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Widespread *Legionella pneumophila* contamination of dental stations in a dental school without apparent human infection

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SUMMARY

Following isolation of *Legionella pneumophila* from a special dental station water circuit, used primarily to cool high-speed dental drills which produce fine aerosols, a case finding and environmental survey was undertaken. Widespread colonization of the dental stations was found and the results suggested that amplification of the background levels of *L. pneumophila* was taking place within the stations. However there was no evidence for transmission causing human infection.

INTRODUCTION

Dental station water is frequently contaminated with microorganisms such as viridans streptococci and *Pseudomonas* species and counts as high as 10^6 colony forming units per ml have been reported (Abel *et al.* 1971; Fitzgibbon *et al.* 1984; Furuhashi & Miyamae, 1985). The isolation of *Legionella pneumophila* from dental stations has not been previously reported. However high speed air turbine dental drills generate aerosol clouds where most particles are 5 μm or less (Cooley, 1984; Madden *et al.* 1969). Thus contaminating *L. pneumophila* could be inhaled into the alveoli of both patients and staff (Macfarlane, 1983).

The London Hospital Dental Institute (LHDI) was housed in an air conditioned building on six floors with 91 dental stations available for use by clinical dental students. There was an average of over 1850 patient attendances weekly. Each dental station had two water-cooled high-speed drills, a '3 in 1' syringe which could blow air, water or spray as well as provide suction, and a water outlet for an ultrasound descaling machine. These outlets were all supplied by a high-pressure circuit which was separate from the drinking-water supply and the water supply to other outlets in the building. The pressure was created by a booster

pump on the rising main. There were two pumps but the second, back-up, pump had not been used for 9 months. Water pipe external surface temperatures, measured on several occasions, were 10 °C for the mains input pipe, 27 °C a half metre distal from the pump and 23 °C after a further 4 metres. Also a temperature gradient probably existed within the dental station electrical control box, but this was not measured.

In July 1985, staff at the LHDI became concerned following reports of debris in water coming from the dental station outlets and an increase in the level of respiratory illness in staff and students. The Safety Officer tested five dental stations after a 64 h period of inactivity, by collecting pooled 20 ml water samples from the three high-pressure outlets of each station. When *L. pneumophila*, serogroups 1 and 4, was isolated from three of the five samples, an investigation was begun to discover whether human infection had occurred and to define the extent and source of the environmental contamination. Measures to eliminate the organism in the dental equipment were undertaken simultaneously, because of the perceived hazard of legionella-contaminated aerosols.

METHODS

Epidemiological

A case finding search in the Health District reviewed all sera submitted since 1983 for measurement of legionella antibody. No cases of legionellosis had been diagnosed. In addition, the names of patients who had sera submitted for a viral screen because of a clinical diagnosis of atypical pneumonia were checked against the dental hospital records. Any patient who had a dental record as well as a medical record had their date of illness onset compared with the dates of dental treatment. If dental treatment had been given within the 2 weeks before illness onset, stored serum specimens were located and tested for legionella antibody.

A survey of the teaching staff and clinical dental students was carried out as these groups had the greatest exposure to dental station aerosols. A questionnaire which asked about respiratory illness within the previous 7 months and about earlier lower respiratory tract illness was distributed and serum specimens collected to ascertain who had evidence of *L. pneumophila* infection. For comparison, stored sera from a group of medical students in their final year of study and from some young doctors were examined.

National surveillance data (Bartlett & Bibby, 1983) on legionellosis from 1979 was reviewed to see whether reported cases had worked as dentists or in a dental-care-related occupation. Data was also reviewed for cases who had a history of dental treatment in the 2 weeks before the onset of symptoms: this information was sought in 1984–5.

Environmental

Eight dental stations that were not previously tested were selected to represent all parts of the separate high pressure water circuit throughout three floors of the building. The following water samples were collected from these stations: 750 ml from the inlet pipe, 20 ml from each high-speed drill and the 'three in one' syringe and 250 ml from the ultrasound descaler outlet. In addition a 750 ml water sample

was obtained from the active booster pump. Some days later a 750 ml sample was collected from the standby booster pump. First draw water samples were placed in sterile plastic containers without added neutralizing agents and processed within 24 h. Sites were not cleansed prior to sampling.

Two of three dental stations found contaminated with legionella at the earlier investigation, had their water supply cut off prior to the general chlorination. They were then dismantled and some parts were removed to a laboratory for detailed examination.

The water system was decontaminated overnight by injecting chlorine at the booster pump until a level of 10 parts per million of free residual chlorine was reached at the furthest outlets. The 360 other outlets on the dental stations were then flushed through and the chlorinated water was allowed to stand for 4 h. Finally unchlorinated water was similarly run through each outlet after a negative chlorine reading was measured at the periphery.

Microbiological surveillance was conducted by taking follow-up 20 ml water samples pooled from each outlet of the eight representative stations. This was done immediately and early on Monday morning at 1,4, 10 and 20 weeks following chlorination.

