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CASE REPORT

Colonisation of the respiratory tract with *Legionella pneumophila* for 63 days before the onset of pneumonia

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Summary

We report the case of a 70-year-old man who was admitted to hospital A 66 days before developing *Legionella pneumophila* pneumonia 6 days after open heart surgery at hospital C. The strain of *L. pneumophila* recovered from the patient's sputum was of the same subtype (monoclonal antibody type, enzyme type, plasmid profile, and restriction endonuclease pattern) as a strain of *L. pneumophila* in the potable water supplied to the room where he stayed in hospital A. We conclude that the patient's respiratory tract became colonised by *L. pneumophila* while he was in hospital A and persisted for at least 63 days until he developed pneumonia requiring antibiotic treatment while in hospital C.

Introduction

Legionnaires' disease is caused by the ubiquitous aquatic bacterium *Legionella pneumophila*.[1] Two forms of legionellosis—pneumonia (legionnaires' disease) and a non-pneumonic type of illness (pontiac fever) are known.[2–4] The pneumonic form has an incubation period of 2–10 days, the non-pneumonic form has a shorter incubation period of 36 h.[2]

Legionellaceae, of which there are now over 30 species and more than 46 serogroups, are not part of the normal flora of human beings. Inhalation of contaminated aerosols or aspiration of contaminated potable water are the usual mechanisms of acquisition of these organisms by human beings from the environment.[5–7]

Colonisation of the oropharynx (or any part of the respiratory tract) by *Legionella* sp. has not yet been demonstrated.[8] Our report of a case suggests that such colonisation may have taken place.

Materials and methods

Culture of respiratory secretions for legionella

Sputum was inoculated on 5 % sheep blood agar (BA), buffered charcoal yeast extract agar (BCYE) containing 0·1 % alpha-ketoglutarate and two selective media: BCYE containing cefamandole, polymyxin B and anisomycin (MPA)

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and BCYE containing polymyxin B, anisomycin and vancomycin[9] (PAV) (Gibco Laboratories, Madison, WI, U.S.A.). All plates were incubated at 37 °C in a humidified atmosphere containing 5 % CO_2 for 7 days and examined daily. Colonies that morphologically resembled *Legionella* sp. were subcultured on blood and BCYE agar. Those that failed to grow on blood agar were examined by a direct fluorescent antibody technique[10] and use of *L. pneumophila* SG 1 antiserum.

Water samples

Water samples (50 ml) were centrifuged at 3000 rpm for 20 min. The supernatant was removed, leaving approximately 10 % of the original volume in which the sediment was resuspended. A sterile cotton tipped swab (Solon Manufacturing Company, Solon, MN, U.S.A.) was then used to inoculate the surface of BA, BCYE, PAV, and MPA plates. Plates were incubated and colonies identified as outlined above.

Antibody titres to *Legionella pneumophila*

Antibody titres to *L. pneumophila* SG 1 were determined on acute and convalescent serum samples by means of an indirect fluorescent antibody technique.[11] A positive and a negative control were included with each run. All reagents for this test were obtained from the Centers for Disease Control, Atlanta, GA. A four-fold rise in antibody titre to $\geqslant 128$ was considered evidence of recent *L. pneumophila* infection.

Monoclonal antibody typing

Isolates from patients and potable water were typed according to their monoclonal antibody reactivity patterns[12] by Dr J. Joly, Universite Laval, Quebec City.

Plasmid profiles

Portions of the growth obtained after 48 h incubation of the isolates on BYCE agar were suspended in 0·5 ml TE buffer (0·5 M Tris-Hcl pH 8·0, 0·02 M EDTA). After pelleting and resuspending in 25 μl TE, plasmid DNA was extracted from the cells by means of a modified alkaline SDS procedure.[13] The contents of the extracts were determined by electrophoresis on vertical 0·75 % agarose gels followed by ethidium bromide straining. Strains without detectable plasmids constituted profile 0; those carrying a 20 MDa plasmid profile II. Profile III was characterised by the presence of 96 and 72 Mda plasmids while profile VI consisted of a 100 MDa plasmid.

Endonuclease restriction analysis

Chromosomal DNA served as substrate for restriction endonuclease digestions. Recovery from pelleted cells was achieved by means of a modified Roussel-Chabbert procedure.[14] The restriction enzymes *EcoR*I and *Bgl*II were used to differentiate the isolates. Digestion continued for 8 h at 37 °C in buffers provided by the supplier of the enzyme (Boehringer Mannheim, Dorval, Quebec). Restriction fragments were separated in vertical, 0·75 %

agarose gels and visualised by ultraviolet irradiation after ethidium bromide staining of the gels. Resultant distinct patterns were assigned letter codes a, b, c, or d.

