

## Second Serogroup of *Legionella hackeliae* Isolated from a Patient with Pneumonia

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**A *Legionella*-like organism (strain 798-PA-H, ATCC 35999) isolated from a lung aspirate was shown by DNA hybridization to belong to the species *Legionella hackeliae*. Growth, gas-liquid chromatographic, and physiologic characteristics were consistent with those of the type strain of *L. hackeliae*, Lansing 2 (ATCC 35250). However, 798-PA-H was minimally reactive with *L. hackeliae* direct immunofluorescence assay conjugate or slide agglutination test antiserum. Cross-absorption studies with 798-PA-H and Lansing 2 antisera supported placing 798-PA-H in a second serogroup of *L. hackeliae*.**

*Legionella hackeliae* is one of 22 species in the genus *Legionella* (1). The type strain of the species, Lansing 2, was isolated from a bronchial biopsy specimen from a patient with pneumonia. We report here on a second serogroup of *L. hackeliae* isolated from a lung aspirate from an immunocompromised woman with pneumonia.

### CASE REPORT

A 52-year old woman was admitted to Montefiore Hospital in March 1982 for an unidentified illness, which included fever and diffuse lymphadenopathy. One month before, she had required endotracheal intubation for congestive heart failure and, during that time, had been placed on 20 mg of prednisone per day for treatment of hypereosinophilia with pulmonary infiltrates. During the present admission, although she was asymptomatic, a new left-upper-lobe infiltrate was found on a chest x ray. A biopsy of an enlarged cervical lymph node revealed immunoblastic lymphadenopathy with transformation to immunoblastic sarcoma. A gram stain of material obtained from a percutaneous lung aspirate of the left upper lobe revealed numerous polymorphonuclear leukocytes and small gram-negative rods. A modified Kinyoun stain was negative, as were direct immunofluorescence assays (DFA) (2) for *L. pneumophila* serogroups 1 to 6, *L. micdadei*, *L. dumoffii*, *L. bozemanii*, *L. gormanii*, and both serogroups of *L. longbeachae*. After the biopsy, the patient was started on cefamandole (1 g every 6 h). Her fever abated and, after 10 days, the antibiotic was discontinued. She was discharged 5 days later. Subsequently, a *Legionella*-like organism not reactive with any available anti-*Legionella* conjugate was grown from the lung aspirate material on buffered charcoal-yeast extract (BCYE) agar. Cultures for routine aerobic and anaerobic bacteria, mycobacteria, and fungi were negative. Follow-up chest x rays over the next 3 months disclosed gradual resolution of the pulmonary infiltrate.

### MATERIALS AND METHODS

The *Legionella*-like organism 798-PA-H was sent to the Immunology Section, Centers for Disease Control, for further characterization. The organism was inoculated onto BCYE agar, BCYE agar without cysteine, and blood agar as previously described (4). The strain was tested with working

dilutions of all available DFA conjugates (2): *L. pneumophila* serogroups 1 to 9 and strains Seattle 1 and San Francisco 9, *L. longbeachae* serogroups 1 and 2, *L. bozemanii* serogroup 1, *L. dumoffii*, *L. micdadei*, *L. gormanii*, *L. wadsworthii*, *L. oakridgensis*, *L. feeleyi* serogroup 1, *L. sainthelensi*, *L. spiritensis*, *L. hackeliae*, *L. jordanis*, and *L. rubrilucens*. Antisera to all known *Legionella* species ( $n = 22$ ) and serogroups ( $n = 34$ ) were used in the slide agglutination test (2a, 3). Antisera to strain 798-PA-H were prepared, and cross-absorption experiments were performed as previously described (3). Gas-liquid chromatography was performed by William R. Mayberry, East Tennessee State University, Johnson City. DNA hybridization and physiologic studies were performed as previously described (1).

### RESULTS

The organism grew on BCYE agar but not on BCYE agar without cysteine or on blood agar. By DFA, strain 798-PA-H stained minimally (1 to 2+) and only with the *L. hackeliae* Lansing 2 conjugate. The only agglutination observed was with antiserum to *L. hackeliae* Lansing 2 (serogroup 1), but the reaction strength (2+) was below the minimal positive level.

DNA studies showed that strain 798-PA-H belonged to the species *L. hackeliae*, with 85% relatedness to Lansing 2 and less than 25% relatedness to all other *Legionella* species. Gas-liquid chromatographic profiles were consistent with those for Lansing 2 (William R. Mayberry, personal communication). Physiologic test results were identical to those for Lansing 2 (1), with the exception of oxidase: strain 798-PA-H was oxidase negative, whereas Lansing 2 was weakly oxidase positive. Both organisms gave positive test results for catalase, gelatinase, beta-lactamase, motility, and browning of tyrosine-supplemented agar and negative test results for urease, nitrate reduction, glucose fermentation, autofluorescence, and hippurate hydrolysis.

Reciprocal absorption studies with the Lansing 2 and 798-PA-H antisera and strains showed that cross-reactive antibodies could be removed by absorption with the heterologous strain without removing antibodies to the homologous strain (Table 1). This supports the designation of 798-PA-H (ATCC 35999) as a second serogroup of *L. hackeliae*.

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TABLE 1. Slide agglutination antibody titers of unabsorbed and absorbed rabbit antisera against *L. hackeliae* strains

| Antiserum         |                              | Slide agglutination antibody titer <sup>a</sup> against indicated antigen |          |
|-------------------|------------------------------|---------------------------------------------------------------------------|----------|
| Immunizing strain | Absorbed with <sup>b</sup> : | Lansing 2                                                                 | 798-PA-H |
| Lansing 2         | Nothing                      | 32                                                                        | 16       |
|                   | 798-PA-H (1)                 | 32                                                                        | 8        |
|                   | 798-PA-H (2)                 | 16                                                                        | <2       |
| 798-PA-H          | Nothing                      | 8                                                                         | 64       |
|                   | Lansing 2 (1)                | 4                                                                         | 64       |
|                   | Lansing 2 (2)                | <2                                                                        | 16       |

<sup>a</sup> Dilution giving 2+ or greater agglutination.<sup>b</sup> Numbers in parentheses represent the number of times undiluted antiserum was absorbed.

## DISCUSSION

Characterization of a second serogroup of *L. hackeliae* brings to five the number of *Legionella* species with multiple serogroups. *L. hackeliae* serogroup 2 is the 35th serogroup within the genus. Of these, 24 have been shown to be associated with human illness by isolation from or DFA staining of tissues or respiratory secretions. To our knowledge, *L. gormanii* is the only species for which antisera have been available that has not yet been directly documented as being associated with human legionellosis. Until recently, reagents were not available for the 11 most recently characterized serogroups; these included only one isolate from

clinical specimens (*L. hackeliae* [1]). The remaining 10 species were isolated from environmental specimens.

The large number of antisera required to identify all known legionellae has made DFA staining with monospecific conjugates impractical. Isolation of the organism should always be attempted. This is especially important in identifying *Legionella*-like organisms that do not react or that react minimally with antisera to presently known species or serogroups. In the present study, a specific diagnosis of the pneumonia and demonstration of *L. hackeliae* serogroup 2 as the etiologic agent were possible only after the organism had been isolated.

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