Microbiological

The 20 ml water samples were centrifuged at 2500 rev./min for 20 min and the deposit resuspended in 0·1 ml of sterile water. The larger samples were filtered through Pall (Ultipore) nylon membrane filters, pore size 0·22–0·45 μm. The material retained on the surface of the membrane was resuspended in a portion of the filtrate and then centrifuged and treated in the same way. All specimens were divided and one set was heated at 50 °C for 30 min. Both sets of samples were then cultured on buffered charcoal yeast extract agar containing vancomycin, polymixin and glycine. Plates were incubated in air at 37 °C for 10 days and colonies resembling legionellae were identified by standard methods (Wright & Dennis, 1985). All serogroup 1 isolates were subgrouped using monoclonal antibodies (Watkins *et al.* 1985).

Estimations of the levels of antibody directed against *L. pneumophila* serogroup 1 were performed using the rapid micro-agglutination test (RMAT) (Harrison & Taylor, 1982*a*) and indirect fluorescent antibody test (IFAT) (Harrison & Taylor, 1982*b*).

RESULTS

Stored serum samples were available from 333 patients admitted with atypical pneumonia over a 22-month period, December 1983 to September 1985. Hospital records were located for 290 (87%) patients and 25 had a dental record of whom only 2 had dental treatment immediately before their illness onset. Legionella antibodies were not detected in the convalescent serum specimens of either of these two cases.

During the investigation period a 53-year-old dental patient informed staff that he was hospitalized 3 weeks earlier with pneumonia and a diagnosis of legionellosis had been suspected. However his convalescent serum did not contain legionella serogroup 1 antibodies.

Table 1. *Serosurvey of exposed dental staff and clinical dental students and unexposed medical students and young doctors*

Group	RMAT ⩾ 8 (%)*	IFAT ⩾ 16 (%)†	Total (100%)
Dental teaching staff	3 (12)	1 (4)	26
Clinical (5th yr)	5 (12)	4 (10)	41
Dental (4th yr)	2 (4)	4 (9)	45
Students (3rd yr)	2 (5)	0	40
Dental group total	12 (8)	9 (6)	152
Medical students and young doctors	0	1 (1)	70

* Micro-agglutination antibody difference: $0{\cdot}05 > P > 0{\cdot}01$.

† Immunofluorescence antibody difference: $P > 0{\cdot}05$.

Comparing the dental group with the medical group, for either serological test, and using Fisher's exact test, one tailed.

Twenty-six (29%) of 91 dental teaching staff and 152 (73%) of 208 clinical dental students completed a questionnaire and gave a blood specimen. Most of those who did not respond were on holiday at the time of the survey. As judged by the RMAT, 8% with antibody present at a titre of 8 or greater, and the IFAT, 6% with antibody present at a titre of 16 or greater, the antibody prevalence in the exposed dental group was marginally increased compared with the unexposed medical students and young doctors (Table 1), but for the immunofluorescence antibodies this difference could have arisen by chance. There was very little concordance between the results of the two serological tests; of the 10 subjects with IFAT antibody present at a titre of 16 or greater only three had RMAT antibody present at a titre of 8 or greater. Of the 12 from the dental group with RMAT antibodies at a titre of 8, in only two were antibodies present at a higher titre, one at 16 and the other at 32.

Half of the dental students and staff reported a flu-like illness or chest infection during the previous 7 months. Illnesses were not clustered in any particular month and there was no difference in legionella antibody prevalence between well and ill people or between ill people with chest symptoms and ill people without chest symptoms. One student was admitted to hospital with an atypical pneumonia in April 1985 but 3 months later legionella antibodies were undetectable. There were eight cases of pneumonia reported before 1985; none of the subjects had detectable legionella antibodies and all of their illnesses had occurred before exposure to the Dental Institute had begun.

Occupational information was available, from surveillance questionnaires and laboratory report forms, on some of the 1121 cases of legionellosis reported between 1979 and 1985 in England and Wales; in 1980 two dentists were ill, one of whom probably acquired the disease while on holiday abroad and the other, a 48-year old man, was reported from a Midlands laboratory. A case was reported in 1983 who had had dental treatment within 14 days of the illness onset but, in the same period, had also returned from travel abroad. Of the 360 cases reported in 1984 and 1985, information on recent dental treatment was available for one sixth and none of these had attended a dentist within 14 days of their illness onset.

