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A field study of the survival of *Legionella pneumophila* in a hospital hot-water system

I. D. FARRELL[1], J. E. BARKER[1]*, E. P. MILES[2] AND J. G. P. HUTCHISON[1]

[1] *Regional Public Health Laboratory, East Birmingham Hospital, Bordesley Green East, Birmingham B9 5ST*

[2] *Works and Estates Department, Mid Staffordshire Health Authority, Corporation Street, Stafford ST16 3SR*

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SUMMARY

The colonization, survival and control of *Legionella pneumophila* in a hospital hot-water system was examined. The organism was consistently isolated from calorifier drain-water samples at temperatures of 50 °C or below, despite previous chlorination of the system. When the temperature of one of two linked calorifiers was raised to 60 °C, by closing off the cold-water feed, the legionella count decreased from *c.* 10^4 c.f.u./l to an undetectable level. However, 10 min after turning on the cold-water feed which produced a fall in calorifier temperature, the count in the calorifier drain water returned to its original level. Investigations revealed that the cold-water supply was continually feeding the calorifiers with *L. pneumophila*. Simple modifications in the design of the system were made so that the cold-water feed no longer exceeds 20 °C; these measures have considerably reduced the number of *L. pneumophila* reaching the calorifiers.

INTRODUCTION

Legionella pneumophila is often found in the piped water of large buildings where it may serve as a source of human infection. Indeed, there have been a number of reports implicating hospital hot- and cold-water systems with outbreaks of Legionnaires' disease [1–3] in addition to those associated with cooling towers [4, 5]. For this reason, most hospitals follow Department of Health advice [6] which recommends circulating hot water should be delivered at the taps not below 50 °C to reduce the risk of infection.

Nevertheless, despite the fact that in many hospitals hot water is distributed to wards above 50 °C, *L. pneumophila* is frequently isolated from taps and showers in little used or remote parts ('dead-legs') of the plumbing system ([7] and Barker and Farrell, unpublished data). A previous report [8] showed that flushing intermittently the entire hot-water supply system of a hospital unit, with water at 77 °C, reduced the number of contaminating legionellae but did not necessarily eradicate them.

* Correspondence and author for reprints.

We found that, in spite of careful maintenance on the calorifiers in a hospital unit using cleaning and flushing, and temporary chlorination of the water to 10 p.p.m. for 10 h, *L. pneumophila* was consistently isolated from the system. Thus the control of this organism in plumbing systems of large buildings continues to pose a problem for those responsible for their maintenance.

This investigation was initiated in 1986 to examine in detail the source of contamination, the dynamics of colonization and the survival of *L. pneumophila* in a hospital hot-water system. Samples were taken from the point at which the Severn Trent Water Authority (STWA) mains enters the establishment, from selected sites in the distribution network, and from the hot and cold outlets in the wards. The efficacy of raising calorifier temperatures as a means of controlling legionellae colonization was also examined.

METHODS

Water sampling protocol

Cold-water samples were taken at specific points in a segregated distribution system supplying two horizontal calorifiers (A and B), operated in parallel to provide domestic hot water for two hospital wards and the pathology department (Fig. 1). Samples were taken, as shown in Fig. 1 from: (1) the inlet of the STWA supply, (2) the hospital cold-water 'break-tank' (which reduces the mains water pressure in this system), (3) the water softener, (4, 5) the cold-water storage tank which feeds the calorifiers.

Calorifier study

After cleaning, flushing and disinfecting the system by drawing chlorinated water from the water storage tank, through the calorifiers and then out of the drain taps at the bottom of the vessels, preliminary samples were taken from each calorifier drain over a 6-month period to establish the level of contamination by legionellae. Thereafter, disinfection was done once more before survival studies were carried out by raising the temperature of the water throughout one calorifier to ascertain the temperature and time required to kill any contaminating legionellae.

The experiments were conducted in the morning (Test 1), when there was a high demand on the system for hot water and repeated when the demand for hot water was at its minimum during the afternoon (Test 2).

