JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1980, p. 395-401
0095-1137/80/09-0395/07$02.00/0
Vol. 12, No. 3

Legionella pneumophila Serogroup Six: Isolation from Cases of Legionellosis, Identification by Immunofluorescence Staining, and Immunological Response to Infection

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Isolates of *Legionella pneumophila* that are serologically different from strains of serogroups 1 through 5 were obtained from lung biopsy tissue or pleural fluid from three renal transplant recipients in Chicago, Ill. These strains were placed in a newly designated *L. pneumophila* serogroup, serogroup 6, on the basis of fluorescent-antibody staining characteristics. An *L. pneumophila* strain obtained from Bethesda, Md., one from Houston, Tex., and one from Oxford, England, also belong to this new serogroup. *L. pneumophila* serogroup 6 appears to be widely distributed geographically.

Legionellosis is caused by a gram-negative bacillus (10) for which the name *Legionella pneumophila* has been proposed (2). Legionellosis is manifested as Legionnaires disease, with pneumonia as the prominent feature, or as Pontiac fever, in which the absence of pneumonia is typical (8-10). Both forms of the disease are probably acquired from environmental sources.

In early studies of *L. pneumophila*, Cherry et al. (3) prepared direct fluorescent-antibody (DFA) conjugates for five strains, and these reagents were evaluated in staining tests with the 10 strains of *L. pneumophila* that were available to them at that time. Although some differences in intensity of staining among the 10 strains were observed, a conjugate prepared for the Knoxville 1 strain stained all strains brightly when used at the appropriate dilution. The first evidence of distinct serogroups of *L. pneumophila* came with isolation of Togus 1, which did not stain with the Knoxville 1 conjugate (12). Shortly thereafter a total of four serogroups were designated (11). Serogroups 1, 2, 3, and 4 comprise strains staining with conjugates for Knoxville 1, Togus 1, Bloomington 2, and Los Angeles 1, respectively. Recently, a fifth serogroup was recognized of which there are presently a number of environmental isolates and the human isolate Cambridge 2 (A. C. England, R. M. McKinney, P. Skaliy, and G. W. Gorman, Ann. Intern. Med., in press).

When serogroup 1 to 4 antigens were used in the indirect immunofluorescence assay (IFA), Wilkinson et al. (20, 21) found that the human antibody response to *L. pneumophila* infection could be serogroup specific. That is, fourfold rises in titer to a significant level often occurred against only one serogroup antigen. In many cases, however, the antibody response appeared to be against an antigen common to *L. pneumophila* serogroups 1 to 4, and in a few other cases, it appeared to be against a nonspecific, gram-negative bacterial antigen. The latter cross-reactions could be inhibited by prior mixing of the sera with a hot aqueous extract of *Escherichia coli* O13:K92:H4, an antigen-blocking fluid (20).

In this report we describe the DFA staining characteristics of six *L. pneumophila* isolates of a new serogroup (serogroup 6) and show the antibody response by IFA of two of the patients from whom the isolates were obtained.

MATERIALS AND METHODS

Isolation and characterization of the organisms. *L. pneumophila* strains Chicago 2, 3, and 4 were isolated from human lung tissues from three renal transplant patients with radiologically documented pneumonia. Houston 2 was obtained as a reference diagnostic culture from the Texas State Public Health Laboratory, Houston, Tex. Oxford 1 was kindly provided by R. J. Fallon. Bethesda 1 was kindly provided by the Microbiology Service of the National Institute of Health Clinical Center, Bethesda, Md. The six strains were identified as *L. pneumophila* by previously described growth requirements and characteristics (19) and by cellular fatty acid composition (14). Chicago 2 and Houston 2 were further characterized as *L. pneumophila* by deoxyribonucleic acid relatedness measurements (2).

