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Serological Cross-Reactions between *Coxiella burnetii* and *Legionella micdadei*

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***Coxiella burnetii* and *Legionella micdadei* are both gram-negative bacteria potentially responsible for identical clinical syndromes resembling upper respiratory infections. These infections, quite common in immunocompromised patients, are usually diagnosed by serology with a microimmunofluorescence assay. We found that 34.5% of Q fever patients had a significant titer of antibodies against *L. micdadei*. Cross-reactions involved immunoglobulin G antibodies and were demonstrated by a cross-adsorption study and protein immunoblotting. Western blot analysis performed after treatment with proteinase K indicated that cross-reactions were probably due to both protein and lipopolysaccharide antigens. It is critical that the existence of this cross-reaction be recognized, as misdiagnosis of either condition may lead to incorrect and ineffective treatment.**

Coxiella burnetii, the etiologic agent of Q fever, is a strictly intracellular bacterium which lives in the phagolysosome of the host cell. The genus *Coxiella* contains only one species: *C. burnetii*. Unique to *C. burnetii* is its antigenic phase variation. The virulent phase I organisms are isolated in natural or laboratory infections of animals and humans, whereas the avirulent phase II organisms are isolated during serial passage of *C. burnetii* in immunoincompetent hosts, such as embryonated hen eggs or cell culture systems. Two forms of the disease, acute and chronic, are currently recognized. The most common clinical presentations of acute Q fever are pneumonia, granulomatous hepatitis, meningoencephalitis, and nonspecific fever (22); acute Q fever may also be asymptomatic (22). The most common clinical manifestations of chronic Q fever are blood culture-negative endocarditis, infections of vascular aneurysms and prostheses, and osteomyelitis (1). Laboratory diagnosis of both acute and chronic Q fever is usually performed by serological methods, with microimmunofluorescence being the method of reference (16, 23). Isolation of *C. burnetii* is possible by inoculation of embryonated eggs, animal inoculation, or use of cell culture. Isolation of *C. burnetii* is not, however, routinely performed.

Legionella micdadei is a gram-negative, aerobic, non-spore-forming bacillus (11). The *Legionellaceae* family includes a total of 39 species made up of more than 50 different serogroups. *L. micdadei* was first isolated in 1979 and designated the "Pittsburgh pneumonia agent" (15). It is the second most frequently reported cause of legionellosis after *Legionella pneumophila. L. micdadei* legionellosis typically presents as pneumonia, although other manifestations, such as cutaneous infections (12), abscesses (10), and endocarditis (14), have also been reported. Most cases of pneumonia due to *L. micdadei* are nosocomial in origin and occur in severely immunocompromised patients, including bone marrow (19) and renal (15) transplant recipients. Laboratory diagnosis of *L. micdadei* infections may be performed by isolation of the organism, by direct fluorescent-antibody examination, or by serology.

These two bacteria may cause clinically identical symptoms, such as fever, upper respiratory infections (15, 22), nonspecific infections in immunocompromised patients (15, 17, 19), and endocarditis (1, 14). It is of great importance to differentiate between these two microorganisms, as their susceptibilities to antibiotics are different. Tetracycline is the standard treatment for Q fever and is ineffective for legionellosis; erythromycin is the standard treatment for legionellosis and is ineffective for Q fever.

Thus, it is relevant both to patient care and to progress in basic science that we have observed serological cross-reactions between *C. burnetii* and *L. micdadei.*

MATERIALS AND METHODS

Sera and patients. One hundred serum samples from blood donors were supplied by the National French Center for Blood Transfusion for determination of seroprevalence of immunoglobulin G (IgG), IgA, and IgM antibodies to *L. micdadei*. This population was considered to be healthy. The seroprevalence of IgG, IgA, and IgM antibodies to phase I and II *C. burnetii* antigens for these sera has been previously reported (23). Eighty-four serum samples collected from previously diagnosed Q fever patients (34 with acute and 50 with chronic Q fever) (1, 22, 23) were tested for IgG, IgA, and IgM antibodies to *L. micdadei* antigens. *C. burnetii* was isolated by using a cell culture system for 17 (5 acute and 12 chronic) of the 84 reported Q fever patients (18). All of the reported Q fever patients were examined by one of us (D.R.), and none of them had a known history of *Legionella* infection or exposure to an epidemic source of *Legionella* species. Sera of patients with documented infection due to *L. micdadei* were not available.