Test 1. The calorifier temperature was raised by closing the cold water feed to one of them (Fig. 1, A), whilst the feed to the second calorifier (B) remained open, so that it could be monitored as a control. Water samples were taken from the drains (Fig. 1, 6 and 7) of both calorifiers at the start of the experiment and then at regular intervals until the drain temperature of the test calorifier (A) reached approximately 60 °C. Then the cold feed valve was opened and 10 min later a further sample was collected.

Test 2. When the first calorifier had been tested as above, the test procedure was reversed, so that the temperature in calorifier (B) was increased to 60 °C.

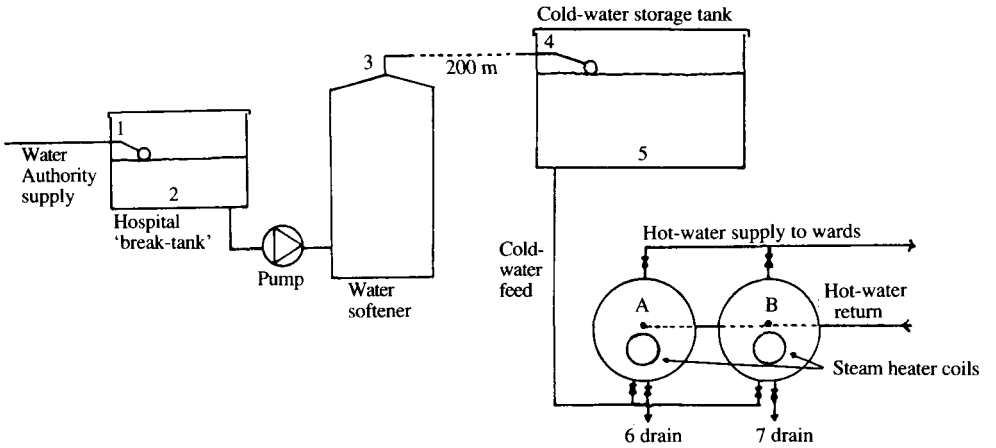

Fig. 1. A diagram (not to scale) of the water-distribution system supplying a hospital unit. Hot water is heated in two horizontal calorifiers A and B. Sample points 1, Water Authority inlet; 2, hospital 'break-tank'; 3, water softener; 4, inlet to the cold-water storage tank; 5, tank water; 6, 7, calorifier drains.

Laboratory methods

The bacterial flora of 1 l water samples was filtered out with a 0·2 μm, 142 mm diameter, nylon membrane under positive pressure. The membranes were then cut into segments and retained debris resuspended in 50 ml of sterile distilled water by vigorous shaking. Before culturing, a 10 ml volume of each suspension was heated to 50 °C for 30 min to reduce the number of bacterial contaminants.

The number of colony forming units per litre (c.f.u./l) of *L. pneumophila* in the concentrated samples were determined on buffered charcoal yeast extract agar (BCYE [9]) with and without the addition of glycine (0·3% w/v), polymyxin (80000 IU/l), vancomycin (3 mg/l), and cycloheximide (80 mg/l).

The inoculated plates were incubated at 37 °C in a moist atmosphere and examined daily for up to 10 days before discarding. Presumptive strains of legionella were tested for cysteine dependence before positive identification by immunofluorescence, using rabbit antisera, prepared against *L. pneumophila* serogroups 1–6 (supplied by the Division of Microbiological Reagents and Quality Control, Colindale, London).

RESULTS

Preliminary samples

Preliminary experiments showed that *L. pneumophila* serogroup 4 was present in 23 of 30 samples taken from calorifiers A and B drainage outlets during a 6-month period. These numbered from 10^2–10^5 c.f.u. of legionella per litre of water. Serogroups other than serogroup 4 were not found when up to six separate colonies from the isolation plates were examined by immunofluorescence for serogroups 1–6.

