APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1995, p. 1208–1213 Vol. 61, No. 4
0099-2240/95/$04.00+0

Legionella Contamination of Dental-Unit Waters

RONALD M. ATLAS,[1]* JEFFREY F. WILLIAMS,[2] AND MARK K. HUNTINGTON[2]

Department of Biology, University of Louisville, Louisville, Kentucky 40292,[1] and Department of Microbiology, Michigan State University, East Lansing, Michigan 48824[2]

Received 3 November 1994/Accepted 9 January 1995

Water samples collected from 28 dental facilities in six U.S. states were examined for the presence of *Legionella pneumophila* and other *Legionella* spp. by the PCR-gene probe, fluorescent-antibody microscopic, and viable-plate-count detection methods. The PCR and fluorescent-antibody detection methods, which detect both viable and viable nonculturable *Legionella* spp., gave higher counts and rates of detection than the plate count method. By the PCR-gene probe detection method, *Legionella* spp. were detected in 68% of the dental-unit water samples and *L. pneumophila* was detected in 8%. Concentrations of *Legionella* spp. in dental-unit water reached 1,000 organisms per ml or more in 36% of the samples, and 19% of the samples were in the category of 10,000/ml or above. *L. pneumophila*, when present in dental-unit water, never reached concentrations of 1,000/ml or more. Microscopic examination with fluorescent-antibody staining indicated that the contamination was in the dental-unit water lines rather than in the handpieces. *Legionella* spp. were present in 61% of potable water samples collected for comparative analysis from domestic and institutional faucets and drinking fountains; this percentage was not significantly different from the rate of detection of *Legionella* spp. in dental-unit water. However, in only 4% of the potable water samples did *Legionella* spp. reach concentrations of 1,000 organisms per ml, and none was in the 10,000 organisms-per-ml category, and so health-threatening levels of *Legionella* spp. in potable water were significantly lower than in dental-unit water. *L. pneumophila* was found in 2% of the potable water samples, but only at the lowest detectable level. The results demonstrate that high concentrations of *Legionella* spp. frequently develop in dental-unit water lines. They suggest that, although *L. pneumophila* is not the dominant component in dental-unit water, heavy exposure to species of *Legionella* should be investigated as a potential health risk for dental personnel and their immunocompromised patients.

Several studies have indicated that dentists and dental staff have higher rates of respiratory infections than the general public (4, 6, 29). Contaminated handpieces are believed to be at least partially responsible for these higher rates of respiratory disease (15). Appropriate procedures to decontaminate handpieces, including autoclaving and handpiece replacement between patients, have been developed and implemented in many dental practices (1, 22, 24, 29). These procedures are aimed at reducing the likelihood of aerosol dissemination of pathogens within dental operatories and the resulting infections. However, decontamination of handpieces such as high-speed drills and syringes does not remove the potential for exposure to pathogens that originate within the water lines of dental units.

It has been suggested that *Legionella* spp. within dental lines may contribute to respiratory illnesses among dentists and dental staff (24). Higher rates of seropositivity for *Legionella* antibodies have been found among dental personnel than among the general public (10, 25, 28), suggesting that aerosols generated in dental operatories are a source of exposure to *Legionella* spp. Water-cooled, high-speed handpieces generate stable aerosols that may contain *Legionella* spp. (1). The complex design of dental-chair equipment results in the stagnation of water within the water lines, where bacteria, including *Legionella* spp., can proliferate within a biofilm (22).

Legionella pneumophila and other *Legionella* spp. have occasionally been isolated from dental operatories (23, 27). *Legionella* spp., however, often are difficult to isolate because of overgrowth by other microorganisms and because *Legionella* spp. often are found within protozoa; detection of *Legionella* spp. by viable-culture methods frequently gives variable results even from sources believed to be responsible for disease outbreaks (19). Therefore, direct fluorescent-antibody detection and PCR detection of *Legionella* spp. have been recommended for epidemiological investigations (19); these approaches proved valuable, for example, when standard culture techniques were unsuccessful in tracking the source of a 1992 outbreak of Pontiac fever in the United States to a resort hot tub (19).

In the present study, we determined the prevalence of *L. pneumophila* and other *Legionella* spp. in dental-unit water samples compared with that in potable water samples, using a detection system based upon the PCR-gene probe detection procedure described by Mahbubani et al. (14). We also used direct fluorescence microscopy to confirm the presence of *Legionella* spp. The PCR method detects all viable cells of the genus *Legionella*, including viable nonculturable organisms that are not detected by conventional selective cultivation. It also identifies the presence of *L. pneumophila* and permits a semiquantitative evaluation of the intensity of contamination.