Antigen production. *L. micdadei* (ATCC 33204) and *C. burnetii* Nine Mile (ATCC VR 615) were obtained from the American Type Culture Collection. Phase II *C. burnetii* Nine Mile was grown in L929 mouse fibroblasts, as previously reported (23). Infection was monitored by Gimenez staining (9). When 90% of the cells were infected, the culture supernatant was pelleted by centrifugation (10,000 × *g* for 10 min), disrupted by sonication, and inactivated in 0.1% formaldehyde. Inactivated antigens were centrifuged (10,000 × *g* for 10 min) and adjusted to a dilution of 2 mg/ml in phosphate-buffered saline (PBS) by using a UV spectrometer (UV 1200 series; Shimadzu, Kyoto, Japan). Phase I organisms were produced by inoculation of mice with phase II organisms (23). Ten days after infection, the spleens were removed and crushed; the resulting suspension was inoculated into L929 mouse fibroblasts. The infected cells were then harvested, and the bacteria were purified by the technique described above (23). *L. micdadei* was grown on biphasic charcoal medium agar (BCYE; Biomerieux, Lyon, France) for 5 days at 37°C in a 5% CO_2 atmosphere. Bacteria were harvested in PBS (sonic disruption was not performed), centrifuged (10,000 × *g* for 10 min), inactivated in 0.1% formaldehyde, centrifuged a second time (10,000 × *g* for 10 min), and adjusted to 2 mg/ml by using a UV spectrometer.

Serological procedure. Ten microliters of antigen (*C. burnetii* phase I or phase II or *L. micdadei*) was applied with a fine-point pen onto a 30-well microscope slide (Dynatech Laboratories, Ltd., Billingshurst, United Kingdom), air dried, and fixed with acetone for 10 min. Sera were serially diluted in PBS with 3% nonfat powdered milk. Then 15 μl of each dilution (doubling dilutions starting at 1/50) was deposited onto each well containing fixed antigen. Slides were incubated for 30 min at 37°C and then washed three times, for 10 min each time, with PBS. After air drying, 15 μl of fluorescein isothiocyanate-conjugated goat anti-

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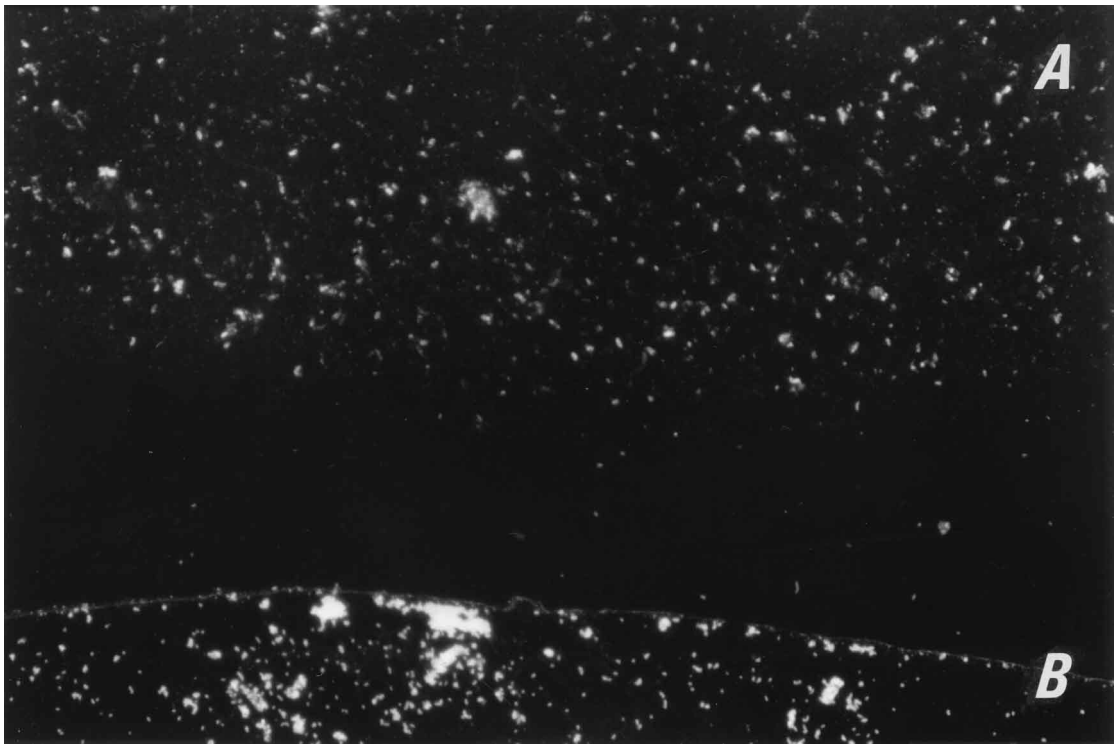