The six strains of serogroup 1 listed in Table 1 were among those studied by Cherry et al. (3). Togus 1 is

TABLE 1. *L. pneumophila strains tested by DFA staining with Chicago 2 (serogroup 6) conjugate*

Serogroup 1[a, b]	Serogroup 2[b]	Serogroup 3[c]	Serogroup 4[b]	Serogroup 5[b]	Serogroup 6[d]
Philadelphia 1	Togus 1	Bloomington 2[e]	Los Angeles 1	Dallas 1E[e]	Chicago 2
Knoxville 1	Atlanta 1	Burlington 4	Baltimore 2	Dallas 2E[e]	Chicago 3
Bellingham 1	Atlanta 2	Detroit 5	SRP20[e]	Dallas 3E[e]	Chicago 4
Flint 1	Atlanta 4		SRP22[e]	Burlington 1E[e]	Houston 2
Albuquerque 1	Macon 1		SRP23[e]	Cambridge 2	Oxford 1
Burlington 1	York 1		SRP26[e]		Bethesda 1

[a] These six plus 39 additional *L. pneumophila* strains of serogroup 1 were tested.
[b] All were negative with the serogroup 6 conjugate at a dilution of 1:8.
[c] Cross-reactivity of 2^+ to 3^+ fluorescence intensity was observed.
[d] Fluorescence of 4^+ intensity was observed with all serogroup 6 isolates.
[e] Environmental isolates.

the reference strain for serogroup 2 (12). The other five serogroup 2 strains (Table 1) were isolated from diagnostic specimens received at the Center for Disease Control Atlanta, Ga. Bloomington 2 is the environmental isolate BL 433 reported by Morris et al. (13). Burlington 4 is a clinical isolate of serogroup 3 kindly provided by W. Winn. Detroit 5 is from the Henry Ford Hospital, Detroit, Mich.; Los Angeles 1 was isolated by Edelstein et al. (4). Baltimore 2 was reported by Ormsbee et al. (16). The serogroup 5 strains Dallas 1E, 2E, and 3E and Burlington 1E are reported by England et al. (England et al., in press.) Cambridge 2 is a clinical isolate reported by Nagington et al. (15).

Antisera and DFA conjugates. Antisera and conjugates were prepared for the Chicago 2 strain by methods described previously (12), except that Freund adjuvant was not used. The serogroup 3 (Bloomington 2) conjugate used in this study was described previously (11). Conjugates were adjusted to contain 10 mg of immunoglobulin G (IgG) per ml.

DFA staining. Heat-fixed cells were stained and examined by fluorescence microscopy as described by Cherry et al. (3). Fluorescence intensities were graded from strong to weak on a diminishing scale from 4^+ to 1^+. Serial twofold dilutions of each conjugate were prepared for testing with strains of the homologous serogroup. The working dilution of the conjugate was taken as one twofold dilution lower than the highest dilution that produced fluorescence of 4+ intensity with all tested strains of the homologous serogroup. Cross-reactions were recorded as that intensity of fluorescence of strains of the heterologous serogroup that was observed upon staining with the working dilution of the conjugate.

Absorption of conjugates. Cells for absorption were grown for 48 h at 35°C on charcoal-yeast extract agar plates (6). The cells were harvested by gentle scraping of the charcoal-yeast extract agar plates and rinsing with 1% Formalin in 0.85% NaCl. The growth was transferred to sterile tubes, and after standing overnight in Formalin-saline, the cells were washed two times by centrifuging from suspension in 0.85% NaCl with 0.2 M sodium borate, pH 7.5. Five milliliters of packed cells was used to absorb 10 ml of conjugate containing 5 mg of IgG per ml. The suspension was maintained at 4°C overnight, after which the absorbed conjugate was separated by centrifuging. Chicago 2 conjugate was absorbed with Bloomington 2 cells and Bloomington 2 conjugate was absorbed with Chicago 2 cells.

