J. Hyg., Camb. (1984), **93**, 349–353
Printed in Great Britain

Legionella pneumophila in aerosols from shower baths

By P. J. L. DENNIS, A. E. WRIGHT*, D. A. RUTTER, J. E. DEATH and B. P. C. JONES

PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 0JG

(*Received* 5 *April* 1984; *accepted* 16 *April* 1984)

SUMMARY

Routine examination of the water system in an establishment where showers were used by staff after work or leisure activities revealed the presence of *Legionella pneumophila*. Descaling and steaming cleared the system but six months later recolonization was found to have occurred. *L. pneumophila* was isolated from air samples collected in the shower room whilst the shower was in operation. No clinical cases have occurred. Serological examination of those using the showers throughout the six month period together with a control group showed no evidence of infection.

INTRODUCTION

Legionella pneumophila and other species of *Legionella* are often found in water systems in large establishments such as hospitals and hotels (Best *et al.* 1983; Tobin, Swann & Bartlett, 1981; Fisher-Hoch, Bartlett & Tobin, 1981 and Wadowsky *et al.* 1982). Recent work in the UK has shown that the organism is widely distributed, but relatively few sites are associated with clinical disease (Bartlett *et al.* 1983).

The mode of transmission of infection with legionellosis remains a matter for speculation but there is good evidence to suggest that it is by inhalation. Cases have occurred downwind from the exhaust vents of cooling towers known to be a source of the organism (Glick, Gregg & Berman, 1978; Dondero *et al.* 1980). Arnow *et al.* (1982) described five cases of nosocomial legionnaire's disease from aerosols created from respiratory devices in which contaminated water was used. Cases have been described (Bartlett *et al.* 1984) in British tourists returning from a continental hotel where the showers were thought to be a source of infection. Isolation was also made from shower heads in a Transplant Unit (Tobin *et al.*, 1980) where several infections had occurred, and baths or showers were suspected as a source in Kingston Hospital (Fisher-Hoch, Smith & Colbourne, 1982). Experimentally it is possible to demonstrate the susceptibility of guinea-pigs to airborne infection (Baskerville *et al.* 1981). Recently Zuravleff *et al.* (1983) demonstrated subclinical infection in guinea-pigs exposed to aerosols from a humidifier contaminated with *L. pneumophila*.

* Correspondence should be addressed to Dr A. E. Wright at the Public Health Laboratory, Institute of Pathology, General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE.

During a routine survey of a building in which some staff used the showers at the end of the day's work and others used them following leisure activities we found that the hot water system contained *L. pneumophila* serogroup 1. The system was descaled and steam treated and was thus cleared of the organism. This method of treatment was chosen because of the simplicity of the plumbing system. We did not chlorinate the system or hold the temperature of the stored water at 60 °C as recommended by the DHSS (1980) and employed in the outbreak of legionnaire's disease at Kingston Hospital (Fisher-Hoch, Bartlett & Tobin, 1981). Subsequent samples of water remained negative until six months later, when follow-up specimens again showed the presence of *L. pneumophila* serogroup 1. Air samples were collected in the shower room and the blood of those using the showers examined for evidence of subclinical infection.

MATERIALS AND METHODS

Water samples were collected from taps and showers in 5 l amounts and filtered through Pall nylon membrane filters of pore size 0·22 μm. The deposit was resuspended in 50 ml of filtrate and two aliquots of 10 ml taken from each sample. Direct cultures were done on selective media (Wadowsky & Yee, 1981) modified by the addition of ACES buffer (Pascule *et al.* 1980), α-ketoglutarate, vancomycin (1 μg/ml) (Edelstein, 1982) and cycloheximide. Individual plates of selective media were inoculated with 0·1 ml of the first aliquot before and after heating (Groothuis & Veenendaal, 1983; Dennis, Bartlett & Wright, 1984) at 50 °C for 30 min and with 0·1 ml of the second after treatment with acid buffer (Bopp *et al.* 1981), pH 2·2. Plates were incubated in a humidified air incubator at 35 °C and read at intervals up to 14 days. Colonies with a grey/blue or purple appearance and which would not grow in the absence of L-cysteine and ferric pyrophosphate were tested for the presence of legionella specific fatty acids by gas–liquid chromatography. Serogrouping was done by an indirect immunofluorescent method based on that described by Harrison & Taylor (1982).