FIG. 1. Microimmunofluorescence of serum from Q fever patient 7C with *L. micdadei* (A) and *C. burnetii* (B) antigens. Magnification, ×400.

human IgG, IgA, and IgM (Fluoline G, A, and M; Biomerieux, Marcy l'Etoile, France) diluted 1/300 was deposited onto each well. Slides were incubated for 30 min at 37°C and then were washed three times for 10 min in PBS, air dried, mounted in buffered glycerol (Boehringer, Mannheim, Germany), and observed by using epifluorescence at a magnification of ×400 (Fig. 1). IgM and IgA antibody levels were determined after removal of IgG antibodies with rheumatoid factor absorbent (Boehringer). Microimmunofluorescence was also performed on cross-reactive sera after purification of the IgG with the MAbTrap G II kit (Pharmacia, Uppsala, Sweden) according to the instructions supplied by the manufacturer.

Enzyme treatment. Outer membrane proteins were removed prior to immunoblotting by incubation of a suspension of *L. micdadei* antigen with solutions of proteinase K (2 mg/ml) (Boehringer) and 5% sodium dodecyl sulfate at a ratio of 4:2:1. The mixture was held for 1 h at 37°C, a further volume of proteinase K solution was added, and the mixture was then incubated for another hour at 37°C.

Western blot analysis. *L. micdadei* and *L. pneumophila* (Philadelphia strain, ATCC 33152) antigens (2 mg/ml) were dissolved in Laemmli solution and boiled for 6 min. The digested *L. micdadei* proteins were resolved by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis according to the Laemmli method (24), with a 3.9% (wt/vol) polyacrylamide stacking gel and a 12% (wt/vol) polyacrylamide gradient gel. Twenty microliters of antigen was loaded into each well and was electrophoresed at 400 V for 1 h. The resolved proteins were transferred to nitrocellulose paper at 40 V for 1 h. The blots were blocked overnight at room temperature with Tris-buffered saline (20 mM Tris-HCl [pH 7.5]) and with 5% nonfat dry milk; they were then washed with distilled water. Sera from Q fever patients were diluted to 1/100 in TBS (Tris-buffered saline, 500 mM HCl, 0.1% Merthiolate) with 3% nonfat milk, overlaid onto the blots, and then incubated overnight at room temperature. After three 10-min washes in TBS, the nitrocellulose paper was incubated separately for 1 h with peroxidase-conjugated goat anti-human IgG and anti-human IgM antibodies diluted at 1/200 in PBS with 3% nonfat dry milk (Immunotech, Marseille, France) and then was washed twice with PBS. Bound conjugate was detected by using a colorimetric reaction (0.015% 4-chloro-1-naphthol–0.015% hydrogen peroxide in 16.7% methanol in TBS) for 10 min. Western blot analysis was performed both before and after treatment of *L. micdadei* antigens with proteinase K and before and after adsorption of sera with a mixture of *C. burnetii* phase I and phase II antigens.

Adsorption of sera. Sera from 3 Q fever patients which cross-reacted with *L. micdadei* (patients 3C, 7C, and 20C) were adsorbed with *L. micdadei* antigen or with a mixture of phase I and phase II *C. burnetii* antigens. Sera were diluted to 1/10 in the antigen suspension, which had been previously adjusted to 2 mg/ml. The mixture was incubated on a rocker for 16 h at 20°C and was centrifuged at 10,000 × *g* for 10 min. Adsorption was performed again on the resulting supernatant. Microimmunofluorescence assays for *C. burnetii* and *L. micdadei* were performed on all adsorbed sera with antibodies to total immunoglobulins (Fluoline H; Biomerieux). Cross-adsorption results were confirmed by Western blot analysis. As a negative control, Western blot analysis was also performed after adsorption of sera with an *L. pneumophila* antigen preparation.