Multilocus enzymes analysis

Electromorphs of constitutive legionella enzymes were detected in starch gels by means of the procedures described by Selander *et al.*[15,16] Enzymes examined were: malate dehydrogenase, isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, leucine dehydrogenase, lysine dehydrogenase, indophenol oxidase, aspartate dehydrogenase, aconitase, peptidase 1, leucine aminopeptidase and adenylate kinase. Enzyme types were designated by arbitrarily selected numbers.

Case report

A 70-year-old man was admitted to hospital A on 18 July 1989 and discharged on 21 July 1989. While there he underwent cardiac catheterisation and was found to have severe aortic stenosis, occlusion of his right coronary artery and critical stenosis of the left anterior descending coronary artery. The left ventricular ejection fraction was 29%. A chest radiograph obtained on 19 July 1989 at this hospital is labelled 19.7.89 A in Plate 1. A right pleural effusion, pulmonary venous congestion and left ventricular hypertrophy are evident. He was afebrile throughout his stay.

Increasing shortness of breath when the patient visited hospital A on 8 September 1989 as an out-patient prompted another chest radiograph (see Plate 1). There was very little change from the previous chest radiograph but on this occasion the radiologist's report indicated possible right basal consolidation with loss of volume. The next day, the patient was admitted to hospital B because of the above symptoms. He also had orthopnoea and ankle oedema but was without a cough. He had smoked 130 packs of cigarettes per year. On examination, he was afebrile, pulse rate 120/min, respiratory rate 28/min. Crackles were heard on auscultation over both lower lung fields extending half way up the chest. Wheezes were present over the right lower lobe. The jugular venous pulse was visible 4 cm above the sternal angle; third and fourth heart sounds were present and a loud crescendo/decrescendo systolic ejection murmur was heard along the left sternal border radiating to the carotid arteries. The congestive heart failure was treated with diuretics while the patient remained in hospital B from 8 September to 15 September 1989. A chest radiograph obtained in hospital B is shown as B in Plate 1. This radiograph is essentially similar to that obtained 3 days previously. The patient remained afebrile during his stay in hospital B.

On 15 September 1989, the patient was transferred to hospital C and underwent aortic valve replacement and two-vessel coronary artery bypass grafting on 16 September 1989. A chest radiograph was obtained post-operatively on this day and is shown in Plate 1 as 16.09.89 C. This radiograph was obtained while the patient was supine so that most of the pleural fluid had shifted to the apex of the right lung. A rounded opacity also is evident at the base of the right lung. The post-operative course was complicated by bleeding

and hypotension. The white blood cell count rose to 23×10^9/l. On 22 September the patient developed a cough with brown sputum. Two doses of hydrocortisone sodium succinate (250 mg/dose) were given intravenously. On 22 September an infectious disease consultant diagnosed pneumonia and obtained sputum for culture for legionella. Four days later, growth of *L. pneumophila* serogroup 1 was reported. A chest radiograph obtained on 25 September 1989 showed diffuse opacities in the left upper lobe and left mid zone (not shown). Blood cultures obtained on 22 September 1989 grew *Enterococcus faecalis* and *Staphylococcus epidermidis*. At this time the white blood cell count was 28×10^9/l. Therapy was begun with erythromycin and ceftriaxone. On 24 September, when results of the blood cultures were available, treatment with vancomycin was started while that with ceftriaxone was stopped. Erythromycin continued to be given until 13 October 1989. The patient's temperature did not rise above 37·3 °C during his stay in hospital.

A chest radiograph obtained on 13 October, 1989, at which time there had been marked clinical improvement, is shown in Plate 1 as 13.10.89 C. Pleural fluid may be seen still to have been present on the right side while the rounded opacity on that side is evident also. A resolving opacity may be seen in the left lower lung field. The patient was discharged from hospital on 22 October 1989 and remained well as of July 1990. Acute stage and 2 week convalescent phase serum antibody titres to *L. pneumophila* serogroup 1 were $\leqslant 64$.