IFA. Detailed protocols for performing the IFA were described previously (21). Antigens were prepared from the following strains that had been grown on charcoal-yeast extract agar: Philadelphia 1 (serogroup 1); Togus I (serogroup 2); Blommington 2 (serogroup 3); Los Angeles 1 (serogroup 4); Cambridge 2 (serogroup 5); and Chicago 2, 3, and 4, Houston 2, Oxford 1, and Bethesda 1 (serogroup 6). Heat-killed antigens were suspended in 0.01 M phosphate-buffered saline, pH 7.6, containing 0.5% normal yolk sac. Patients' sera were diluted 1:16 in 3% buffered normal yolk sac and subsequently twofold in phosphate-buffered saline or, for some experiments, in blocking fluid. Each serum was tested with polyvalent (anti-human immunoglobulin) and immunoglobulin class-specific conjugates (20). Sera were available only from patient cases 1 and 3.

Case histories. Case 1. Case 1 was a 30-year-old white female on chronic renal dialysis who was admitted to the hospital on 6 November 1978 to evaluate for a renal transplant. Three days later she received a kidney from a living related donor and made an uneventful recovery. On hospital day (HD) 22 she suddenly developed a fever of 101.6°F (ca. 38.6°C), had shaking chills, and, on chest X ray, was found to have an infiltrate in the upper lobe of her left lung. On HD 27 an open lung biopsy was performed. DFA staining of the lung biopsy with the Knoxville 1 conjugate was found to be positive. The patient was started on erythromycin and showed rapid improvement, making a steady recovery with preservation of renal function. She was discharged on HD 54. Culture of the lung biopsy on Muell-Hinton-IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.)-hemoglobin agar recovered two small colonies of *L. pneumophila* after 9 days of incubation (Chicago 2).

Case 2. Case 2 was a 53-year-old male with chronic polycystic kidney disease on long-term dialysis. He was hospitalized on 10 November 1978 for a cadaver renal transplant, and 6 days later he suffered an episode of acute rejection. This was successfully treated with increased steroid therapy. On HD 30 the patient suddenly developed signs of acute rejection which progressed, resulting in a transplant nephrectomy on HD 44. After surgery the patient developed pulmonary infiltrates, and on HD 51, an open biopsy of the lower lobe of the right lung was obtained along with pleural

fluid for culture and microscopy. The lung biopsy was felt to be histologically consistent with *Pneumocystsi carinii* but negative for *L. pneumophila* by culture. However, the pleural fluid was positive for *L. pneumophila* by culture (Chicago 3). Despite therapy with erythromycin and trimethoprim-sulfamethoxazole, signs and symptoms of infection persisted, and the patient died on HD 59. Autopsy revealed organizing pneumocystis pneumonia, dissemianted candidiasis, and pulmonary cytomegalovirus infection. No histological evidence of bacterial pneumonia was present.

Case 3. Case 3 was a 44-year-old white male in chronic renal failure from chronic pyelonephritis since 1973. He had first received a cadaver kidney in 1976. Nine months later the kidney was removed because of chronic rejection. On 20 June 1979 he received a second cadaver kidney and did well until HD 19, when he began to complain of chills, malaise, pleuritic chest pain, and cough. He developed a fever of 101°F, and a chest X ray showed an infiltrate in the left lung. His condition deteriorated rapidly, and later, on HD 21, he suffered a cardiorespiratory arrest while being transferred to the intensive care unit. He was revived, but sustained resultant irreversible ischemic cerebral damage. On HD 22, an open lung biopsy was recommended and the patient was strated on erythromycin. On HD 25, a lung biopsy was performed. The chest X ray showed resolution of the left pulmonary infiltrate with erythromycin therapy by HD 25; however, the patient remained comatose and flaccid, and he died on HD 64.

A DFA stain of imprints from the lung biopsy showed two to three bacilli per field when stained with either the Knoxville 1 or the Togus 1 conjugate. Culture on Mueller-Hinton-IsoVitaleX-hemoglobin agar revealed several small colonies of *L. pneumophila* after 8 days of incubation (Chicago 4).

