was used to evaluate potential risk factors. After receiving approval from the UIHC Institutional Review Board, a single investigator (P.A.M.) interviewed all cases and controls. Questions were designed to address known risk factors for AK and potential exposure to water contaminated by the flood.

STUDY POPULATION

During the study—July 1, 1993, through December 31, 1994—all cases were diagnosed by 1 of 2 cornea specialists (W.D.M. or J.E.S.), who also performed all TSCM examinations. To ascertain whether cases of AK were being diagnosed elsewhere, we surveyed all ophthalmologists and optometrists in the state; all cases known to them had been diagnosed at UIHC. Controls were selected from outpatients who were seen in the ophthalmology clinic for diagnoses other than AK. They were matched to cases by age (within 5 years) and date of clinic visit (within 4 months). For each incident case, a list of potential controls was provided by medical records personnel. Those controls who were eventually interviewed were selected from the list of appropriately matched eligible controls using a random number table. When telephoned, only 1 potential control declined to participate in the study, and a second control was selected.

MEASUREMENT METHODS

Ascertainment of Disease

The diagnosis of AK required 3 components: (1) the diagnosis of keratitis, (2) identification of the 10- to 25-µm ovoid refractile bodies with TSCM, and (3) demonstration of amoebic trophozoites or cysts on histopathologic examination of corneal scraping. After locating plausible organisms with TSCM, the ophthalmologist (W.D.M. or J.E.S.) used a Kimura spatula to remove the epithelium and to drag it across a glass slide to dislodge any organisms. The specimen was fixed in 10% formalin and stained with hematoxylin-eosin. The ocular pathologist (R.F.) screened 2 or more slides per case. Organisms were identified as trophozoites if the cytoplasmic borders were sharply defined and the structure contained a nucleus with a well-defined karyosome. Cysts were identified on the basis of size and a polygonal shape.

Initially, corneal specimens were submitted only for bacterial and viral cultures. Once the outbreak was recognized, specimens were also cultured for *Acanthamoeba* using nonnutrient agar that was supplemented with enteric flora.[19] A positive *Acanthamoeba* culture specimen was not required for the diagnosis.

Ascertainment of Exposure

Our questionnaire was designed to assess 3 primary exposure variables: contact lens use, corneal trauma, and exposure to contaminated water, particularly flood-related exposures. According to 2 major reviews on *Acanthamoeba*,[5,20] these organisms are ubiquitous in the environment and may resist freezing, drying, and the usual levels of chlorine used in drinking water and swimming pools. With this in mind, we constructed more than 30 questions about various types of water exposure. Furthermore, because *Acanthamoeba* often coexist with *Legionella* species,[21] we included items thought to affect *Legionella* growth, such as recent plumbing work and air conditioning use. Regarding the flood, we asked each person whether their house or job site had been flooded and whether they had participated in flood cleanup activities.

Ascertainment of Potential Confounding Variables

We matched cases and controls by age because corneal integrity declines with age, making older individuals more susceptible to infections. We also asked about glaucoma, systemic diseases (diabetes and kidney disease), and immunosuppression that may alter corneal integrity. Previous research suggested that gender plays a role in the development of AK, perhaps because certain occupations or recreational activities place men at increased risk.[14] Therefore, we included questions about gender, occupation, and recreational activities.

STATISTICAL ANALYSIS

When statistical comparisons were made, continuous variables were analyzed using the Student t test or the nonparametric equivalent, and categorical variables were analyzed with a χ^2 or z statistic, all with 2-tailed tests of significance. Potential risk factors were first analyzed using a univariate conditional (matched) logistic regression program (SAS, SAS Institute Inc, Cary, NC). Using Wald χ^2 criterion in the univariate analysis, we entered putative risk factors with P values of less than or equal to .20 stepwise into the conditional logistic regression model. Parameter estimates, SEs, odds ratios (ORs), 95% confidence intervals (CIs), and P values were calculated using the SAS conditional nominal logistic regression program. Population-based estimates of the incidence of AK in the state of Iowa were used to determine the relative risk of exposure to the flood of 1993 for a subsequent diagnosis of AK. These risk ratios and 95% CIs were calculated using computer software (Epi Info, version 6, Centers for Disease Control and Prevention, Atlanta, Ga).

with keratitis, we also considered that recognition (ascertainment) bias might explain the increase. With tandem scanning confocal microscopy (TSCM), an ophthalmologist can inspect the cornea and actually visualize organisms in vivo; its use in diagnosing keratitis has been described previously.[15-18] Herein, we describe the risk factors associated with this presumed epidemic of AK.