Results of typing potable water and clinical isolates of *Legionella pneumophila*

As soon as the results of the sputum culture became available, water samples were obtained from the rooms in hospitals A, B, and C where our patient had stayed. *Legionella pneumophila* SG 1 was isolated from the potable water samples obtained from hospitals A and B. These isolates were then characterised according to their reactivity with monoclonal antibodies specific to serogroup 1; plasmid complements; possession of structural variants of constitutive enzymes and comparison of their endonuclease digestion patterns (Table I).

Discussion

Legionnaires' disease has occurred sporadically at hospital A since 1983 (T. J. Marrie, unpublished observations) and its potable water has been contaminated by *L. pneumophila* since then. Isolates of *L. pneumophila* associated with this hospital have been consistently of only one subtype: plasmid negative, endonuclease pattern C and monoclonal antibody type, France (both patient and water isolates). So too, was the isolate recovered on 26 September 1989 from the potable water in the room occupied by our patient during the period 18–21 July 1989 (Table I). Hospital B has had an intermittent low rate of detection of *L. pneumophila* in its potable water. Three isolates of *L. pneumophila* from this hospital's water in early October 1989 belonged to subtypes distinct from that of hospital A (Table I). The water of hospital C was also contaminated by *L. pneumophila*. Potable water taken from the area occupied by the patient in hospital C on the day his sputum was found to be positive was negative for *Legionella sp. Legionella pneumophila* recovered from