RESULTS

DFA staining. As a stringent test of specificity, the Chicago 2 conjugate was tested at a 1:8 dilution against all strains of *L. pneumophila* serogroups 1 through 5 listed in Table 1. The 45 strains of serogroup 1, six strains of serogroup 2, six strains of serogroup 4, and five strains of serogroup 5 were all negative in cellular staining with this conjugate. Stained flagella were observed in about 50% of the strains, but this did not interfere with interpretation of cellular staining.

Reciprocal cross staining between conjugates and strains of serogroup 3 and serogroup 6 of 2^+ to 3^+ fluorescence intensity was observed with the Chicago 2 (group 6) and the Bloomington 2 (group 3) conjugates at a 1:8 dilution. Therefore, the conjugates were tested further at the working dilution (1:256) for cross-reactivity between these two serogroups. Results are shown in Table 2. Significant cross-reactivity was observed with both of the unabsorbed conjugates at the working dilution. The cross-reactivity was completely removed by absorption with cells of the cross-reactive strain, but working dilutions were reduced in each case from 1:256 with the unabsorbed conjugate to 1:32 with the absorbed conjugate.

Cells of the six *L. pneumophila* serogroup 6 strains were negative in DFA staining with conjugates for serogroups 2 and 5 and produced weak, irregular fluorescent staining patterns with conjugates for serogroup 1. A small percentage of the cells fluoresced brightly with serogroup 4 conjugate.

IFA. Thirteen serum samples drawn from case 1 showed a diagnostic rise in titer (≥4-fold increase to ≥128 during convalescence) against all *L. pneumophila* antigens tested (Table 3). Titers obtained against the serogroup 6 antigens with the polyvalent (anti-human immunoglobulin) conjugate, however, were 64-fold greater than those obtained against serogroup 1 to 5 antigens 4 weeks after onset of illness, an indication that this patient's antibody response to infection was primarily serogroup 6 specific. Nine months after onset her serogroup 6 titer remained at an elevated level, 1,024. Similarly serum drawn from case 3 approximately 5 weeks after onset had a serogroup 6 titer that was at least 32-fold greater than those obtained against the heterologous antigens. It is interesting that this patient's titer 13 days after onset had been the same (4,096) against all antigens tested, an indication that his initial antibody response was against a common *L. pneumophila* antigen (20, 21). Serogroup 6 strains were isolated from both patients: Chicago 2 from case 1 and Chicago 4 from case 3.

To determine which immunoglobulin class(es) contributed to the titers obtained with the polyvalent conjugate and to establish further the specificity of the test, titers obtained with class-specific conjugates and with blocking fluid were compared. Blocking fluid has been used successfully to inhibit nonspecific titers in legionellosis patients' sera against a variety of gram-negative bacteria (20). Serogroup 6-specific and *L. pneumophila* common antigen responses of case 1 were in the IgG, IgM and IgA fractions, with maximal titers 22 days after onset of illness for the specific antigen and 14 days after onset for the common antigens (Fig. 1). Serogroup-specific titers remained elevated for at least 7 weeks in the IgG and IgM fractions but not in the IgA fraction, which had dropped to a titer of 64 in 5 weeks. Case 3 also had maximal common antigen responses within 2 weeks after onset in the IgG, IgM, and IgA classes (Fig. 2). However, in contrast to case 1, his IgM serogroup-specific response also peaked at this time, whereas his maximal IgG and IgA serogroup-specific re-

TABLE 2. *DFA staining intensities of homologous and cross-reactive antigens with serogroup 3 and 6 conjugates*

Serogroup	Conjugate			Fluorescence intensity	
	Vaccine strain	Absorbing strain	Working dilution[a]	Bloomington 2,[b] serogroup 3	Chicago 2,[c] serogroup 6
3	Bloomington 2	None	1:256	4	2
		Chicago 2	1:32	4	
6	Chicago 2	None	1:256	1	4
		Bloomington 2	1:32		4

[a] One twofold dilution lower than the maximum dilution that still gave 4^+ (very bright) staining of all strains of the homologous serogroup.