RESULTS

Seroprevalence for antibodies against *L. micdadei* from the panel of 100 blood donors. For IgG antibodies, 99 of 100 serum samples from healthy blood donors were negative at a dilution of 1/100 and 1 was positive at 1/200. For IgA and IgM antibodies, all serum samples were negative at a dilution of 1/50.

***L. micdadei* serological results for acute and chronic Q fever patients.** Of the 84 serum samples from Q fever patients investigated for *L. micdadei* antibodies, 34.5% (29 of 84) had antibodies against *L. micdadei* at levels significantly higher than those in the healthy population ($P < 0.05$ [χ^2]) (Table 1); the other 55 serum samples from Q fever patients had no antibodies to *L. micdadei*. Of acute and chronic Q fever patients, 26.5% (9 of 34) and 40% (20 of 50), respectively, had IgG antibodies to *L. micdadei*. Two serum samples were available for patient 5A; the first contained no antibodies to either *C. burnetii* or *L. micdadei*, and the second contained IgG antibodies to both *C. burnetii* and *L. micdadei* at a dilution of 1/400 and IgM antibodies to *C. burnetii* at 1/800. No serum samples from Q fever patients had IgM or IgA antibodies to *L. micdadei.*

IgG purified from cross-reacting sera reacted strongly with *L. micdadei* in the microimmunofluorescence assay. Sera from which IgG had been removed did not, however, demonstrate any microimmunofluorescence activity, confirming that the IgG isotype alone was responsible for cross-reaction.

Western blot analysis. Western blot analysis performed with sera from patients 3C, 7C, and 20C against *L. micdadei* anti-

TABLE 1. Titers of IgG, IgM, and IGA antibodies against *C. burnetii* phase I and phase II antigens and against *L. micdadei* antigens for acute and chronic Q fever patients[a]

Patient	Titer of antibody to:						
	Phase II *C. burnetii*			Phase I *C. burnetii*			*L. micdadei*
	IgG	IgM	IgA	IgG	IgM	IgA	IgG
1A	800	50	50	200	50	50	200
2A	400	1,600	50	400	50	200	100
3A	16,400	3,200	<50	<50	<50	<50	400
4A	400	<50	<50	<50	<50	<50	100
5A[b]	400	800	<50	<50	<50	<50	400
6A[b]	200	<50	200	100	<50	50	100
7A[b]	800	200	50	100	<50	50	800
8A[b]	400	50	<50	200	<50	<50	100
9A[b]	1,600	1,600	<50	100	1,600	<50	100
1C	409,600	800	<50	204,800	400	<50	1,600
2C	3,200	<50	800	3,200	<50	800	100
3C[b]	819,200	50	1,600	409,600	50	1,600	1,600
4C	1,600	<50	<50	800	<50	<50	400
5C	1,600	<50	50	1,600	<50	50	1,600
6C	1,600	200	800	1,600	200	800	1,600
7C[b]	102,400	800	25,600	102,400	400	25,600	1,600
8C	12,800	<50	1,600	6,400	<50	800	400
9C	12,800	50	800	12,800	100	800	800
10C	6,400	50	800	3200	<50	400	200
11C[b]	102,400	50	12,800	102,400	50	12,800	1,600
12C[b]	51,200	800	25,600	51,200	800	12,800	1,600
13C[b]	51,200	3,200	12,800	25,600	1,600	6,400	400
14C[b]	51,200	1,600	12,800	102,400	6,400	25,600	6,400
15C[b]	25,600	200	6,400	25,600	200	6,400	1,600
16C[b]	102,400	50	6,400	102,400	50	6,400	800
17C[b]	51,200	800	3,200	51,200	800	6,400	1,600
18C[b]	102,400	400	25,600	102,400	200	25,600	200
19C[b]	102,400	6,400	100	102,400	6,400	100	800
20C[b]	6,400	<50	100	3,200	<50	100	200

[a] Patients 1A to 7A were diagnosed with acute Q fever, and patients 1C to 12C were diagnosed with chronic Q fever.
[b] *C. burnetii* isolated by cell culture.

gens yielded several bands with conjugated anti-human IgG antibodies and no bands with conjugated anti-human IgM antibodies. After treatment with proteinase K, only one band, at 45 kDa, was present (Fig. 2). Adsorption of sera from patients 3C, 7C, and 20C with *L. pneumophila* antigen did not change the Western blot profile (Fig. 3).