MATERIALS AND METHODS

Samples. A total of 265 dental-unit water samples were collected from 28 dental facilities in California, Massachusetts, Michigan, Minnesota, Oregon, and Washington. Included in the study were eight institutional clinics and 20 private practices. Samples included water from high-speed drill handpiece lines, dental-syringe lines, and scaler lines. For comparison, 126 potable water specimens were also collected through convenience sampling of domestic and institutional facilities and water fountains readily accessible to the participants in Kentucky, Michigan, and California. Generally, 50- to 100-ml water samples were collected in sterile containers and shipped on ice to the laboratories in Louisville, Ky., or East Lansing, Mich. Locally collected specimens were brought directly to the laboratory and stored on ice before processing.

* Corresponding author. Mailing address: Department of Biology, University of Louisville, Louisville, KY 40292. Phone: (502) 852-8962. Fax: (502) 852-0725. Electronic mail address: rmatla01@ulkyvm.louisville.edu.

Additional samples were collected from the home and office of a dentist who died of legionellosis. A formalin-preserved portion of lung tissue collected on autopsy from the deceased dentist was also analyzed.

PCR detection of *Legionella* spp. Each water sample was filtered through a Durapore filter to trap bacterial cells. The trapped bacteria were lysed either by boiling in Chelex resin or by treatment with EnviroAmp lysing reagent (Perkin-Elmer-Roche Molecular Systems, Nutley, N.J.). An aliquot of the sample was transferred to a reaction vessel for amplification of the diagnostic gene sequences. The EnviroAmp detection kit was used for PCR amplification and gene probe detection. The kit includes PCR buffer, *Taq* DNA polymerase, and biotinylated primers for amplification of a genus-specific region of the *Legionella* 5S rRNA gene and of the *L. pneumophila mip* gene. Perkin-Elmer Cetus (Norwalk, Conn.) or Quarterbath (Inotech Biosystems, Inc., Lansing, Mich.) thermal cyclers were used for amplification with a 30-cycle program of 0.5 min at 95°C for denaturation and 1 min at 63°C for primer annealing and DNA extension.

The PCR-amplified 5S rRNA and *mip* DNA sequences were detected by reverse dot blot strip analysis with an immobilized probe. Specific probes complementary to internal sequences of the amplified regions are immobilized on nylon membrane strips in the detection kit. Biotinylated PCR products generated by amplification with biotinylated primers are specifically hybridized to the immobilized probes. After stringent washing of the strips, the presence of hybridized biotinylated PCR products is detected by incubating the strips with a streptavidin-horseradish peroxidase conjugate, washing them, and adding to them the substrate for horseradish peroxidase. A blue dot appearing on the nylon membrane indicates the presence of bound PCR product. The entire PCR detection process, including sample processing, DNA amplification, and hybridization detection, takes about 3 h.

An internal positive control (IPC) is included as a means for detecting poor amplification or hybridization. The IPC is a synthetic DNA sequence that is coamplified by the primers for the *mip* gene, and its template is included in the *Legionella* PCR mixture. When the IPC failed to yield the positive blue dot indicating poor amplification, such as may be caused by the presence of inhibitors of PCR in the environmental specimen, the sample was diluted and the PCR DNA amplification was repeated.

An internal negative control is also included as a means of ensuring appropriate hybridization stringency. The positive control probe is perfectly complementary to a sequence in the IPC amplification product. The negative control probe has a 1-bp mismatch with this sequence. When the hybridization reactions are done correctly, the PCR product generated from amplification of the IPC will hybridize with the positive control probe but not with the negative control probe. The hybridization conditions have been optimized in this system to be stringent enough to allow detection of a 1-bp mismatch between the probe and a PCR product. The positive control also provides a semiquantitative basis for estimating the number of *Legionella* cells in the sample. The IPC corresponds to 1,000 copies of the *mip* gene sequence, and thus the intensity of the sample hybridization signal can be graded on a scale to determine the relative number of *Legionella* cells.

Fluorescent-antibody detection of *Legionella* spp. Seventy samples (30 from dental offices and 40 from domestic potable waters) that were tested by PCR were also examined for the presence of *Legionella* spp. by fluorescent-antibody staining. For fluorescent-antibody staining, 10-ml water samples were filtered through 0.2-μm-pore-size black Nuclepore filters. Five milliliters of 0.05% formalin was passed through the filters to fix and preserve the cells. Cells collected on replicate filters were examined by epifluorescence microscopy after indirect polyclonal fluorescent-antibody staining for *Legionella* spp. with a commercial kit (Merifluor-Meridian Diagnostics) and monovalent fluorescent-antibody staining for specific *Legionella* spp. with a commercial kit (Mardx Diagnostics, Carlsbad, Calif.). Also, formalin-preserved lung tissue from a California dentist who died of Legionnaires' disease was examined by fluorescence-antibody staining. The flexible dental-unit water lines from this dentist's operatory also were sectioned, and the biofilms were stained with fluorescent antibody to detect the presence of *Legionella* spp.