[b] The other serogroup 3 strains, Burlington 4 and Detroit 5, were identical to Bloomington 2 in staining reactions.

[c] The other serogroup 6 strains, Chicago 3, Chicago 4, Houston 2, Oxford 1, and Bethesda 1, were identical to Chicago 2 in staining reactions.

TABLE 3. *IFA titers of sera from cases 1 and 3 against L. pneumophila serogroup 1 to 6 antigens, using polyvalent anti-human immunoglobulin conjugate*

Serum specimen		IFA antigen titer	
Patient	Day drawn before (−) or after onset	Serogroup 6[a] (Chicago 2)	Serogroup 1-5[b]
Case 1	−4, −6, −21	128	≤64
	14	4,096	512
	22, 26	16,384	512
	29	32,768	512
	35	16,384	256
	37, 45, 49	8,192	128
	254	2,048	64
	267	1,024	64
Case 3	2	≤64	≤64
	13	4,096	4,096
	37	32,768	1,024
	43[c]	16,384	1,024

[a] Titers obtained against all other serogroup 6 strains agreed within one doubling dilution.

[b] Titers obtained against serogroup 1 (shown in table) 2, 3, 4, and 5 antigens (see text for strains used) agreed within one doubling dilution for case 1. Case 3 titers against serogroup 1 (shown) were higher than those serogroups 2, 3, and 5.

[c] Autopsy specimen.

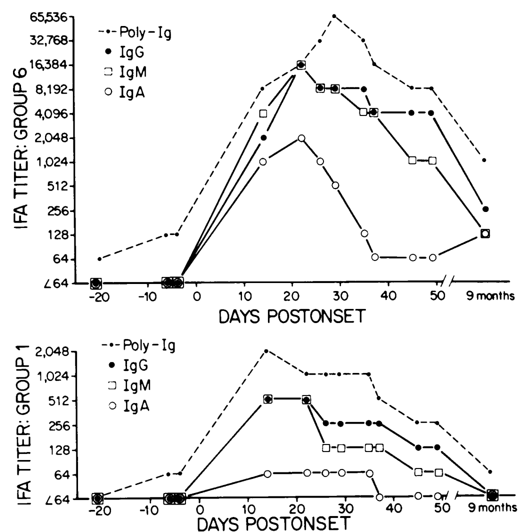

FIG. 1. *IFA titers of case 1 sera against L. pneumophila (top) serogroup 6 (Chicago 2) and (bottom) serogroup 1 (Philadelphia 1) antigens with anti-human immunoglobulin (Poly-Ig), anti-human IgG, anti-human IgM, and anti-human IgA conjugates. (Top) Specific response; (bottom) common antigen response.*

sponses were at 5 weeks postonset. Titers obtained when sera from both cases were diluted in blocking fluid were within the acceptable one-tube variation of those obtained when the sera were diluted in phosphate-buffered saline, an indication that the titers were specific for *Legionella* antigens.

DISCUSSION

In this report, we describe the isolation of a new serogroup of *L. pneumophila* from cases of Legionnaires disease. Although the isolated Chicago 2, 3, and 4 were obtained on Mueller-Hinton–IsoVitaleX–hemoglobin agar, charcoal-yeast extract agar is currently the growth medium of choice because of its greater sensitivity for primary isolation of *L. pneumophila* from infected tissue (6). *L. pneumophila* strains Chicago 2, 3, and 4, Houston 2, Oxford 1, and Bethesda 1 are serologically similar to each other and distinct from serogroups 1 to 5 as shown by DFA staining tests. The cross-reactivity of these strains with the serogroup 3 conjugate is of moderate intensity, and strains of this new serogroup could lead to confusion in interpreting diagnostic staining with group 3 conjugate or