Serological cross-adsorption. Cross-adsorption studies demonstrated a reduction of homologous and heterologous antibodies when adsorption was performed with the *C. burnetii* antigen but a reduction only of homologous antibodies when adsorption was performed with the *L. micdadei* antigen (Table 2).

DISCUSSION

We found that 34.5% of Q fever patients had significant titers of antibody against *L. micdadei*. Seroconversion was observed for both *C. burnetii* and *L. micdadei* antigens in serum samples collected 3 weeks apart from an acute Q fever patient. Cross-reactions involving IgG antibodies were noted in sera from both acute and chronic Q fever patients. The cross-reaction was demonstrated by a cross-adsorption study and Western blot analysis. The cross-adsorption study indicated that reactivity with *L. micdadei* resulted from cross-reaction with *C.*

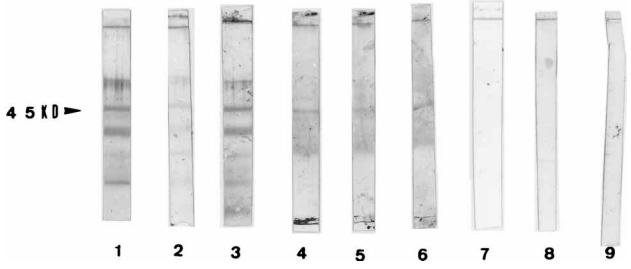

FIG. 2. Western blot of sera from Q fever patients 3C, 7C, and 20C with *L. micdadei* antigens (lanes 1 to 3, respectively), with proteinase K digest antigens of *L. micdadei* (lanes 4 to 6, respectively), and after adsorption of sera with a mixture of phase I and phase II *C. burnetii* antigens (lanes 7 to 9, respectively).

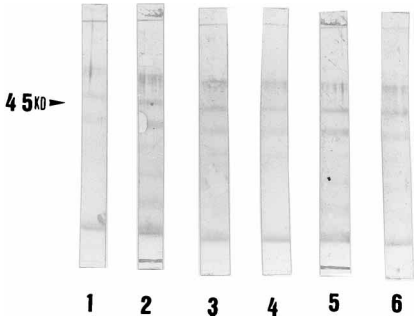

FIG. 3. Western blot of sera from Q fever patients 3C, 7C, and 20C with *L. micdadei* antigen before (lanes 1 to 3, respectively) and after (lanes 4 to 6, respectively) adsorption of sera with *L. pneumophila* antigen.

TABLE 2. Results of the serological cross-adsorption study of Q fever sera with both phase I and phase II *C. burnetii* antigens and with *L. micdadei* antigens

Serum	Titer of total immunoglobulins (proportion of unadsorbed serum titers) in serum adsorbed with:					
	Nothing (control)		*L. micdadei* antigen		*C. burnetii* phase I + phase II antigens	
	Anti-*C. burnetti*	Anti-*L. micdadei*	Anti-*C. burnetii*	Anti-*L. micdadei*	Anti-*C. burnetii*	Anti-*L. micdadei*
3C	409,600	1,600	409,600 (1)	<50 (<0.03)	25,600 (0.06)	<50 (<0.03)
7C	102,400	1,600	102,400 (1)	<50 (<0.003)	800 (0.003)	<50 (<0.03)
20C	6,400	200	6,400 (1)	<50 (<0.25)	100 (0.015)	<50 (<0.25)

burnetii antibodies, because prior adsorption of these sera with *C. burnetii* removed reactivity. Enzyme treatment was performed in order to investigate proteinic involvement in the cross-reactions. Western blot analysis performed after treatment with proteinase K indicated that cross-reactions were probably due to both protein and lipopolysaccharide antigens, because removal of protein antigens prior to immunoblotting removed all bands but one.