Viable-plate-count detection of *Legionella* spp. The same 70 water samples examined by fluorescent-antibody testing were also tested by viable-plate-count methods for the detection of *Legionella* spp. For viable-culture detection, 100-ml water samples were concentrated on 0.2-μm-pore-size polycarbonate filters. The cells were resuspended in 10 ml of the original sample; 1 ml of the suspension was treated with HCl for 15 min, neutralized with KOH, and plated on buffered charcoal-yeast extract-α medium by following the procedure recommended by the Centers for Disease Control and Prevention for the detection of viable *Legionella* spp. in potable waters (5). The plates were incubated at 35°C for up to 7 days, and colonies typical of *Legionella* spp. were counted.

RESULTS

Legionella spp. were detected by the PCR amplification-DNA gene probe method in 68% of the dental-unit water samples (Fig. 1), and *L. pneumophila* was detected in 8%. Of the 207 dental-unit water samples from California, Michigan, Oregon, and Washington, 18% contained concentrations of *Legionella* spp. of 1,000 to 10,000 organisms per ml, whereas of 122 domestic potable water samples from California, Kentucky, and Michigan, only 4% contained concentrations of *Legionella* spp. of 1,000 to 10,000 organisms per ml (Table 1). Sixty-one percent of domestic potable water samples had detectable levels of *Legionella* spp. (Fig. 1), but only 2% of domestic potable water samples tested positive for *L. pneumophila*. Moreover, only 4% of the 126 domestic potable water specimens had 1,000 or more *Legionella* organisms per ml. *L. pneumophila*, when present, was in the range of the lowest detectable concentrations. None of the domestic potable water samples contained concentrations of *Legionella* spp. of ≥10,000 organisms per ml; however, 19% of the dental-unit waters examined were in the category of ≥10,000 organisms per ml. None of the domestic potable water or dental-office water samples had concentrations of *L. pneumophila* of ≥1,000 organisms per ml. Overall, *Legionella*-positive samples were detected in collections from seven of eight institutional dental clinics and 16 of 20 private dental offices. *L. pneumophila*-positive samples came from four institutions and seven private offices.

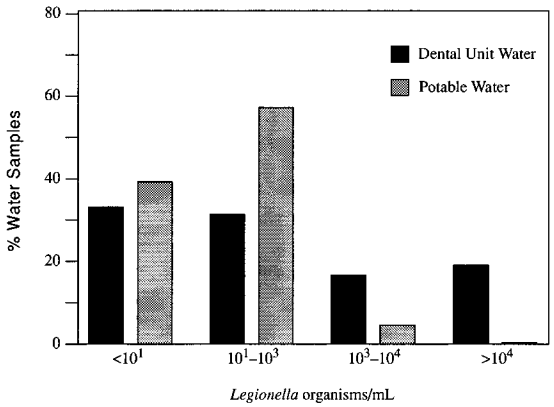

FIG. 1. PCR-DNA probe test results for *Legionella* spp. in 265 dental-unit water samples and 126 potable water samples.

The percentage of samples positive for *Legionella* spp. at different dental sites was highly variable (Table 2). For example, of 10 locations around Seattle, Wash., and Portland, Oreg., 8 provided *Legionella*-positive dental-unit water samples, with 4 of these sites showing evidence of *L. pneumophila* (Table 2). The proportion of *Legionella*-positive samples at each site ranged from 0 to 100%. None of 40 samples from two large teaching institutions was positive for *L. pneumophila*, whereas 8 of 19 from another school were positive, several at high levels (≥1,000 organisms per ml).

TABLE 1. Concentrations of *Legionella* spp. and *L. pneumophila* in water from dental offices and domestic sources

Concn (no. of organisms/ml)	% of samples positive for organism(s)			
	Dental office water		Domestic potable water	
	Legionella spp.	*L. pneumophila*	*Legionella* spp.	*L. pneumophila*
<1	36	94	39	98
1–100	15	3	38	2
100–1,000	14	3	19	0
1,000–10,000	18	0	4	0
>10,000	17	0	0	0

TABLE 2. *Legionella* spp. and *L. pneumophila* in dental-unit water[a]

Site no.	No. of samples tested	No. positive for *Legionella* spp.	No. positive for *L. pneumophila*
1	9	2	1
2	28	19	2
3	12	0	0
4	6	4	0
5	6	6	1
6	6	0	0
7	12	12	0
8	6	6	2
9	4	4	0
10	3	2	0

[a] Samples collected from 10 locations in Portland, Oreg., and Seattle, Wash.