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Table 1. *The effects of time and temperature on the survival of* L. pneumophila *in drain waters of Calorifier A (Test 1, high hot-water demand)*

Time of water sample collection (min)	Calorifier A (cold-water feed closed)			Calorifier B (cold-water feed open)		
	Calorifier temperature (°C)		Viable count c.f.u./l (drain water)	Calorifier temperature (°C)		Viable count c.f.u./l (drain water)
	Top	Drain		Top	Drain	
Start	76·5	33	4×10^3	73	31	5×10^3
5	76	34	$3·5 \times 10^3$	72	28	$6·5 \times 10^3$
15	76	40·5	$3·0 \times 10^3$	71	28	5×10^3
30	75	49	1×10^4	70	27	$1·25 \times 10^4$
60	74·5	65	Legionella not isolated	66·5	27·5	$2·5 \times 10^4$
10 min after cold supply to calorifier A opened	74	35	$1·5 \times 10^4$	67	29	$1·5 \times 10^4$

Table 2. *The effects of time and temperature on the survival of* L. pneumophila *in drain waters of Calorifier B (Test 2, low hot-water demand)*

Time of water sample collection (min)	Calorifier A (cold-water feed open)			Calorifier B (cold-water feed closed)		
	Calorifier temperature (°C)		Viable count c.f.u./l (drain water)	Calorifier temperature (°C)		Viable count c.f.u./l (drain water)
	Top	Drain		Top	Drain	
Start	55	32	$1·2 \times 10^4$	60	32	$1·8 \times 10^4$
15	—	—	NT	60	34·5	$3·5 \times 10^4$
35	45	40·5	$1·4 \times 10^4$	59	37	1×10^3
47	—	—	NT	63	40	2×10^3
80	—	—	NT	71	50·5	2×10^3
100	50	32	4×10^3	65	60·5	Legionella not isolated
15 min after opening cold supply to calorifier B	68	40	2×10^3	61	39	6×10^3

NT, not tested.

Survival studies

As preliminary samples taken from the calorifiers had shown them to be consistently contaminated with *L. pneumophila*, the effect on the organisms survival of raising the temperature in one of them (calorifier A) was studied. Table 1 shows that before the cold water supply had been shut off (in order to increase the temperature in the vessel), 4×10^3 c.f.u./l of *L. pneumophila* serogroup 4 were found in the drain water, the temperature of which was 33 °C. When, 30 min after closing off the cold-water supply, the drain temperature had reached 49 °C, 1×10^4 c.f.u./l of *L. pneumophila* were found – this difference in bacterial count is thought to be within experimental error.

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However, 30 min later when the drain temperature had increased to 65 °C, legionellae were no longer detected in the sample. At this stage the cold-water feed to calorifier A was reopened; after only 10 min the drain water temperature had fallen to 35 °C and contained $1{\cdot}5 \times 10^4$ c.f.u./l *L. pneumophila* serogroup 4. The temperature at the top of the calorifier remained fairly constant throughout the experiment at about 75 °C. The count of *L. pneumophila* in the control calorifier (B), where the cold feed was left open, varied between 5×10^3 and $2{\cdot}5 \times 10^4$ c.f.u./l and the highest temperature recorded at the drain was 31 °C.

Table 2 shows the results obtained after closing the cold water supply to calorifier B (Test 2). Five samples of the drain water taken over an 80-min period, contained *L. pneumophila*, ranging in number from $1{\cdot}0 \times 10^3$–$3{\cdot}5 \times 10^4$ c.f.u./l. The temperature of the drain water at the start of the experiment was 32 °C and after 80 min it had reached 50·5 °C. Twenty minutes later the temperature had increased to 60·5 °C and legionellae were no longer detected. At this point the cold-water feed to calorifier B was turned on again and within 15 min the temperature had fallen to 39 °C and the legionella count had increased to 6×10^3 c.f.u./l.

High or low demand for hot water on the wards had no significant effect on the results of the survival studies. For Test 2, a more gradual increase in the drain temperature of calorifier (B) was achieved by regulating the amount of steam passing through the heating coil, to try and ascertain more precisely the temperature required to kill *L. pneumophila* under these conditions (Table 2).