Table 2. *Isolation of* L. pneumophila *from high-pressure water system*

Site	Number sampled	Number with growth	Serogroup	Approx. count (c.f.u./l)
Mains booster pumps				
Active	1	0	—	—
Standby	1	0	—	—
Dental station survey				
Inlet water	8	4	5	10^2–10^3
High-speed drills	16	7	1	10^3–10^5
'3 in 1' syringes	8	4	1	10^3–10^5
Ultrasound descalers	8	1	1	10^3
Dismantled stations				
Tubing to high speed drills	2	0	—	—
Solenoid valve	1	0	—	—

Legionellae were found throughout the periphery of the water system (Table 2). They were in low numbers in the inlet water samples, and could not be isolated from 20 ml aliquots, but were found when the entire 750 ml volumes were filtered. Legionellae were readily isolated from the 20 ml samples of outlet water and here the counts were approximately 100-fold greater than those in the inlet samples. Strains from serogroups 1 and 5 were recovered and all the serogroup 1 strains belonged to the Bellingham 1 subgroup.

Legionellae were not isolated from the two specimens of high-speed drill tubing which were tested, nor from a solenoid valve. In addition, they were not found in the water samples drained from both the active and standby booster pumps. The organisms were not recovered from any of the samples tested after chlorination.

DISCUSSION

We were unable to attribute a single local case of legionellosis to recent attendance at the Dental Institute. While recognizing that the catchment population for dental treatment was different from that for hospital admissions, it was unlikely to be sufficiently different that all pneumonia cases, which might have resulted from contact with the Dental Institute, were hospitalized elsewhere.

None of the staff members and clinical dental students most exposed had evidence of legionella pneumonia and their reported respiratory symptoms were not serologically attributable to legionella exposure. Because of the relative absence of suspect clinical cases and the low prevalence of serogroup 1 antibodies in those studied, it was decided not to look for antibodies to other legionella serogroups.

The 6% prevalence of IFAT antibodies at a titre of 16 or over in this group was not significantly different from the control group. Also our survey did not establish whether the observed antibody prevalence was related to dental station exposure: parts of the building were air conditioned and we did not survey non-clinical staff members who were exposed to the building but not the dental stations. In contrast, a recent serosurvey in the United States reported a significantly increased level of

antibodies in dental clinic personnel and those with longest exposure tended to have the greatest antibody level (Fotos *et al.* 1985). As the RMAT was developed to improve early diagnosis of Legionaires Disease (Harrison & Taylor, 1982*a*) and in view of the low prevalence of antibodies found with both serological tests, the lack of concordance between the tests was not surprising.

Using sufficiently sensitive techniques, *L. pneumophila* can occasionally be isolated from taps in buildings connected directly to the rising mains (Colbourne & Trew, 1986). However, it was not possible to demonstrate the presence of the organisms in the boosted mains supply during the course of this investigation and the apparent elimination of the organism from the plumbing system for 20 weeks after chlorination was not indicative of low level seeding from the mains supply. Although the role of the booster pumps as an amplification site could not be demonstrated, the temperature profile of the water was within the range which permits legionella colonization (Voss *et al.* 1986).

The total volume of water in each dental station distal to the inlet filter was only 40 ml, so the small volumes collected from the drills and '3 in 1' syringes were representative of water from within the station (although the third 20 ml sample would have been diluted with water from the main circuit) and legionellae were grown from 11 of these 24 samples. The larger volumes taken from the descaler outlets consisted almost entirely of main circuit water. As legionellae were only isolated from one of these eight specimens and in numbers comparable with the inlet water samples, it seems possible, therefore that multiplication occurred within the dental stations.

The failure to isolate the organism from the tubing and solenoid valve of stations which were previously shown to be contaminated was surprising. The initial sampling may have flushed organisms out or the possibility existed that some chlorine may have reached these stations before they were disconnected from the decontamination process.

If transmission of legionella was possible during dental treatment undertaken before the elimination measures a number of factors could account for our failure to find evidence of infection; the level of legionella contamination was low and most nosocomial legionellosis cases attributed to tap water have involved systems in which quantitative cultures have yielded 10^5–10^9 cfu/l (Arnow, Weil & Para, 1985); the *L. pneumophila* serogroup 1 strains isolated belonged to a less virulent subgroup (Brown *et al.* 1982; Plouffe *et al.* 1983; Watkins *et al.* 1985); patients receiving conservative dental treatment on whom drills were used were generally younger and if infected they were less likely to develop disease, rubber 'dams' were frequently used on patients during dental procedures and finally our investigation may not have been sensitive enough to observe a very low incidence of infection.

Legionellosis associated with dentistry has never been reported. Certain current dental station designs may be amplifying environmental legionella and the use of dental drills may be generating airborne droplet nucleii containing legionella, leading to inhalation by some patients or operators. If, within England and Wales, nosocomial legionellosis occasionally results from dental treatment, the incidence is probably so low that the most efficient means to assess the hazard would be for clinicans to enquire about recent dental treatment and the occupation of all

diagnosed legionella cases and to report this information nationally. Until such transmission is recognized, elimination measures such as we undertook are probably not justified. In anticipation of the possibility of an association, it would seem prudent to encourage experimental work on the ecology of *L. pneumophila* within the micro water systems of dental stations. Meanwhile, routine testing of dental station water for the presence of *L. pneumophila*, in the absence of associated proven clinical cases cannot be recommended.

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