There was no significant difference in the rates of detection of *Legionella* spp. in water samples collected from various dental instruments (determined for the first 100 samples collected in this study), as detected by the PCR amplification-gene probe method, which is consistent with the theory suggesting dental-unit water lines as the source of *Legionella* contamination (Table 3). Approximately two-thirds of all the specimens collected came from dental air or water syringe lines, with most of the remainder collected from high-speed handpiece lines. Thirty samples from scalers were processed. The samples from lines to high-speed dental handpieces were positive only slightly less frequently than those from lines to syringes (rates of positivity for both, >50%). Although the number of scaler line samples was much lower, the positivity rate was disproportionately high (85%).

Microscopic observation of both potable and dental-unit waters by direct epifluorescent microscopy revealed numbers of *Legionella* spp. in the same range as those estimated by PCR (Fig. 2). The direct counts were somewhat higher than the estimations by PCR, indicating that (i) there may have been some antibody cross-reactivity with non-*Legionella* spp., (ii) the fluorescent-antibody counts may have included some dead bacteria not detected by PCR, or (iii) the PCR may have slightly underestimated the numbers of *Legionella* spp. The viable plate counts were lower than the numbers estimated by PCR detection and the direct counts of fluorescent-antibody-stained samples. (Note that the higher percentage of samples with fewer than 10 cells of *Legionella* spp. detected by viable counting, as shown in Fig. 2, reflects the fact that a lower percentage of samples with higher numbers of *Legionella* organisms was detected by this method than by the other detection methods.) In several cases the plate count procedure failed to detect any *Legionella* spp., even when the PCR and fluorescent-antibody procedures detected high concentrations (>1,000 organisms per ml) of *Legionella* spp. Both PCR and direct fluorescent counts can detect viable nonculturable bacteria which are not counted by plating procedures. *Legionella* spp. commonly form such viable nonculturable cells (11), and it is likely that they contributed to the difference between plate count results and

TABLE 3. *Legionella* spp. and *L. pneumophila* in water from various dental instruments

Instrument	No. of samples tested	No. positive for *Legionella* spp.	No. positive for *L. pneumophila*
High-speed drill	46	20	2
Syringe	48	29	4
Other	6	5	0

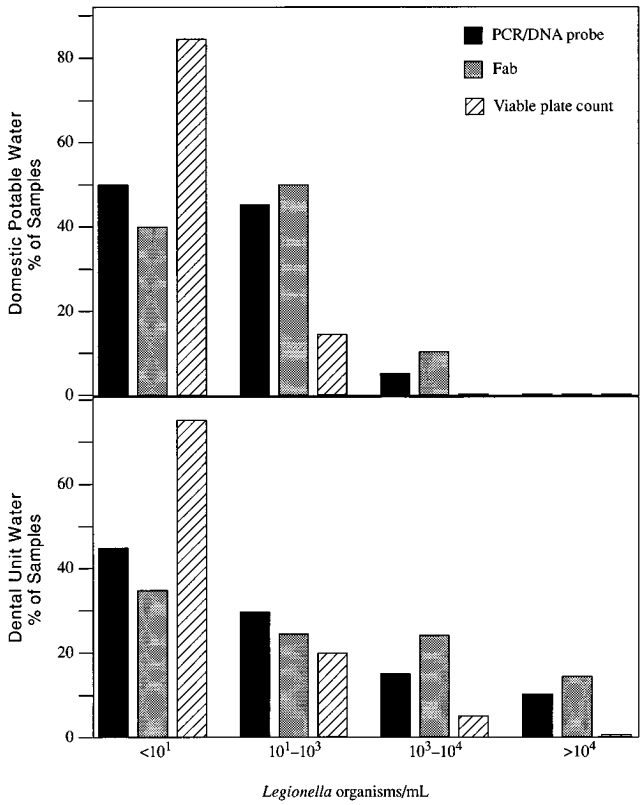

FIG. 2. Comparison of PCR-DNA probe, fluorescent-antibody (Fab), and viable-plate-count methods for detecting *Legionella* spp. in 30 dental-unit water samples and 40 domestic potable water samples.

those of PCR and fluorescent-antibody detection. Also, plate counts of *Legionella* spp. often underestimate the presence of *Legionella* spp. in water samples because of interference by other heterotrophic bacteria or because the *Legionella* spp. are within protozoa. Many of the *Legionella* spp. observed by fluorescence microscopy appeared to be within or on the surfaces of protozoa in the biofilm lining the flexible thin lines used in the dental-unit water systems. (In such cases, the protozoa fluoresced slightly and the legionellae were in clusters and fluoresced brightly.) No special plating procedures were used in this study to recover viable *Legionella* spp. from protozoa.