Although these experiments revealed that the drain waters (below 50 °C) of calorifier A and B consistently contained legionella, it was reassuring to find that from samples of the hot water, at 52 °C from taps in the wards, *L. pneumophila* serogroup 4 was not isolated. It was also found that a temperature of about 60 °C killed legionella in the calorifier, because the organism was not isolated from drain water samples. However, as the organism reappeared in the drain water within 10 min of turning on the cold-water feed, it seemed likely that this feed to the calorifier was the source and therefore a detailed examination of the cold-water supply was made.

Legionellae were not isolated from cold-water samples, 1, 2 and 3, taken from the STWA inlet, the hospital 'break-tank' and the water softener (Fig. 1). There was 200 m of pipe between the water softener and the water-storage tank feeding the calorifiers and the only convenient sampling point for this length of pipe was at the ball valve inlet to the tank itself (Fig. 1, sample point 4). Three samples of this water were negative, but a fourth sample contained a small number of *L. pneumophila* serogroup 4; the count was 5×10^2 c.f.u./l of water. Four of four samples of the cold-tank water (Fig. 1, sample point 5), yielded *L. pneumophila* serogroup 4, with counts ranging from 6×10^2–$4{\cdot}4 \times 10^4$ c.f.u./l.

DISCUSSION

Our findings suggest, as has been shown by others [10] that *L. pneumophila* gets into plumbing systems in very low numbers from mains water, but it cannot be detected by the usual laboratory methods until multiplication has taken place in warm conditions of 20–45 °C.

In the present system there was a long length of ducted pipework, creating a

raised ambient water temperature, between the water softener and the calorifier water storage tank, so it was not surprising that the first positive sample was found at end of this pipework (sample point 4). The temperature of the water at the ball valve inlet was *c.* 12 °C and contained only 500 c.f.u./l of *L. pneumophila* serogroup 4, i.e. the lower limit of detection by our method. However, higher legionellae counts (up to $4{\cdot}5 \times 10^4$ c.f.u./l) were found in the tank water itself, multiplication being stimulated by a water temperature of 25 °C, and probably also by stagnation at the warm bottom of the tank. This cold tank was sited about 6 m above the calorifiers and was not adequately insulated, consequently heat gain was considerable. Moreover, the cold water feed to calorifiers was complex, with a 'dead-leg' containing water at about 25 °C; suitable conditions for multiplication of legionellae.

As the cold-water storage tank and interconnecting pipework was a reservoir of legionellae, and these in turn continually fed the calorifiers, a critical problem could arise in the event of a steam shut down. If the temperature at the top of the calorifiers fell, where water is drawn off, then legionellae would not be killed and consequently they would contaminate the hot-water distribution system and no doubt colonize some areas if the shutdown was for a long period.

In order to rectify some of the above defects, simple modifications in the design of the plant have been made to ensure that the cold-water feed to the calorifiers does not exceed 20 °C: a 'dead-leg' between the water-storage tank and the calorifiers has been removed; the cold-water storage tank has been insulated and the calorifier room below provided with a false ceiling to reduce bottom heating. These measures have considerably reduced the number of *L. pneumophila* reaching the calorifiers from the cold-water feed because, in the 2 years since they were introduced, the counts in repeat samples of calorifier drain water have been consistently in the order of 500 c.f.u./l.

Heating water to 60 °C and above is an effective way of killing *L. pneumophila* both *in vitro* [11] and, as this investigation has shown, also under environmental conditions. It was difficult to maintain an even temperature throughout the calorifiers because the steam heater coils were positioned towards the centre of the vessels, thus creating temperature stratification ranging from *c.* 30 °C at the bottom to > 60 °C at the top. It proved impossible in this system to achieve a temperature of > 60 °C at the drains without closing off the cold water supply. However, in practice this will usually not matter during normal operational conditions, as the draw-off point is taken from the top of the calorifiers. The temperature there was sufficiently high (*c.* 65 °C) to destroy legionellae and to ensure that ward tap temperatures were > 50 °C; these outlets were clear of the organisms.

