

## Recognition of a Second Serogroup of *Legionella longbeachae*

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A strain of *Legionella longbeachae* (Tucker 1) that was isolated from the postmortem lung tissue of a pneumonia patient was serologically distinct from four other strains of *L. longbeachae*. The recognition of a second serogroup of *L. longbeachae* represents the first reported instance of serogroup diversity within a species of *Legionella* other than *L. pneumophila*. The disease caused by the Tucker 1 strain does not seem to be readily distinguishable from that of pneumonia caused by other legionellae.

A newly recognized species of *Legionella*, *L. longbeachae*, was recently described by McKinney et al. (12) and was shown to cause respiratory illness. The recognition of this organism extends the number of species in the genus *Legionella* to six. Previously described *Legionella* species shown to be etiological agents causing pneumonia are: *L. pneumophila* (3, 11), *L. dumoffii* (2), *L. bozemanii* (2), and *L. micdadei* (10). Another species, *L. gormanii* (16), is represented by an environmental isolate (6). This species has been serologically, although not yet culturally, implicated in human pneumonia (16). Of these, only *L. pneumophila* has been subdivided into discrete serogroups on the basis of direct fluorescent antibody testing (7, 13, 15).

Like other *Legionella* species, *L. longbeachae* is a fastidious gram-negative bacillus, which grows on charcoal yeast extract (CYE) agar (8) and fails to grow on either Trypticase soy (BBL Microbiology Systems, Cockeysville, Md.) or blood agar. The organism is serologically distinct but reacts similarly to *L. pneumophila* in biochemical tests, except that *L. longbeachae* is negative in a hippurate hydrolysis test, whereas 98% of *L. pneumophila* strains tested are positive (9). The cellular fatty acid composition of *L. longbeachae* is not readily distinguished from that of *L. pneumophila*.

In the short time since it was recognized, *L. longbeachae* serogroup 1 has been implicated in at least six cases of pneumonia. Four of these have been previously described (12). One of the other two cases was in a patient in Ohio; an isolate from a bronchial washing from this patient was identified as *L. longbeachae* serogroup 1. The other case was in a patient in North Carolina; two sequential sputum specimens from

this patient were positive by direct fluorescent antibody testing for *L. longbeachae* serogroup 1 (unpublished data). Thus, it appears that *L. longbeachae* may be an important cause of legionellosis.

This report describes the isolation from a fatal case of pneumonia of another strain of *L. longbeachae* with serological characteristics distinct from those strains (Long Beach 4, Los Angeles 24, Concord 1, and Atlanta 5) reported by McKinney et al. (12).

### CASE REPORT

A 74-year-old white male was admitted to Doctors Hospital, Tucker, Ga., on 27 June 1980, with progressive dyspnea, fever, and chills which began 3 days before admission. During the 3-day hospitalization, the patient's condition continued to deteriorate, and despite aggressive therapy, he became comatose and died on 1 July 1980.

The patient's medical history included a diagnosis of empyema of the gall bladder in 1971, at which time a cholecystectomy was performed. In 1975, the patient was diagnosed as having squamous cell carcinoma of the tongue for which cobalt irradiation therapy was given. Also during this year, diabetes mellitus was diagnosed. Since 1975, the patient had repeated bouts of congestive heart failure, secondary to atherosclerotic heart disease. In November 1979, he had an acute myocardial infarction.

At the time of this admission, the patient's vital signs were: temperature, 40.0°C; blood pressure, 120/50 mmHg; respiration, 28/min; and pulse rate, 120/min. The admitting leukocyte count was 32,000/mm<sup>3</sup>, with 84% polymorphonuclear leucocytes, 11% bands, 1% metamyelocytes, 1% promyelocytes, 1% mononuclear cells, and 2% lymphocytes. Toxic granulation (2+) was noted. A chest X-ray on admission revealed extensive left basilar pneumonia and congestive heart failure.

During the 3-day hospitalization, the leukocyte

count rose progressively to 78,900/mm<sup>3</sup>, with an increasing left shift, toxic granulation, and Dohle bodies. Chest X-rays revealed progressive bilateral pneumonia infiltrates. The patient continued to have a markedly elevated temperature, ranging as high as 41.5°C the day before he died.

Two sequential sputum cultures showed *Staphylococcus epidermidis* and normal flora. A bronchial washing taken on 29 June 1980 showed no bacterial growth from inoculations onto chocolate, thioglycolate, blood, and eosin methylene blue agars.

Initially, the patient was treated with 80 mg of gentamicin intravenously every 8 h; keflin (2 g) was added intravenously every 6 h; and lanoxin (0.25 mg per day) and acetaminophen (10 grains every 2 h) were also administered. After 48 h, erythromycin (1 g every 6 h) was added intravenously. The patient received a total of six doses of erythromycin before his death.