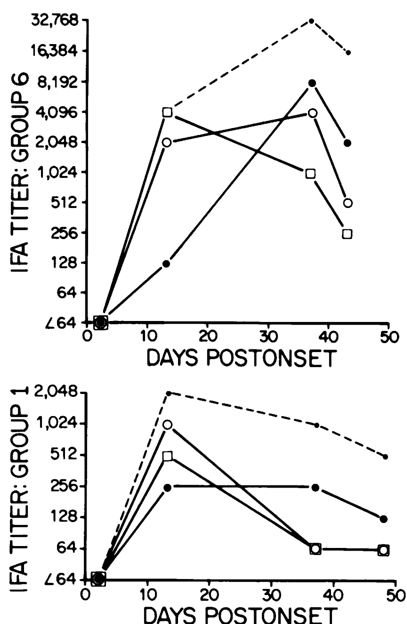

FIG. 2. *Case 3 sera tested as indicated for case 1 (see legend to Fig. 1). Symbols: •, anti-human immunoglobulin; ●, IgG; □, IgM; ○, IgA.*

with *L. pneumophila* polyvalent conjugates that are currently in use. The Oxford 1 strain has been reported as a clinical isolate of serogroup 3 (5, 17). These authors recognized both serological difference and similarity between Oxford 1 and Bloomington 2, and indeed the serogroup 6 strains could be considered as variants of serogroup 3. However, we believe that both DFA and IFA reactivities with the group of isolates Chicago 2, 3, and 4, Houston 2, Oxford 1, and Bethesda 1 are sufficiently different from the serogroup 3 strains to warrant the designation of a new serogroup. We recommend that these strains be designated *L. pneumophila* serogroup 6.

Although appropriate absorption of conjugates for serogroups 6 and 3 renders them completely group specific, it is doubtful that absorbed conjugates are necessary for diagnostic purposes. A clear distinction can be made between these two serogroups on the basis of intensity of fluorescence with working dilutions of unabsorbed conjugates for the two serogroups.

The curious pattern of staining of group 6 stains with conjugates for serogroups 1 and 4 was first suspected to be due to a mixed culture. However, the staining pattern persisted in numerous cultures from single-colony picks, and the fact that it is common to all six strains of serogroup 6 indicates that it is a characteristic of this serogroup. The observation of positive DFA staining of lung biopsy material from cases 1 and 3 with the Knoxville 1 (serogroup 1) conjugate could be explained on the basis of the characteristic spotty staining pattern of serogroup 6 isolates with this conjugate. However, positive staining of biopsy material in case 3 with Togus 1 (serogroup 2) conjugate is not as readily explained since cross-reactivity was not observed with serogroup 2 conjugate with any of the serogroup 6 strains from pure cultures on charcoal-yeast extract plates. This phenomenon of *L. pneumophila* bacteria staining in tissue with conjugate for a heterologous serogroup, but failure to stain with the same heterologous serogroup conjugate after isolation on artificial media, was observed previously with the Togus 1 isolate (12). The reason for the occasional observation of serogroup cross-reactivity of this nature is not understood. In any case, experience has shown that it is necessary to use the homologous serogroup conjugate to consistently reveal *L. pneumophila* organisms in tissue. Retrospective DFA staining of biopsy tissues from cases 1 and 3 with serogroup 6 conjugate revealed many more organisms and much more brightly fluorescent organisms than were initially observed with either the serogroup 1 or the serogroup 2 conjugate.

Staining of flagella of *L. pneumophila* with low dilutions of conjugates has been observed previously (18). The specificity of flagellar staining among these organisms has not been studied in detail, but it obviously has no relationship to cellular staining. Laboratorians should be aware of the possibility of common flagellar staining which can be readily distinguished from the serogroup-specific cellular staining. The relationship of the various staining patterns as seen in the DFA test and the common antigens as shown in the IFA have not yet been established.

In previous studies of the immune response to legionellosis, diagnostic rises in titer during convalescence were found that were specific for serogroup antigen 1, 2, 3, or 4 or that were specific for antigens common in multiple-serogroup strains (20, 21). The IFA results in this study support this concept. The patient from whom the Chicago 2 strain was isolated had a rise in titer against all six serogroup 6 strains, to a level that was 64-fold greater than her titers against the serogroup 1 to 5 antigens. Although primarily serogroup specific, this patient's antibody response also occurred against a common antigen as shown by the fact that initially her seroconversion was detected with the serogroup 1 antigen used routinely at the time of her illness. Similarly, the patient from whom the Chicago 4 strain was isolated had both serogroup 6-specific

and common antigen titer rises, but the former was 32-fold greater than the latter.