Air samples were collected on three consecutive days using an all-glass cyclone similar to that described by Decker *et al.* (1969) at a rate of 500 litres of air per minute. Page's saline (Page, 1967) was used as a collecting fluid because tests had shown that legionella organisms survived well in it for several hours prior to culture. Each sample consisted of 2500 l air in approximately 10 ml of fluid collected over a five minute period. The sampler was set up in the shower room close to the shower but so placed as to avoid direct entrainment of water droplets from the shower head. Three samples were collected before and six after the shower was turned on. The samples were concentrated by centrifugation at 1500 *g* for 30 min and 9 ml of supernatant removed.

The three samples collected as controls prior to turning on the shower were pooled, and the six samples collected after the shower was turned on were pooled in two batches of three. Each pool contained particles collected from 7500 l air. The deposits were placed on BCYE (buffered charcoal yeast extract) and GVPC (glycine, vancomycin, polymyxin, cycloheximide) media and incubated in the same manner as the water samples. Temperature and relative humidity (r.h.) measurements were made using a sling psychrometer.

Although no clinical illness resembling legionnaire's disease was known to have occurred, 90 members of staff agreed to be bled for serological studies. Sixty-five members never used the showers, fourteen were intermittent users of the order of once per week, and eleven were frequent users, three or more times per week. The sera were examined by the indirect immunofluorescent method using a formalin-killed yolk sac antigen prepared by the PHLS Division of Microbiological Reagents and Quality Control (MRQC) and by a heat-killed antigen prepared from the organism isolated from the water system. The method used was that described by Harrison & Taylor (1982) and our results using the yolk sac antigen were kindly checked on several sera randomly chosen by Dr A. G. Taylor (MRQC).

RESULTS

Cultures of water collected from the taps and showers showed the presence of *L. pneumophila* serogroup 1 at a level of 10^3 c.f.u. l^{-1}.

Air samples collected in the shower room before the showers were started were negative. On the first day, 2 c.f.u. and on the second day, 3 c.f.u. were isolated from 7500 l air collected in the first 15 min after turning on the shower. No legionella were found between 15 and 30 min. No legionella were recovered on the third day.

The temperature in the shower room was 15 °C prior to turning on the shower and 27 °C after the shower had been allowed to run for 15 min. During this period the rh. rose from 55 to 100 %.

After recleaning and treating with free steam the water system was cleared of legionella.

None of the sera from the 90 members of staff fluoresced with the yolk sac antigen prepared by DMRQC but two did so with a heat-killed antigen which we prepared from the local isolate. These two sera gave titres of 32, but the two members of staff concerned had not suffered from a Legionellosis-like illness nor had they used the showers.

DISCUSSION

The recovery of viable legionella from air samples confirms the suspicion that aerosols are created by showers and hence may be a source of infection to humans when domestic water systems are contaminated. The fact that the number of organisms present in the system was low may be the reason why no one became infected. If it is assumed that each c.f.u. arose from a single cell the mean concentration of legionella in 7500 l air collected during the first 15 min of operating the shower is 0·0003 l^{-1}. The length of time needed for a man breathing at a rate of 12 l min^{-1} to inhale one viable cell would be:

$$\frac{1}{12 \times 0{\cdot}0003} = 277 \text{ minutes.}$$

Nevertheless, it is well established that in abattoirs where cases of undulant fever are known to have occurred only low numbers of brucellae have been isolated. Kauffmann *et al.* (1978) quote the isolation of a single c.f.u. of *Brucella suis* from 15592 l air over a five day period in one abattoir and 4 c.f.u. from 84664 l sampled

over a 30-day period. Although survival conditions may be relatively unimportant in this situation the temperature and humidity prevailing in the shower rooms would favour survival of legionella in aerosols (Berendt, 1980).