Postmortem examination revealed moderate gastrointestinal hemorrhage, hepatomegaly (2,600 g) with advanced macronodular cirrhosis, massive splenomegaly (900 g), and advanced generalized atherosclerosis. A single prominent verrucous vegetation (0.6 by 0.15 cm) was noted adhering to the pulmonary artery endothelium immediately above the valve cusp. A similar smaller vegetation was attached to the right atrial endocardium. The 1,600-g left lung revealed complete consolidation of the lower lobe and partial consolidation of the upper lobe. The 1,300-g right lung revealed patchy consolidation and congestive changes in all three lobes. The left hemithorax contained 300 cm<sup>3</sup> of a clear yellow fluid, and the right hemithorax contained a smaller amount of a similar fluid. Microscopy revealed an extensive lobar pneumonia. Major bronchi in the left lower lobe were occluded with amorphous mucinous material admixed with acute and chronic inflammatory elements. The alveolar spaces were filled with degenerating polymorphonuclear leukocytes and mononuclear inflammatory cells along with various amounts of eosinophilic proteinaceous material and fibrin. Other portions of the lung were representative of an earlier consolidation stage of pneumonia wherein the distended alveoli were filled with fibrin and mixed inflammatory elements, including numerous histiocytes. Dieterle silver impregnation stain revealed numerous small bacilli in the lung parenchyma, both extracellularly and within histiocytes and polymorphonuclear leukocytes. Several acid-fast stains (Ziehl-Neelsen, Kinyoun's, and Fite-Faraco) and tissue Gram stains (Brown and Brenn and Brown-Hopps) failed to reveal these organisms in lung tissue sections. Microscopic examination of the verrucous vegetations showed a coagulum of fibrin and blood cells, with ulceration of the underlying endothelial surface. No organisms were revealed when the lesions were examined by using the above staining procedures.

#### MATERIALS AND METHODS

Scrapings of Formalin-fixed lung, homogenates of fresh lung, and sections of Formalin-fixed tissues were examined by direct fluorescent antibody tests as previously described (5). Impression smears of fresh lung were made on CYE agar and streaked for isolation.

Dilutions of the lung homogenate were also inoculated to CYE agar. All cultures were incubated in a moist chamber at 35°C. Antibody to the isolate, Tucker 1, was produced in rabbits as described in previous studies of *L. pneumophila* (14), except that Freund adjuvant was not used. The immunoglobulin G (IgG) portion of the antiserum was purified by affinity chromatography on a staphylococcal protein A-Sepharose CL4B (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) column, and a fluorescein isothiocyanate conjugate was prepared by methods previously described (14). Biochemical testing and DNA relatedness studies were carried out by procedures used in the characterization of *L. pneumophila* (1, 4, 9, 18). Sections of the lung and other organs obtained at autopsy were cut at 4 µm in thickness, deparaffinized, and stained with routine histological stains and Dieterle silver impregnation stain (17).

#### RESULTS

Fresh and Formalin-fixed tissues from the lung obtained at autopsy were negative when tested with fluorescent antibody conjugates for *L. pneumophila* serogroups 1 to 6, *L. micdadei*, *L. dumoffii*, *L. bozemanii*, and *L. gormanii*.

Within 3 to 4 days, colonies morphologically resembling *Legionella* species grew on all CYE plates inoculated with the fresh lung tissue. The isolate (Tucker 1) proved to be a *Legionella*-like organism, failing to grow on Trypticase soy or blood agar and producing browning on tyrosine yeast extract agar. Tucker 1 exhibits a cellular fatty acid composition similar to the compositions of *L. longbeachae* strains Long Beach 1, Los Angeles 24, Concord 1, and Atlanta 5 (C. Wayne Moss, personal communication). The organism is positive for catalase, oxidase, and β-lactamase, liquifies gelatin, fails to reduce nitrate, and is negative in a hippurate hydrolysis test. The isolate was negative when tested with fluorescent antibody conjugates to those *Legionella* species and serogroups mentioned above.

The high relative binding ratios obtained in both 60 and 75°C reactions with unlabeled deoxyribonucleic acid (DNA) from the Tucker 1 isolate and labeled DNA of Long Beach 4 indicate near identity between the two strains. In contrast, the relative binding ratios of DNAs from the type strains of *L. pneumophila*, *L. bozemanii*, *L. micdadei*, *L. dumoffii*, and *L. gormanii* to Long Beach 4 are quite low at 60°C and essentially disappear at 75°C (Table 1).