With regard to the water samples obtained from the dental operatory of the California dentist who died of pneumonic legionellosis, high levels of *Legionella* spp. (>10,000 organisms per ml) were detected by both PCR and fluorescent-antibody methods; only low levels (1 to 100 organisms per ml) were detected by viable-count procedures. Only low levels (<100 organisms per ml) of *Legionella* spp. were detected in potable water samples from his home by all three detection procedures. Monovalent fluorescent-antibody staining showed the presence of *L. dumoffii*, *L. pneumophila*, and *L. longbeachae* in both the formalin-preserved lung tissue obtained on the dentist's autopsy and the water samples from his dental operatory. *L. pneumophila* serogroup 1 (SG1) was present in only very low numbers in both the lung tissue and water samples. *L. longbeachae* was the dominant fluorescing *Legionella* sp. in the lung tissue, although it had not been cultured from the patient prior to his death; it was not the dominant *Legionella* sp. in the water samples from his dental operatory. *L. dumoffii* was identified in both the lung tissue and the water samples by the same mono-

valent fluorescent-antibody staining procedure as had been used by the clinical laboratory to identify the culture obtained from the patient prior to his death. *L. dumoffii* organisms were especially abundant within protozoa in the biofilms of the dental-unit water lines of his dental operatory.

DISCUSSION

Legionella species are ubiquitous in aquatic environments, including cooling towers and potable water supplies (7, 9, 30). *Legionella* species are causative agents of *Legionella* pneumonia (Legionnaires' disease) and nonpneumonic legionellosis (Pontiac fever) (17, 32). Infection most often is initiated through inhalation by susceptible individuals of aerosols containing high levels of *Legionella* spp. (7, 9, 30); exposure to low numbers of *Legionella* organisms generally is not viewed as a health risk for immunocompetent individuals (32). From the first clinically recognized outbreak of Legionnaires' disease in Philadelphia in 1976, air-conditioning cooling towers have been implicated most often as the source of infection (7, 17). Cooling towers are monitored and biocides are added to reduce levels of *Legionella* species (20). While air-conditioning cooling towers have been considered the likeliest sources of heavy exposure, potable water supplies, hospital showerheads, and even vegetable moisturizers in produce markets have been implicated in some outbreaks of disease due to infections with *Legionella* spp. (2, 7, 12, 30, 32).

Dental-unit water is a potential source of exposure to *Legionella* species, especially since dental instruments form aerosols (31). A longitudinal study that examined 20 dental offices with 53 units for hygiene-related problems in dental practices in Dresden, Germany, found that viable (culturable) *Legionella* species could only sporadically be detected; only 40% of the dental-unit and potable water samples examined yielded culturable *Legionella* spp. (3). A separate examination of 42 dental units in 35 dental practices in Germany found *L. pneumophila* SG1 in only 9% of the dental units examined (27). While culturable *Legionella* spp. were only sporadically detected, serological surveillance of a dental staff and a control group in Dresden, Germany, showed an elevated level of anti-*L. pneumophila* SG6 antibodies among dentists (13), particularly among those working in dental operatories where *L. pneumophila* SG6 was isolated, suggesting that the dental-unit water aerosols were the source of exposure.

Similarly, examination of serum samples from 107 dentists, dental assistants, and dental technicians in Austria by an indirect immunofluorescence test for antibodies to *L. pneumophila* SG1 to SG6, *L. micdadei*, *L. bozemanii*, *L. dumoffii*, *L. gormanii*, *L. jordanis*, and *L. longbeachae* SG1 and SG2 revealed a significantly higher rate of seropositivity to *Legionella* antigens than occurs in the general population (28). There was a strong correlation between *Legionella*-seropositive individuals and the extent to which those individuals were exposed to aerosols from high-speed drills and dental syringes. Thirty-four percent (36 employees from 13 dental practices) of the sample group showed a positive reaction for antibodies to *L. pneumophila*, compared with only 5% testing positive in a control group of nonmedical workers. Of the 36 positive serum samples, 13 (36%) reacted with SG6, 12 (33%) with SG1, 12 (33%) with SG5, and 3 (8%) with SG4; 8 samples were positive for antibodies to other *Legionella* species. Among the sample population, dentists had the highest prevalence (50%) of *L. pneumophila* antibodies, followed by dental assistants (38%) and technicians (20%). In an analogous study in the United States, 20% of the students and employees at a dental clinic in West Virginia were seropositive for *Legionella* antibodies (10). These results suggest that dental personnel are at an increased risk of *Legionella* exposure and suggest that the water lines of the dental practice are the likely source.

Our findings, obtained by PCR detection to estimate concentrations of *Legionella* spp. (14), indicate that high levels of *Legionella* contamination of water may be encountered in dental operatories. The actual concentration of *Legionella* spp., however, varied from one dental operatory to another. Similarly, the rates of isolation of *Legionella* spp. and *L. pneumophila* serotypes in dental-unit water samples studied at the London University Clinic were quite variable (23), suggesting that the microbial ecological conditions in dental lines may fluctuate and affect *Legionella* populations.