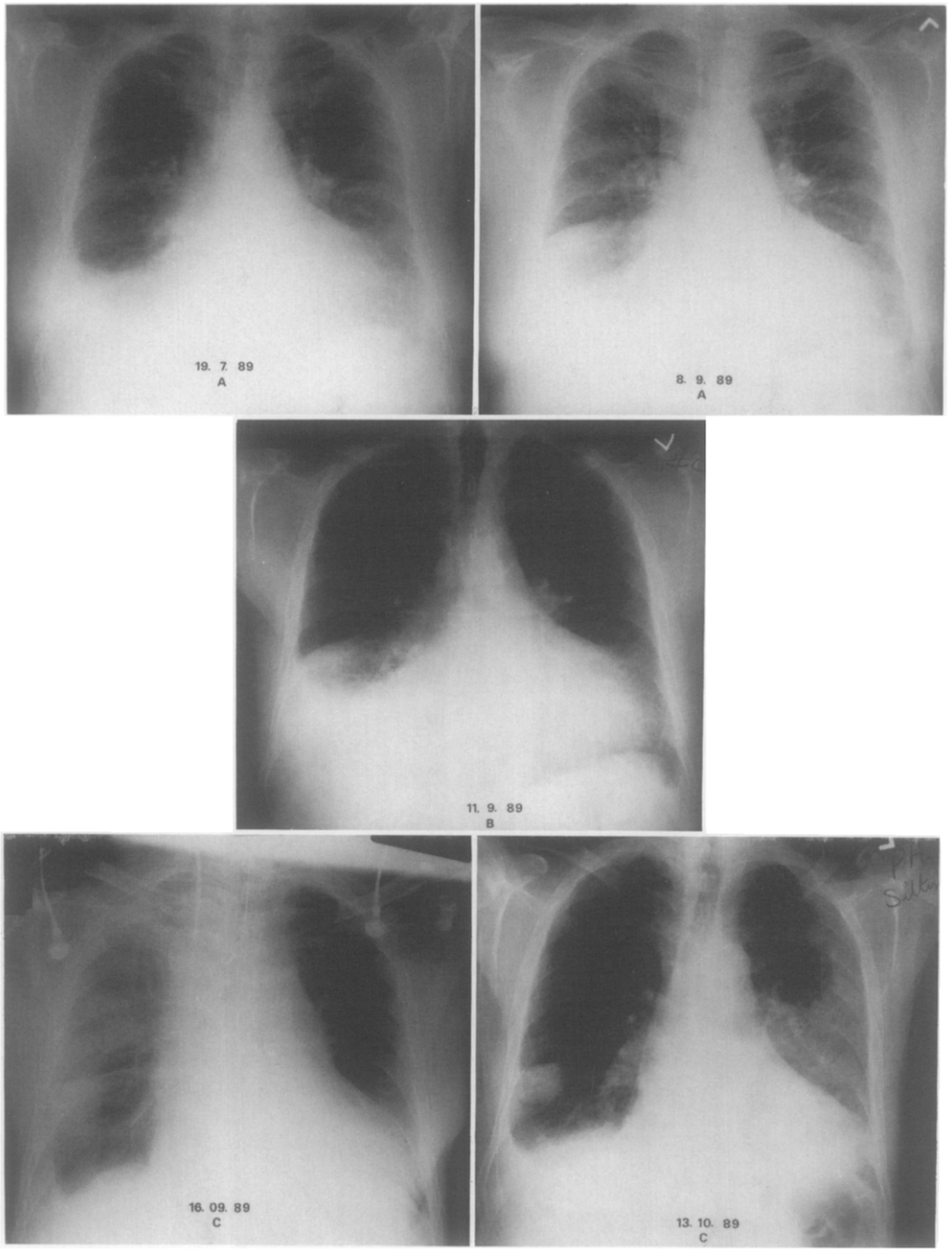

Plate 1. Chest radiographs obtained during the patient's stay in hospitals A, B and C on the dates indicated. The one designated 8.9.89A was obtained while the patient was attending as an outpatient. Radiographs marked 19.7.89A; 8.9.89A; 11.9.89B were obtained while the patient was afebrile and did not have any symptoms or signs of pneumonia. The radiograph marked 16.09.89C was obtained immediately after aortic valve replacement and coronary artery bypass grafting. The radiograph marked 13.10.89C shows a resolving left pulmonary opacity. Note the right pleural effusion on all radiographs and the non-resolving rounded opacity in the right lower lobe (interpreted as a pseudotumour).

Table I *Results of various methods of subtyping the clinical and potable water isolates of* Legionella pneumophila *serogroup* 1

Isolate		Typing method			
Source	Date	Monoclonal antibody type	Plasmid profile	Restriction endonuclease analysis	Allo-enzyme
Patient	23 September 1989	France	O	c	3
Potable water					
Hospital A	26 September 1989	France	O	c	3
Hospital B	5 October 1989	OLDA	VI	b	N.D.
		Oxford	III	b	N.D.

N.D. = Not done.

other sites in hospital C during this period belonged to subtypes different from those associated with hospital A.

The patient's isolate was the same subtype of *L. pneumophila* as that recovered from the potable water in the room in hospital A which he occupied 63–66 days previous, i.e. 18–21 July 1989 (Table I). This room was positive for *Legionella* sp. before his admission and water from the same wing in this hospital was contaminated with the identical subtype in June 1988. The patient's only other contact with hospital A was to obtain a chest radiograph as an out-patient on 8 September 1989. This chest radiograph shows only a minor change from the one obtained on 19 July 89 (see Plate 1)—although the radiologist did report possible right basal consolidation with loss of volume. The patient was asymptomatic at that time. We have not found any patients with *Legionella* sp. following exposure to the radiology department or any of the outpatient departments of hospital A despite 8 years of observation. Thus, the evidence points to acquisition of *Legionella* sp. during his stay in hospital A rather than during the visit to the radiology department. Our failure to detect *Legionella* sp. in potable water from the room occupied by the patient in hospital C further implicates hospital A as the source of the organism. We have typed over 200 isolates of *L. pneumophila* SG 1 from hospital C over the past 4 years but have never recovered monoclonal antibody type France.

The maximum incubation period for Legionnaires' disease is believed to be 10 days. The patient described here was exposed to hospital A 63–66 days before isolation of *L. pneumophila* from his sputum. The only reasonable explanation is that he became colonised somewhere in his respiratory tract by *L. pneumophila* during his stay in hospital A. It is likely that inoculation of his lower respiratory tract by the colonising *Legionella* sp. took place during surgery or ventilator therapy. He was discharged from hospital A on 21 July 1989 and developed pneumonia on 22 September thereby indicating an incubation period of at least 63 days.

The question arises as to whether or not the patient did have legionella pneumonia. He was bacteraemic with *E. faecalis* and *S. epidermidis* at the time *Legionella* sp. was isolated but he did not seroconvert to *Legionella* sp. The

interval between the acute and convalescent stage samples of serum in this case was only 2 weeks. Six or more weeks are required for seroconversion by some patients and up to 20% patients with Legionnaires' disease do not seroconvert.[2] If he did not have legionella pneumonia, this would further strengthen the argument for long-term colonisation.

It is remotely possible that he could have acquired the organism at home. Unfortunately, we did not culture the potable water at his home but have cultured potable water from 20 private homes throughout the city of Halifax and all samples have been negative.

One implication of our observation is that patients believed to have 'community-acquired' Legionnaires' disease should be asked about any visit to hospital during the 2 months before onset of their current symptoms.

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