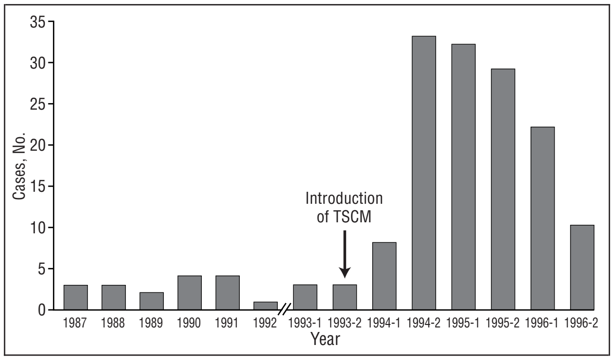

Figure 1. *Epidemic curve of* Acanthamoeba *keratitis at the Univeristy of Iowa Hospitals and Clinics, Iowa City. Cases diagnosed before July 1993 were based on results of histopathologic examination of corneal specimens. For calendar years 1987 through 1992, each bar represents the number of cases diagnosed during the entire year; for calendar years 1993 through 1996, the number of cases diagnosed during a 6-month period. The labels for years 1993 through 1996 are followed by "-1" and "-2," referring to the first 6 months and second 6 months of the year, respectively. TSCM indicates tandem scanning confocal microscopy.*

RESULTS

DESCRIPTION OF THE OUTBREAK

As shown on the epidemic curve, the increase in the number of cases of AK began in late 1993, after the flood of 1993 and the introduction of TSCM (Figure 1). Total number of ophthalmology clinic visits were similar in 1992 (n = 60 365), 1993 (n = 59 575), and 1994 (n = 60 296). In the cornea center, however, the proportion of total visits that were coded "keratitis" increased from 25.4% in 1992 to 35.2% in 1994 ($z = 12.78$, $P < .001$), in part because of additional, often multiple (or repeated) visits by patients with AK. During the study, 39 (28%) of 139 patients who were examined with TSCM had positive examination results; 38 (97%) of these had confirmatory histopathologic findings. Only 1 patient, who had repeatedly negative examination results but positive histopathologic findings, was considered to have a false-negative TSCM result.

Between 1987 and 1994, there were 123 positive corneal specimens (overall positivity rate = 47.5%) from 98 unique patients, two thirds from the UIHC. The positivity rate was similar before and after TSCM was introduced (43.7% vs 55.3%, $\chi^2_1 = 2.25$, $P = .13$).

DESCRIPTION OF CASES

Forty-three patients met our case definition. Of these, 31 patients (72%) could be located, agreed to participate, and had an appropriately matched control. Those not interviewed were somewhat older (57 vs 40 years) but did not differ by gender, contact lens use, or state of residence. Although the average time from onset of symptoms to the first medical evaluation was only 2 days, the median time to diagnosis was 57 days. All cases were seen elsewhere first, and most (84%) already failed empirical therapy for viral or bacterial keratitis.

Table 1. Univariate Analysis of Risk Factors for Presumed *Acanthamoeba* Keratitis*

	Cases, No.	Controls, No.	Odds Ratio	*P*
Contact lens wear	14	4	11.00	.02†
Fishing	11	3	5.00	.04†
Gardening	5	13	0.27	.05
Private well	4	10	0.29	.06
Hot tub	6	1	6.00	.09
History of lens wear	17	11	2.50	.12
Humidifier	9	15	0.40	.12
History of eye disease	12	18	0.46	.14
Smoking	9	5	3.00	.18
Boating	9	5	3.00	.18
Air conditioning (car)	11	15	0.33	.18

**Because of significance at $P \leq .20$, all variables were eligible for entry into the multivariate model. Variables tested but with $P > .20$ include sex, previous eye disease, previous eye surgery, previous eye trauma, diabetes, glaucoma, renal disease, corticosteroid use, immunosuppression, swimming, scuba diving, sailing, water skiing, canoeing, shower use, gas waterheater, plumbing work, home air conditioning, job air conditioning, use of oral hygiene device (Water Pik), nosedrops, aquarium, reported exposure to contaminated water, residence flooded, job flooded, flood cleanup activities, household plant, landscaping, reported soil exposure, and recent eye trauma.*
†Significant at $P < .05$.