Monitoring water systems for *L. pneumophila* has limitations because of the uncertainties inherent in laboratory concentration methods which may result in wide variations in the apparent number of organisms in the same sample. For example, we found as much as a 10^3 difference in c.f.u./l of *L. pneumophila* in sequential water samples taken every 10 min from the same calorifier drain. The question is whether this is a true reflection of the level of contamination, as influenced by the demand for hot water by the wards, turbulence in the calorifier, or merely a reflection of laboratory inaccuracy. The recovery of *L. pneumophila*

from seeded water samples can vary by up to 50%, presumably because of problems of eluting bacteria from the surface of membrane filters (J. E. Barker, unpublished data). For this reason the estimation of legionella numbers using this method should be regarded as an approximation only.

Regular monitoring of water systems for *L. pneumophila* has little value other than to establish a rough numerical baseline. Moreover, routine bacteriological surveillance could justifiably be questioned because, as this study has shown, effective maintenance and temperature controls are simple methods of controlling colonization by this organism, except where danger of scalding has to be considered. There is a case for fitting calorifiers with alarm systems, to alert engineers to significant changes in temperature (be they high or low), which may be unsafe.

Nevertheless, temperature control alone may not be sufficient to eradicate legionellae from plumbing systems. Cold-water feed to the calorifiers must be protected against heat gain to keep the number of legionellae entering calorifiers to a minimum. The calorifier temperature should never fall below 60 °C, at the draw-off point, which should in great measure protect the peripheral plumbing network from contamination once the system has been cleared.

Many of the recommendations made in this report have now been incorporated into various Codes of Practice [12–14] which if followed in detail, should minimize the risk of legionella infection from domestic water systems.

REFERENCES

1. Tobin JO'H, Dunhill MS, French M, et al. Legionnaires' disease in a transplant unit: Isolation of the causative agent from shower baths. Lancet 1980; ii 118–21.
2. Helms CM, Massanari RM, Zeitler R, et al. Legionnaires' disease associated with a hospital water system: a cluster of 24 nosocomial cases. Ann Intern Med 1983; **100**: 333–8.
3. Edelstein PH. Control of Legionella in hospitals. J Hosp Inf 1986; **8**: 109–15.
4. Timbury MC, Donaldson JR, McCartney AC et al. Outbreak of Legionnaires' disease in Glasgow Royal Infirmary: Microbiological aspects. J Hyg 1986; **97**: 393–403.
5. Fisher-Hoch SP, Bartlett CLR, Harper GJ, et al. Legionnaires' disease at Kingston Hospital. Lancet 1981; i: 1154.
6. Department of Health and Social Security: Health Notice; HN(80)39, 1980. Legionnaires' disease and hospital water systems.
7. Colbourne JS, Pratt DJ, Smith MG, et al. Water fittings as sources of *Legionella pneumophila* in a hospital plumbing system. Lancet 1984; i: 2120–3.
8. Best M, Stout J, Muder M, et al. Legionellaceae in the hospital water supply. Lancet 1983; ii: 307–10.
9. Edelstein PH. Improved semi-selective medium for isolation of *Legionella pneumophila* from contaminated clinical and environmental specimens. J Clin Microbiol 1981; **14**: 298–303.
10. Hsu SC, Martin R, Wentworth BB. Isolation of *Legionella* species from drinking water. Appl Environ Microbiol 1984; **48**: 830–2.
11. Dennis PJ, Green D, Jones BPC. A note on the temperature tolerance of Legionella. J Appl Bact 1984; **56**: 349–50.
12. Health and Safety Executive: Guidance Note (HE 48). Legionnaires' disease. London: HMSO, 1987.
13. Chartered Institution of Building Service Engineers: Technical Memorandum (TM 13). Minimising the risk from Legionnaires' disease. London, 1987.
14. Department of Health and Social Security and the Welsh Office. The control of *Legionellae* in health care premises: A Code of Practice. London: HMSO, 1988.