The sequence of antibody formation after onset of Legionnaires disease has not been studied extensively due in part to a lack of sequentially collected specimens but also because of the scarcity of sera from culture-confirmed cases. In the present study two such cases were available. Case 1 had peak IgG, IgM, and IgA responses to the *Legionella* common antigen(s) 14 days after onset of Legionnaires disease, whereas her serogroup 6-specific IgG, IgM, and IgA titers peaked in 22 days. In 5 weeks, her serogroup 6-specific IgA titer was below the level of diagnostic significance. In contrast, her serogroup 6-specific IgG and IgM titers declined slowly to 256 or 128, respectively, over the ensuing 8 months. Case 3 also had maximal common antigen titers in all three immunoglobulin classes 13 days after onset of Legionnaires disease. Unlike case 1, the IgM serogroup 6-specific response of case 3 also peaked 13 days postonset. His IgG and IgA responses peaked at 5 weeks postonset, a time when the IgA level of case 1 had declined. The 3- to 5-week period to reach maximal antibody synthesis found in this study of serogroup 6 disease is similar to that found in Legionnaires disease of serogroup 1 etiology (10). It is tempting to speculate that the 2-week peak in the common antigen response represents an anamnestic response because of prior exposure to these patients to *L. pneumophila*, but further data are needed to test this hypothesis. The fact that the patients received immunosuppressive therapy may have influenced their antibody responses to *L. pneumophila* infection.

L. pneumophila has been studied previously as a cause of Legionnaires disease in renal transplant recipients (1, 7). In the present study, we have presented evidence that a new serogroup of *L. pneumophila*, serogroup 6, causes Legionnaires disease in renal transplant patients and that the new group has a wide geographical distribution. Tests for L. *pneumophila* and legionellosis should include reagents for serogroup 6.