When the fluorescent antibody conjugate for *L. longbeachae* strain Long Beach 4 became available, the isolate and sections and homogenates of the autopsy lung were retested with this reagent. Clinical specimens were negative, and a 1:8 dilution of the conjugate, which contained an IgG concentration of 10 mg/ml when undi-

TABLE 1. Relative binding ratios of DNA from *L. longbeachae* strain Long Beach 4

Unlabeled DNA strain source <sup>a</sup>	RBR <sup>b</sup> , 60°C	RBR, 75°C
Long Beach 4	100	100
Tucker 1	99	94
Philadelphia 1	8	2
WIGA	17	4
TATLOCK	4	1
NY-23	12	1
LS-13	25	

<sup>a</sup> Long Beach 4, type strain of *L. longbeachae*; Philadelphia 1, type strain of *L. pneumophila*; WIGA, type strain of *L. bozemanii*; TATLOCK, type strain of *L. micdadei*; NY-23, type strain of *L. dumoffii*; LS-13, type strain of *L. gormanii*.

<sup>b</sup> Relative binding ratio (RBR) = (% heterologous DNA bound to hydroxyapatite)/(% homologous DNA bound to hydroxyapatite) × 100.

TABLE 2. Direct fluorescent antibody staining characteristics of five strains of *L. longbeachae*

Isolate	Conjugate <sup>a</sup>		
	Tucker 1	Long Beach 4	Tucker 1 absorbed with Long Beach 4 cells
Tucker 1	256	— <sup>b</sup>	128
Long Beach 4	8 <sup>c</sup>	1,024	—
Los Angeles 24	8 <sup>c</sup>	1,024	—
Atlanta 5	8 <sup>c</sup>	512	—
Concord 1	—	512	ND <sup>d</sup>

<sup>a</sup> Reciprocal of highest dilution to give a very bright (4+) staining intensity.

<sup>b</sup> —, Negative staining with a 1:8 dilution of the conjugate.

<sup>c</sup> Staining intensity, 3+; very dim (1 to 2+) staining at a 1:64 dilution.

<sup>d</sup> ND, Not done.

luted, stained the cells of Tucker 1 very dimly (2+). The fluorescent antibody conjugate prepared to Tucker 1 gave very bright (4+) staining of the homologous antigen to a dilution of 1:256. Some cross-reactivity was found between this conjugate and the cells of *L. longbeachae* strains Long Beach 4, Los Angeles 24, and Atlanta 5 at lower than diagnostic dilutions. No reaction was observed with Concord 1 cells. Specificity of the conjugate was easily obtained by absorption with Formalin-killed cells of the strain Long Beach 4. Only a one-tube loss in sensitivity for the Tucker antigen resulted from the absorption (Table 2). All other species and serogroups of *Legionella* and 400 strains of other organisms representing 57 genera and 140 species were negative when tested with the Tucker 1 conjugate.

Sections and homogenates of the autopsied lung examined with the Tucker 1 conjugate revealed numerous brightly stained rods both extracellularly and within polymorphonuclear leukocytes. No organisms were seen in sections of liver, spleen, kidney, brain, heart, and pulmonary artery (specifically including the verrucous lesions noted grossly), which were similarly stained with the Tucker 1 conjugate.

## DISCUSSION

The disease caused by the *L. longbeachae* Tucker 1 strain does not seem to be readily distinguishable from that of pneumonia caused by other legionellae. Infection with the Tucker 1 strain appears to have been confined to the respiratory system, as judged by direct fluorescent antibody staining results with the Tucker 1 conjugate. The generally poor physical condition of the patient before his pneumonia, the history of chronic alcoholism, and the history of atherosclerotic heart disease are sufficient to account for most of the abnormalities observed at autopsy in organs outside of the respiratory system.

DNA hybridization studies show that Tucker 1 and Long Beach 4 were related at the species level. The Tucker 1 isolate was also similar to the other *L. longbeachae* strains with regard to cultural characteristics, biochemical tests, and cellular fatty acid composition. The organism was, however, readily distinguished from Long Beach 4, Los Angeles 24, Atlanta 5, and Concord 1 by direct fluorescent antibody staining. Specificity of the Tucker 1 conjugate can be obtained by absorbing the cross-reacting antibodies with the heterologous Formalin-killed antigen or, more easily, by using a dilution of the conjugate which fails to stain Long Beach 4 cells. We designate Tucker 1, ATCC no. 33484, as the reference strain for *L. longbeachae* serogroup 2. This represents the first recognized instance of serogroup diversity within a species of *Legionella* other than *L. pneumophila*.

Direct and indirect fluorescent antibody tests are the most widely used criteria for confirming a diagnosis of legionellosis. Thus, it is important that, as new species and serogroups of *Legionella* are recognized, the antigens and conjugates for these organisms be included in indirect and direct fluorescent antibody tests, respectively. Further, the recognition of serogroups within species provides a valuable tool in epidemiological studies. The awareness of physicians and clinical personnel concerning isolation techniques and treatment of clinical specimens submitted to reference laboratories is imperative to the continued isolation of new species and sero-

groups of *Legionella*. Specimens should be cultured on CYE (13) agar or quick-frozen in aseptic containers and sent packed in dry ice to the appropriate reference laboratory.

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