Compared with contamination of domestic potable waters, the extent of the *Legionella* contamination of dental-unit water samples was much higher. Our results point to aerosols generated from water within dental operatories as the source of exposure responsible for the elevated seropositivity reported for dental staff both in Europe (28) and in the United States (10). The levels found in dental-unit water were higher than have been recorded previously; this may be a reflection of the performance characteristics of the PCR-DNA detection system relative to those of conventional cultivation procedures (14, 30). Given the greater sensitivity of the PCR-DNA probe detection method (14), it appears that low counts (in the $<$10,000/ml range) may prove clinically significant only for individuals whose exposure to the aerosol occurs over prolonged periods, for example, for dental office personnel or immunocompromised individuals.

The higher concentrations of *Legionella* spp. detected by PCR than by viable-plate-count methods also raise the question as to whether nonviable *Legionella* spp. that have no clinical significance are detected by the PCR method. Relatively short amplicons, as in the EnviroAmp detection system, can be used to detect nonviable *Legionella* spp. (16). Detection of *Legionella* spp. by direct fluorescent-antibody detection gave results similar to those by PCR detection; both methods gave results that were higher than those obtained by viable-culture methods, and both could detect nonviable rather than exclusively viable *Legionella* spp. The viable-culture methods for *Legionella* sp. detection, however, often fail, and the Centers for Disease Control and Prevention has turned to PCR for epidemiological investigations of Legionnaires' disease and Pontiac fever (19). Furthermore, viable nonculturable *Legionella* spp. have been shown to be capable of causing pneumonic legionellosis (6); exposure to high concentrations of viable nonculturable *Legionella* spp. may also be an important cause of Pontiac fever (19).

The high prevalence of heavy contamination with *Legionella* spp. in dental-unit water samples may be a reflection of the rich microbial biofilms commonly present along the length of the fine-bore dental water hoses (31). In the survey of *Legionella* spp. in dental operatories in Britain (23), *Legionella* organisms appeared to be growing within biofilm in the dental-unit water supply. No variability among *Legionella* spp. isolated from dental units in Germany over a 3-year period was found by using monoclonal antibodies or pulsed-field gel electrophoresis analysis, suggesting that stable populations of *Legionella* spp. can be maintained within biofilms in the water lines of the dental units (13). *Legionella* spp. often are found within protozoa in biofilms. Almost all samples in a survey of protozoal infections in dental-unit water in Germany (18) contained *Naegleria* spp. or other amebae which might serve as host cells for intracellular proliferation of *Legionella* spp.; amebae have also been seen frequently in dental-unit biofilms in the United States (31). Virtually all dental-unit water samples examined in our

study contained significant concentrations of protozoan species that can harbor *Legionella* spp. (unpublished observations). Fluorescent cells of *Legionella* spp. were observed in biofilms examined in the present study, especially within protozoa in the biofilms.

In view of the extent of exposure of patients and staff to this source of *Legionella* organisms, it is surprising that no definitive clinical associations have thus far emerged. There were no related cases of human infection detected in two studies at dental institutions in Britain where *Legionella* spp. were isolated from dental-unit water (21, 23). There may be several reasons for the lack of association of dental-unit waters and occurrences of Legionnaires' disease. The sources of most cases of community-acquired pneumonic legionellosis are never identified (30), so the potential implication of dental exposure may represent a hitherto-unrecognized element of the medical history of a proportion of current clinical cases. Nonpneumonic legionellosis of the Pontiac fever type may occur in dental personnel or their patients and cause seroconversion but may be indistinguishable clinically from other flu-like episodes experienced by the general population. *Legionella* spp. vary with regard to their virulence; even though 18 of the 30 species of *Legionella* have been implicated in human disease (8), the Centers for Disease Control and Prevention estimates that *L. pneumophila*, which has a macrophage infectivity potentiator that contributes to its virulence, is the causative agent for 85% of all cases of *Legionella* pneumonia in the United States (26). On the basis of guinea pig infectivity studies, it has been suggested that the strains commonly present in dental-unit water may have limited invasive capacity (13). Most *Legionella* spp. detected in our study were not serogroups of *L. pneumophila*, which would be expected to be less virulent than *L. pneumophila* (32) because they lack the macrophage infectivity potentiator gene of *L. pneumophila* which is involved in cell invasion and subsequent intracellular growth. While *L. pneumophila* was present at a higher rate than in potable water supplies, concentrations of *L. pneumophila* generally were much lower than those of the predominant *Legionella* spp. and *L. pneumophila* was detected at levels below those normally considered to present a health risk for immunocompetent individuals.