LITERATURE CITED

1. **Bock, B. V., B. D. Kirby, P. H. Edelstein, W. L. George, K. M. Snyder, M. L. Owens, C. M. Hatayama, C. E. Haley, R. P. Lewis, R. D. Meyer, and S. M. Finegold.** 1978. Legionnaires' disease in renal-transplant recipients. Lancet i:410–413.
2. **Brenner, D. J., A. G. Steigerwalt, and J. E. McDade.** 1979. Classification of the Legionnaires' disease bacterium. *Legionella pneumophila*, genus novium, species nova, of the family Legionellaceae familia nova. Ann. Intern. Med. **90**:656–658.
3. **Cherry, W. B., B. Pittman, P. P. Harris, G. A. Hebert, B. M. Thomason, L. Thacker, and R. E. Weaver.** 1978. Detection of Legionnaires disease bacteria by direct immunofluorescence staining. J. Clin. Microbiol. **8**:329–338.
4. **Edelstein, P. H., R. D. Meyer, and S. M. Finegold.** 1978. Isolation of a new serotype of Legionnaires' disease bacterium. Lancet ii:1172–1174.
5. **Fallon, R. J., and W. H. Abraham.** 1979. Legionnaires' disease caused by *Legionella pneumophila* serogroup 3. Lancet ii:304.
6. **Feeley, J. C., R. J. Gibson, G. W. Gorman, M. C. Langford, J. K. Rasheed, D. C. Mackel, and W. B. Baine.** 1979. Charcoal-yeast extract agar: primary isolation medium for *Legionella pneumophila*. J. Clin. Microbiol. **10**:437–441.
7. **Foster, R. S., W. C. Winn, W. Marshall, and D. W. Gump.** 1979. Legionnaires' disease following renal transplantation. Transplant. Proc. **11**:93–95.
8. **Fraser, D. W., D. C. Deubner, D. L. Hill, and D. K. Gilliam.** 1979. Nonpneumonic short-incubation-period legionellosis (Pontiac fever) in men who cleaned a steam turbine condenser. Science **205**:690–691.
9. **Glick, T. H., M. B. Gregg, B. Berman, G. Mallison, W. W. Rhodes, Jr., and I. Kassanoff.** 1978. Pontiac fever. An epidemic of unknown etiology in a health department. I. Clinical and epidemiological aspects. Am. J. Epidemiol. **107**:149–160.
10. **McDade, J. E., C. C. Shepard, D. W. Fraser, T. R. Tsai, M. A. Redus, W. R. Dowdle, and the Laboratory Investigation Team.** 1977. Legionnaires' disease. Isolation of a bacterium and demonstration of its role in other respiratory disease. N. Engl. J. Med. **297**:1197–1203.
11. **McKinney, R. M., L. Thacker, P. P. Harris, K. R. Lewallen, G. A. Hebert, P. H. Edelstein, and B. M. Thomason.** 1979. Four serogroups of Legionnaires' disease bacteria defined by direct immunofluorescence. Ann. Intern. Med. **90**:621–624.
12. **McKinney, R. M., B. M. Thomason, P. P. Harris, L. Thacker, K. R. Lewallen, H. W. Wilkinson, G. A. Hebert, and C. W. Moss.** 1979. Recognition of a new serogroup of Legionnaires disease bacterium. J. Clin. Microbiol. **9**:103–107.
13. **Morris, G. K., C. M. Patton, J. C. Feeley, S. E. Johnson, G. Gorman, W. T. Martin, P. Skaliy, G. F. Mallison, B. D. Politi, and D. C. Mackel.** 1979. Isolation of the Legionnaires' disease bacterium from environmental samples. Ann. Intern. Med. **4**:664–667.
14. **Moss, C. W., R. E. Weaver, S. B. Dees, and W. B. Cherry.** 1977. Cellular fatty acid composition of isolates from Legionnaires disease. J. Clin. Microbiol. **6**:140–143.
15. **Nagington, J., T. G. Wreghitt, and D. J. Smith.** 1979. How many Legionnaires? Lancet ii:536–537.
16. **Ormsbee, R. A., M. G. Peacock, G. L. Lattimer, L. A. Page, and B. Fiset.** 1978. Legionnaires' disease: antigenic peculiarities, strain differences, and antibiotic sensitivities of the agent. J. Infect. Dis. **138**:260–264.
17. **Taylor, A. G., and T. G. Harrison.** 1979. Legionnaires' disease caused by *Legionella pneumophila* serogroup 3. Lancet ii:47.
18. **Thomason, B. M., F. W. Chandler, and D. G. Hollis.** 1979. Flagella on Legionnaires' disease bacteria: an interim report. Ann. Intern. Med. **91**:224–225.
19. **Weaver, R. E., and J. C. Feeley.** 1979. Cultural and biochemical characterization of Legionnaires' disease bacterium, p. 20–25. *In* G. L. Jones and G. A. Hebert (ed.), "Legionnaires':" The disease, the bacterium and methodology. Center for Disease Control, Atlanta.
20. **Wilkinson, H. W., C. E. Farshy, B. J. Fikes, D. D. Cruce, and L. P. Yealy.** 1979. Measure of immunoglobulin G-, M-, and A-specific titers against *Legionella pneumophila* and inhibition of titers against nonspecific gram-negative bacterial antigens in the indirect immu-

nofluorescence test for legionellosis. J. Clin. Microbiol. **10**:685-689.
21. **Wilkinson, H. W., B. J. Fikes, and D. D. Cruce.** 1979. Indirect immunofluorescence test for serodiagnosis of Legionnaires disease: evidence for serogroup diversity of Legionnaires disease bacterial antigens and for multiple specificity of human antibodies. J. Clin. Microbiol. **9**:379-383.