UNIVARIATE ANALYSES

Cases and controls were similar with regard to history of eye disease, eye surgery, underlying medical conditions, and recent eye trauma (**Table 1**). Cases were more likely to wear contact lenses than were controls (univariate OR = 11.00, 95% CI = 1.48-228.10; $P = .02$), but a variety of lens types were worn. There were too few lens wearers in the control group to make statistical comparisons, but it seemed that cases and controls had similar cleaning practices. No one used homemade saline to clean their lenses, but 6 cases (43%) and 1 control (25%) used tap water for rinsing.

Except for fishing (univariate OR = 5.00, 95% CI = 1.05-33.00; $P = .04$), cases and controls had similar recreational water exposures. They also had similar household water exposures, except that cases were less likely (OR = 0.29; $P = .06$) to receive their household water from a private well. All variables with *P* values of less than or equal to .20 were eligible for entry into the multivariate model and are shown in Table 1. Those variables with *P* values that exceeded .20 are included in the footnote.

MULTIVARIATE ANALYSIS

Contact lens wear and fishing were independent, positive predictors of the development of AK. Contact lens use was the single best predictor, and for all models tested it was entered first (**Table 2**). The best model, one that we considered both parsimonious and plausible, was the 4-variable model that contained contact lens use (OR = 44.16, 95% CI = 2.02-966.60; $P = .02$), fishing (OR = 22.62, 95% CI = 1.35-380.38; $P = .04$), presence of a humidifier in the home (OR = 0.08, 95% CI = 0.008-0.844; $P = .03$), and well water as the household water supply (OR = 0.12, 95% CI = 0.11-1.29; $P = .08$). This 4-variable model resulted in an overall χ^2 of 15.18 ($P = .004$).

Table 2. Multivariate Analysis of Risk Factors for *Acanthamoeba* Keratitis*

	Parameter Estimate	SE	Odds Ratio	95% Confidence Interval	*P*
Contact lens wear	3.79	1.57	44.16	2.02-966.6	.02
Humidifier	−2.53	1.20	0.08	0.008-0.844	.04
Fishing	3.12	1.44	22.62	1.35-380.38	.03
Water supply from private well	−2.14	1.22	0.12	0.11-1.29	.08

**The model was constructed using conditional logistic regression analysis.*

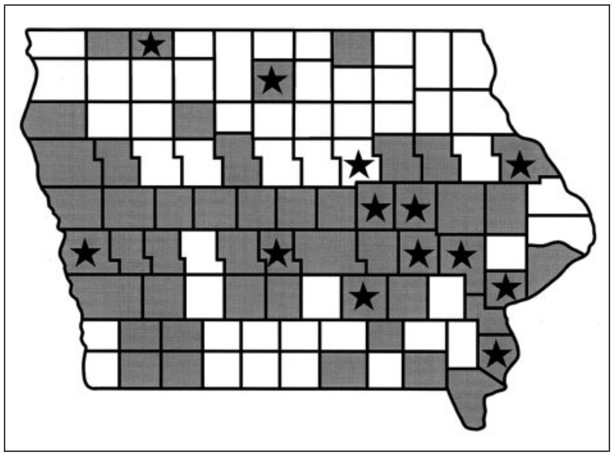

Figure 2. *Cases of presumed* Acanthamoeba *keratitis stratified according to Iowa counties in which the water facilities were affected by the flood of 1993 (shaded areas). Stars indicate counties with 1 or more cases.*

Having a humidifier in the home and using well water instead of the municipal water supply were protective. Stated differently, having household water that originated from the municipal water supply was an independent risk factor for AK. Although marginally significant, we retained household water source in the model because it supports the ecological analysis discussed below and improves plausibility. Because of sample size constraints, we did not consider models with more than 4 variables.

POPULATION INCIDENCE ESTIMATES

Because the entire state of Iowa was declared a flood disaster area, cases and controls were similarly exposed. Thus, we could not use the case-control method to evaluate the flood as a risk factor for AK. However, we postulated that not all counties were equally ravaged. We stratified counties using data from the Department of Natural Resources, Des Moines, that specified whether a county's water facilities had been affected. We noted that only 1 patient with AK resided in 1 of the 48 counties in which the water supplies were not affected (**Figure 2**). The incidence of AK was 10 times higher in the affected counties (1.43 per 100 000) than in the unaffected counties (0.13 per 100 000), a relative risk of 10.83 (95% CI = 1.48-79.49; $P<.003$).