We have been investigating the death of a California dentist from legionellosis (unpublished data). In his case a culture was obtained and identified with monovalent antibodies as *L. dumoffii*. Subsequent to the dentist's death, we examined water samples from his dental operatory and home for *Legionella* spp. by the PCR assay and found high levels (>10,000 organisms per ml) in the dental units and low levels (<100 organisms per ml) in his domestic water. Using polyvalent anti-*Legionella* sp. fluorescent antibodies, we also confirmed the presence of high levels of *Legionella* spp. in dental-unit water samples from his operatory. We further detected cells that stained with monovalent anti-*L. dumoffii* fluorescent antibodies in those water lines and in the formalin-preserved lung tissue collected during the autopsy of the dentist; the lung tissue also contained *L. pneumophila* and *L. longbeachae*, which were also found in water samples from his dental operatory. In the absence of cultivation and species identification it is difficult to ascribe the case described here definitively to dental-unit water line exposure to *Legionella* species. Nevertheless, in view of the high levels of *Legionella* spp. in the dental-unit water lines of the deceased dentist's operatory, it is likely that aerosols from those dental units were the source of the fatal *Legionella* infection.

Regardless of the lack of specific clinical association, exploration of possible preventive measures against *Legionella* spp. and other opportunistic waterborne pathogens (31) in the dental health care setting would be prudent. Preventive strategies increasingly adopted by hospitals faced with nosocomial legionellosis outbreaks have ranged from chemical disinfection to steam sterilization to rid water lines of contaminating biofilm. Hyperchlorination and charcoal filtration have been shown to be ineffective in controlling *Legionella* sp. contamination of dental lines (23), but the application of other measures based on microfiltration or other germicidal flushes would clearly be appropriate.

ACKNOWLEDGMENTS

We thank Martin Boyce and Shawn McCarty for expert technical assistance and the many dental practitioners and hygienists who were kind enough to collect the samples used in this study.

REFERENCES

1. **Abel, L. C., R. L. Miller, R. E. Micik, and G. Gyge.** 1971. Studies on dental aerobiology. IV. Bacterial contamination of water delivered by dental units. J. Dent. Res. **50:**1567–1569.
2. **Bollin, G. E., J. F. Plouffe, M. F. Para, and B. Hackman.** 1985. Aerosols containing *Legionella pneumophila* generated by shower heads and hot-water faucets. Appl. Environ. Microbiol. **50:**1128–1131.
3. **Borneff, M.** 1986. Hygiene problems in dental practice with special attention to dental units. Zentralbl. Bakteriol. Mikrobiol. Hyg. B **183:**130–152.
4. **Burton, W. E., and R. L. Miller.** 1963. The role of aerobiology in dentistry, p. 87–94. *In* First International Symposium on Aerobiology. Naval Biology Laboratory, Naval Supply Center, University of California, Berkeley.
5. **Centers for Disease Control and Prevention.** 1992. Procedures for the recovery of *Legionella* from the environment. Centers for Disease Control and Prevention, Atlanta.
6. **Cuthbertson, W. C.** 1954. Causes of death among dentists: a comparison with the general male population. J. Calif. Nev. State Dent. Soc. **30:**159–160.
7. **Dondero, T. J., R. C. Rendtorff, and G. F. Mallison.** 1980. An outbreak of Legionnaires' disease associated with a contaminated air-conditioning cooling tower. N. Engl. J. Med. **302:**365.
8. **Fang, G. D., V. L. Yu, and R. M. Vickers.** 1989. Disease due to the legionellaceae (other than *Legionella pneumophila*). Medicine (Baltimore) **68:** 116–132.
9. **Fliermans, C. B., W. B. Cherry, L. H. Orrison, S. J. Smith, D. L. Tison, and D. H. Pope.** 1981. Ecological distribution of *Legionella pneumophila*. Appl. Environ. Microbiol. **41:**9–16.
10. **Fotos, P. G., H. N. Westfall, I. S. Snyder, R. W. Miller, and B. M. Mutchler.** 1985. Prevalence of *Legionella*-specific IgG and IgM antibody in a dental clinic population. J. Dent. Res. **64:**382–385.
11. **Hussong, D., R. R. Colwell, M. O'Brien, et al.** 1987. Viable *Legionella pneumophila* not detectable by culture on agar media. Bio/Technology **5:**947–950.
12. **LaMaine, W., H. Jackson, and L. McFarland.** 1990. Legionnaires' disease outbreak associated with a grocery store mist machine—Louisiana, 1989. Morbid. Mortal. Weekly Rep. **39:**108–110.
13. **Lück, P. C., L. Bender, M. Ott, J. H. Helbig, W. Witzleb, and J. Hacker.** 1993. Analysis of *Legionella pneumophila* serogroup 6 strains isolated from dental units, p. 240–242. *In* J. M. Barbaree, R. F. Breiman, and A. P. Dufour (ed.), *Legionella*: current status and emerging perspectives. American Society for Microbiology, Washington, D.C.
14. **Mahbubani, M. H., A. K. Bej, R. Miller, L. Haff, J. DiCesare, and R. Atlas.** 1990. Detection of *Legionella* with polymerase chain reaction and gene probe methods. Mol. Cell. Probes **4:**175–187.
15. **Martin, M. V.** 1987. The significance of the bacterial contamination of dental unit water systems. Br. Dent. J. **163:**152–154.
16. **McCarty, S. C., and R. M. Atlas.** 1993. Effect of amplicon size on polymerase chain reaction detection of bacteria exposed to chlorine. PCR Methods Applications **3:**181–185.
17. **McDade, J. E., C. C. Shepard, D. W. Fraser, T. F. Tsai, M. A. Redus, W. R. Dowdle, et al.** 1977. Legionnaires' disease: isolation of a bacterium and demonstration of its role in other respiratory disease. N. Engl. J. Med. **297:**1197–1203.
18. **Michel, R., and M. Borneff.** 1989. Über die Bedeutung von Amöben und anderen Protozoen in wasserführenden Systeme von Dentaleinheiten. Zentralbl. Bakteriol. Mikrobiol. Hyg. B **198:**312–313.
19. **Miller, L. A., J. L. Beebe, N. C. Butler, H. Martin, R. Benson, R. E. Hoffman, and B. S. Fields.** 1993. Use of polymerase chain reaction in an epidemiologic investigation of Pontiac fever. J. Infect. Dis. **168:**769–772.
20. **Muraca, P. W., V. L. Yu, and A. Goetz.** 1990. Disinfection of water distribution systems for *Legionella*: a review of application procedures and methodologies. Infect. Control Hosp. Epidemiol. **11:**79–88.