We do not know how long the water system had been contaminated but we know that several staff used the showers daily. We know nothing of the dose of these organisms required to infect man nor what markers to look for to judge the pathogenicity of any strain. In view of the ubiquitous nature of legionella in the environment more work is required to determine the factors that contribute to virulence. Subgroups of *L. pneumophila* defined by plasmid content and monoclonal antibody binding power have been suggested (Plouffe *et al.* 1983) as differing in their pathogenicity. Aerosol stability may be a contributory factor. The results of serological investigations showed two anomalies which require comment. The two concerned had titres of <16 against a formalin-killed yolk sac antigen and 32 against a heat-killed locally isolated strain. The difference in titre may have been due to strain differences, but Wilkinson & Brake (1982) have drawn attention to the fact that formalin-killed antigens tend to have lower titres than heat-killed. Titres of 32 are not considered significant and were found in 20 of 2023 normal individuals in the Nottingham area using the Colindale antigen (Macrae, Appleton & Laverick, 1979).

REFERENCES

Arnow, P. M., Chou, T., Weil, D., Shapiro, E. N. & Kretzschmar, C. (1982). Nosocomial Legionnaires' disease caused by aerosolized tap water from respiratory devices. *Journal of Infectious Diseases* **146**, 460–467.

Bartlett, C. L. R., Hutchison, J. G. P., Kurtz, J. B., Turner, G. C. & Wright, A. E. (1983). Legionella in hospital and hotel water supplies. *Lancet* ii, 1315.

Bartlett, C. L. R., Swann, R. A., Casal, J., Canada-Royo, L. & Taylor, A. G. (1984). Recurrent Legionnaire's disease from a hotel water system. *Legionella: Proceedings of the 2nd International Symposium*. American Society for Microbiology, Washington D.C., 237–239.

Baskerville, A., Fitzgeorge, R. B., Broster, M., Hambleton, P. & Dennis, P. J. (1981). Experimental transmission of Legionnaires' disease by exposure to aerosols of *Legionella pneumophila*. *Lancet* ii, 1389–1390.

Berendt, R. F. (1980). Survival of *Legionella pneumophila* in aerosols: effect of relative humidity. *Journal of Infectious Diseases* **141**, 689.

Best, M., Stout, J., Muder, R. R., Yu, V. L., Goetz, A. & Taylor, F. (1983). Legionellaceae in the hospital water-supply. *Lancet* ii, 307–310.

Bopp, C. A., Sumner, J. W., Morris, G. K. & Wells, J. G. (1981). Isolation of Legionella spp. from environmental water samples by low pH treatment and use of a selective medium. *Journal of Clinical Microbiology* **13**, 714–719.

Decker, H. M., Buchanan, L. M., Frisque, D. E., Filler, M. E. & Dahlgren, C. M. (1969). Advances in large-volume air sampling. *Contamination Control* **8**, 13–17.

Dennis, P. J., Bartlett, C. L. R. & Wright, A. E. (1984). A comparison of isolation methods for Legionellaceae. *Legionella: Proceedings of the 2nd International Symposium*. American Society for Microbiology, Washington, D.C., 294–296.

DHSS (1980). *Legionnaire's Disease and hospital water systems*. Health Notice HN (80) 30, Department of Health and Social Security, London.

Dondero, T. J., Rendtorff, R. C., Mallison, G. F., Weeks, R. M., Levy, J. S., Wong, E. S. & Schaffner, W. (1980). An outbreak of Legionnaires' disease associated with a contaminated air-conditioning cooling tower. *New England Journal of Medicine* **302**, 365–370.

Edelstein, P. H. (1982) Comparative study of selective media for isolation of *Legionella pneumophila* from potable water. *Journal of Clinical Microbiology* **16**, 697–699.

Fisher-Hoch, S. P., Bartlett, C. L. R., Tobin, J. O'H., Nelson, A. M., Smith, M. G., Talbot,

J. M., GILLETT, M. B., PRITCHARD, J. E., SWANN, R. A. & THOMAS, J. A. (1981). Investigation and control of an outbreak of Legionnaires' Disease in a District General Hospital. *Lancet* i, 932–936.