To determine whether this purported risk was confounded by differences in the UIHC referral patterns, we classified counties as "low-referral" or "high-referral" counties based on the median number of UIHC visits per 100 population. Twenty-five (51%) of the 49 counties in the low-referral category had water supplies affected by the flood, nearly identical to the 26 counties (52%) in the high-referral category. Six cases of AK were diagnosed in patients from low-referral counties, all 6 from counties in which water facilities were affected. The remaining cases were diagnosed in patients from high-referral counties, all but 1 of whom were from counties with affected water facilities. The fact that the relative risk of developing AK if one lived in a high-referral, flood-impacted county (RR = 8.35) was less than the relative risk for all flooded counties (RR = 10.83) suggests that more cases originated from the low-referral, flood-impacted counties than would be anticipated by our referral patterns.

COMMENT

We describe an epidemic of keratitis in which the clinical, pathologic, and epidemiological evidence supports the diagnosis of AK. Although we were unable to culture *Acanthamoeba* from clinical specimens, we have no alternative laboratory data to suggest that these cases were caused by infection with herpes simplex virus, fungus, or bacteria. Eighty-four percent of cases had failed initial therapy for viral or bacterial keratitis. Furthermore, 29 of 31 patients described responded clinically to the administration of anti-amoebic medications, which when withdrawn, often resulted in recurrence of symptoms. Although multiple courses of treatment were often required, symptoms and TSCM findings resolved completely in 22 patients. There are several potential explanations for our failure to culture *Acanthamoeba* from clinical specimens: (1) the infections were caused by a new species with different growth requirements, (2) the inoculum was insufficient, (3) an inhibitor was present, (4) the organisms were present but nonviable, or (5) the infections were caused by an organism other than *Acanthamoeba*. For the latter to be true, however, both TSCM and histopathologic findings would have to have been misinterpreted.

There are several possible explanations for this outbreak. One explanation is that we identified a true epidemic that was caused by exposure to contaminated water. All cases but 1 came from counties in which the municipal water supplies were affected by the flood, a fact that was not explained by our hospital referral patterns. Aggregate estimates such as these are subject to ecological fallacy bias, which holds that individuals in these counties may not themselves have been exposed to the contaminated water. However, the findings of our case-control study strengthen the evidence for the role of contaminated water in this outbreak. In the multivariate analysis, fishing and living in a household in which the water came from the municipal water system were independent risk factors for keratitis. This seems plausible because rivers and other surface water sources, which can be easily contaminated during a flood, serve not only as sites of recreational activities but as sources of water for many cities.

In our study, fewer patients wore contact lenses than in previous studies,[12-14] but lens use was still the single best predictor of keratitis. Contact lens use can predispose to keratitis by disrupting corneal integrity,[2] and when ubiquitous pathogens like *Acanthamoeba* are present, infection may result. Because not all *Acanthamoeba* isolates produce severe disease,[22] perhaps there was a shift in the distribution of virulent strains after the flood. Our failure to find associations for some of the other study variables may in part be because of our small study size, which was limited by the number of incident cases.

To evaluate a large number of potential risk factors, controls were matched to cases only by age and date of visit to the clinic. The potential for selection bias in choosing controls is acknowledged, particularly because those patients who were attending the clinic for conditions other than AK may not be representative of the population as a whole in Iowa. Given the number of potential risk factors evaluated and tests of significance performed, it is also possible that some of the variables we found to be significant might be because of chance. Recognizing these potential limitations, we present our final multivariate model that was constructed using only 4 variables appropriately commensurate to our sample size. It proved to be highly statistically significant, plausible, and consistent with our ecological analysis.

One final explanation for the presumed epidemic is that the sharp increase in AK cases that we report may be the result of the coincidental introduction of TSCM at our hospital. Perhaps by screening patients with keratitis early with TSCM, some cases that would have gone unrecognized in previous years are now being diagnosed. However, if this were true and recognition bias was the sole cause of this dramatic increase, we would expect that we would diagnose a similar number of cases over time in contrast to the abrupt rise and gradual decrease that we observed. Future studies of flood-related causes of keratitis may confirm our observations.

Accepted for publication April 24, 1998.

The views expressed in this article are those of the authors and do not reflect the official policy of the US Department of Defense or other departments of the US Government.

We thank Dennis Alt, Department of Natural Resources, Des Moines, for sharing his data on the impact of the flood of 1993 on water supplies in the state of Iowa. We especially thank those patients, both cases and controls, who donated their valuable time for this research project.

Corresponding author: Patricia A. Meier, MD, MSc, Hospital Epidemiologist (CMI), Wilford Hall Medical Center, 2200 Bergquist Dr, Suite 1, Lackland AFB, TX 78236-5300 (e-mail: meier@whmc-lafb.af.mil).