21. **Oppenheim, B. A., A. M. Sefton, O. N. Gill, J. E. Tyler, M. C. O'Mahoney, J. M. Richards, et al.** 1987. Widespread *Legionella pneumophila* contamination of dental stations in a dental school without apparent human infection. Epidemiol. Infect. **99:**159–166.
22. **Pankhurst, C. L., and J. N. Philpott-Howard.** 1993. The microbiological quality of water in dental chair units. J. Hosp. Infect. **23:**167–174.
23. **Pankhurst, C. L., J. N. Philpott-Howard, J. H. Hewitt, and M. W. Casewell.** 1990. The efficacy of chlorination and filtration in the control and eradication of *Legionella* from dental chair water systems. J. Hosp. Infect. **16:**9–18. (Erratum, **16:**393.)
24. **Paszko-Kolva, C.** 1991. Risk of infection from dental handpieces. ASM News **57:**287.
25. **Paszko-Kolva, C., M. Shahamat, J. Keiser, and R. R. Colwell.** 1993. Prevalence of antibodies against *Legionella* species in healthy and patient populations, p. 24–26. *In* J. M. Barbaree, R. F. Breiman, and A. P. Dufour (ed.), *Legionella*: current status and emerging perspectives. American Society for Microbiology, Washington, D.C.
26. **Reingold, A. L., B. M. Thomason, B. J. Brake, L. Thacker, H. W. Wilkinson, and J. N. Kuritsky.** 1984. *Legionella* pneumonia in the United States: the distribution of serogroups and species causing human illness. J. Infect. Dis. **149:**819.
27. **Reinthaler, F., and F. Mascher.** 1986. Demonstration of *Legionella pneumophila* in dental units. Zentralbl. Bakteriol. Mikrobiol. Hyg. B **183:**86–89.
28. **Reinthaler, F. F., F. Mascher, and D. Stunzer.** 1988. Serological examinations for antibodies against *Legionella* species in dental personnel. J. Dent. Res. **67:**942–943.
29. **Scheid, R. C., C. K. Kim, J. S. Bright, M. S. Whitely, and S. Rosen.** 1982. Reduction of microbes in handpieces by flushing before use. J. Am. Dent. Assoc. **105:**658–660.
30. **Stout, J. E., V. L. Yu, P. Muraca, J. Joly, N. Troup, and L. S. Tompkins.** 1992. Potable water as a cause of sporadic cases of community-acquired Legionnaires' disease. N. Engl. J. Med. **326:**151–155.
31. **Williams, J. F., A. M. Johnston, B. Johnson, M. K. Huntington, and C. D. Mackenzie.** 1993. Microbial contamination of dental unit water lines: prevalence, intensity and microbiological characteristics. J. Am. Dent. Assoc. **124:**59–65.
32. **Winn, W. C., Jr.** 1988. Legionnaires disease: historical perspective. Clin. Microbiol. Rev. **1:**60–81.