FISHER-HOCH, S. P., SMITH, M. G. & COLBOURNE, J. S. (1982) *Legionella pneumophila* in hospital hot water systems. *Lancet* i, 1073.

GLICK, T. H., GREGG, M. B., BERMAN, B. (1978). Pontiac fever: an epidemic of unknown etiology in a health department. 1. Clinical and epidemiological aspects. *American Journal of Epidemiology* **107**, 149–160.

GROOTHUIS, D. G. & VEENENDAAL, H. R. (1983). Heat treatment as an aid for the isolation of *Legionella pneumophila* from clinical and environmental samples. In *Proceedings of Workshop Conference on Legionnaires' Disease* (ed. F. J. Fehrenbach), Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene **255**, 1, 1–55.

HARRISON, T. G. & TAYLOR, A. G. (1982). Diagnosis of *Legionella pneumophila* infection by means of formalised yolk sac antigens. *Journal of Clinical Pathology* **35**, 211–214.

KAUFMAN, A. F., FOX, M. D., BOYCE, J. M., ANDERSON, D. C., POTTER, M. E., MARTONE, W. J. & PATTON, C. M. (1980). *Airborne Spread of Brucellosis*. Annals of the N.Y. Academy of Sciences: Airborne Contagion, ed. Ruth B. Kundsin.

MACRAE, A. D., APPLETON, P. N. & LAVERICK, A. (1979). Legionnaires' disease in Nottingham, England. *Annals of Internal Medicine* **90**, 580–583.

PAGE, F. C. (1967). Taxonomic criteria for limax amoebae with descriptions of three new species of Hartmanella and three of Vollkampfia. *Journal of Protozoology* **14**, 499–521.

PASCULLE, A. W., FEELEY, J. C., GIBSON, R. J., CORDES, L. G., MYEROWITZ, R. L., PATTON, C. M., GORMAN, G. W., CARMACK, C. L., EZZELL, J. W. & DOWLING, J. N. (1980). Pittsburgh pneumonia agent: direct isolation from human lung tissue. *Journal of Infectious Diseases* **141**, 727–732.

PLOUFFE, J. F., PARA, M. F., MATHER, M. D., HACKMAN, B. & WEBSTER, R. N. (1983). Subtypes of *Legionella pneumophila* serogroup 1. *Lancet* ii, 649–650.

TOBIN, J. O'H., DUNNILL, M. S., FRENCH, M., MORRIS, P. J., BEARE, J., FISHER-HOCH, S. P., MITCHELL, R. G. & MUERS, M. F. (1980). Legionnaires' Disease in a transplant unit: isolation of the causative agent from shower baths. *Lancet* ii, 118–121.

TOBIN, J. O'H, SWANN, R. A. & BARTLETT, C. L. R. (1981). Isolation of *Legionella pneumophila* from water systems; method and preliminary results. *British Medical Journal* **282**, 515–517.

WADOWSKY, R. M. & YEE, R. B. (1981). Glycine containing selective medium for the isolation of Legionellaceae from environmental water supplies. *Journal of Applied and Environmental Microbiology* **14**, 768–772.

WADOWSKY, R. M., YEE, R. B., MEZMAR, L., WING, E. J. & DOWLING, J. B. (1982). Hot water systems as sources of *Legionella pneumophila* in hospital and non-hospital plumbing fixtures. *Applied and Environmental Microbiology* **43**, 1104–1110.

WILKINSON, H. W. & BRAKE, B. J. (1982). Formalin-killed versus heat-killed *Legionella pneumophila* serogroup 1 antigen in the indirect immunofluorescence assay for legionellosis. *Journal of Clinical Microbiology* **16**, 979–981.

ZURAVLEFF, J. J., YU, V. L., SHONNARD, J. W., RIHS, J. D. & BEST, M. (1983). Demonstration of aerosol transmission and subsequent subclinical infections in guinea pigs. *American Review of Respiratory Diseases* **126**, 657–661.