REFERENCES

1. Mader TH, Stulting RD. Viral keratitis. *Infect Dis Clin North Am.* 1992;6:831-850.
2. Liesegang TJ. Bacterial keratitis. *Infect Dis Clin North Am.* 1992;6:815-829.
3. Poggio EC, Glynn RJ, Schein OD, et al. The incidence of ulcerative keratitis among users of daily-wear and extended-wear soft contact lenses. *N Engl J Med.* 1989; 321:779-783.
4. Visvesvara GS. Free-living pathogenic amoeba. In: Lennette EH, ed. *Manual of Clinical Microbiology.* Washington, DC: American Society for Microbiology; 1980: 704-708.
5. Ma P, Visvesvara GS, Martinea AJ, Theodore FH, Daggett PM, Sawyer TK. Naegleria and *Acanthamoeba* infections: review. *Rev Infect Dis.* 1990;12:490-513.
6. Wilhelmus KR. Introduction: the increasing importance of *Acanthamoeba*. *Rev Infect Dis.* 1991;13(suppl 5):367-368.
7. Bacon AS, Frazer DG, Dart JK, Matheson M, Ficker LA, Wright P. A review of 72 consecutive cases of *Acanthamoeba* keratitis, 1984-1992. *Eye.* 1993;7(pt 6): 719-725.
8. Garner A. Pathogenesis of acanthamoebic keratitis: hypothesis based on histologic analysis of 30 cases. *Br J Ophthalmol.* 1993;77:366-370.
9. Dougherty PJ, Binder PS, Mondino BJ, Glasgow BJ. *Acanthamoeba* sclerokeratitis. *Am J Ophthalmol.* 1994;117:475-479.
10. Witschel H, Sundmacher R, Seitz HM. Amebic keratitis: clinico-histopathologic case report. *Klin Monatsbl Augenheilkd.* 1984;185:46-49.
11. Moore MB, McCulley JP, Luckenbach M, et al. *Acanthamoeba* keratitis associated with soft contact lenses. *Am J Ophthalmol.* 1985;100:396-403.
12. Centers for Disease Control and Prevention. *Acanthamoeba* keratitis associated with contact lenses—United States. *MMWR Morb Mortal Wkly Rep.* 1986;35: 405-408.
13. Stehr-Green JK, Bailey TM, Brandt FH, Carr JH, Bond WW, Visvesvara GS. *Acanthamoeba* keratitis in soft contact lens wearers. *JAMA.* 1987;258:57-60.
14. Stehr-Green JK, Bailey TM, Visvesvara GS. The epidemiology of *Acanthamoeba* keratitis in the United States. *Am J Ophthalmol.* 1989;107:331-336.
15. Mathers WD, Sutphin JE, Folberg R, Meier PA, Wenzel RP, Elgin RG. Outbreak of keratitis presumed to be due to *Acanthamoeba. Am J Ophthalmol.* 1996;121: 129-142.
16. Winchester K, Mathers WD, Sutphin JE, Daley TE. Diagnosis of *Acanthamoeba* keratitis in vivo with confocal microscopy. *Cornea.* 1995;14:10-17.
17. Auran JD, Starr MB, Koester CJ, LaBombardi VJ. In vivo scanning slit confocal microscopy of *Acanthamoeba* keratitis. *Cornea.* 1994;13:183-185.
18. Cavanagh HD, Petroll WM, Alizadeh HA. Clinical and diagnostic use of in vivo confocal microscopy in patients with corneal disease. *Ophthalmology.* 1993; 100:1444-1454.
19. Fritsche TR, Gautom RK, Seyedirashti S, Bergeron DL, Lindquist TD. Occurrence of bacterial endosymbionts in *Acanthamoeba* spp. isolated from corneal and environmental specimens and contact lenses. *J Clin Microbiol.* 1993;31: 1122-1126.
20. Bottone EJ. Free-living amebas of the genera *Acanthamoeba* and *Naegleria*: an overview and basic microbiologic correlates. *Mt Sinai J Med.* 1993;60:260-270.
21. Rowbotham TJ. Preliminary report on the pathogenecity of *Legionella* pneumophila for freshwater and soil amoebae. *J Clin Pathol.* 1980;33:1179-1183.
22. van Klink KF, Alizadeh H, Stewart GL, et al. Characterization and pathogenic potential of a soil isolate and an ocular isolate of *Acanthamoeba* castellanii in relation to *Acanthamoeba* keratitis. *Curr Eye Res.* 1992;11:1207-1220.