As cross-reactions involved only IgG antibodies, patients with IgM or IgA antibodies against *C. burnetii* are infected with *C. burnetii* only. If patients have only IgG antibodies against both bacteria (as did patients 4A and 4C), cross-adsorption is necessary to differentiate infection with *C. burnetii* alone from a mixed *C. burnetii-L. micdadei* infection. Cross-adsorption demonstrated that patients 4A and 4C were infected with *C. burnetii* alone (data not shown).

Serological cross-reactions between *Legionella* species and *Coxiella* species have been reported previously (5, 6, 8), but reported results have been inconclusive. One publication reported a case of Q fever pneumonia in which the presence of cross-reacting antibodies to *L. pneumophila* serogroup 4 was demonstrated by a cross-adsorption study (6). However, a cross-adsorption study and protein immunoblotting performed retrospectively on 154 Q fever serum samples demonstrated a lack of serological cross-reaction between *C. burnetii* and *L. pneumophila* (8). One coinfection with *C. burnetii-L. pneumophila* was reported, but a cross-adsorption study was not performed; in addition, different serological methods were employed (a complement fixation assay for *C. burnetii* and microimmunofluorescence for *L. pneumophila*) (5). Serological cross-reactions between *C. burnetii* and *Bartonella quintana*, detected by a passive hemagglutination test, have also been reported (4), as have serological cross-reactions among *C. burnetii*, *B. quintana*, and *Bartonella henselae*, detected by a microimmunofluorescence assay (13).

Cross-reactive antibodies against *Legionella* species have been reported for infections due to several gram-negative bacteria, including *Pseudomonas* species, *Bordetella pertussis*, *Haemophilus influenzae*, *Salmonella typhi*, *Neisseria meningitidis*, *Chlamydia* species, and *Campylobacter jejuni* (2, 3, 7). Serological cross-reactions occur between the different *Legionella* species (including *L. micdadei*). *L. micdadei* shares only 43% common antigens with *L. pneumophila* serogroup 1, as determined by quantitative immunoelectrophoretic techniques (3). In patients diagnosed as having legionellosis, a pool of antigens representing *L. pneumophila* serogroups 1 to 6 detected the largest number of reactive serum samples, whereas single-serogroup antigens detected less than half of this number of positive serum samples (27). However, a monoclonal antibody directed against a 60-kDa *Legionella* heat shock protein reacted in an immunoblot with all *Legionella* species but not with other bacteria (21).

Serological cross-reactions between *L. micdadei* and *C. burnetii* are in fact not surprising because phylogenetic studies based on 16S ribosomal RNA sequencing indicate that the *Legionella* species are closely related to *C. burnetii* (20). In addition, the fatty acid composition of *C. burnetii* is similar to the fatty acid composition of some *Legionella* species (*L. bozemanii* and *L. dumoffii* rather than *L. pneumophila* and *L. micdadei*) (25), and the biochemical features of the peptidoglycan-associated protein of both bacteria are similar (26).

We previously reported the lack of serological cross-reaction between *L. pneumophila* and *C. burnetii* (8). As *L. pneumophila* and *L. micdadei* are antigenically different (3), it is probable that the epitope involved in the serological cross-reaction between *C. burnetii* and *L. micdadei* is not present on *L. pneumophila*. Thus, these results are not contradictory.

It is important to investigate the possibility of cross-reactions between these bacteria because both may cause identical clinical syndromes which are not treated by the same antibiotics.

REFERENCES

1. **Brouqui, P., H. Tissot Dupont, M. Drancourt, Y. Berland, J. Etienne, C. Leport, F. Goldstein, P. Massip, M. Micoud, A. Bertrand, and D. Raoult.** 1993. Chronic Q fever. Ninety-two cases from France, including 27 cases without endocarditis. Arch. Intern. Med. **153:**642–648.
2. **Chessbrough, J. S., T. Makin, B. C. Taxman, N. J. Beeching, and K. J. Mutton.** 1992. False-positive *Legionella* serology in campylobacter infection. Lancet **339:**429.
3. **Collins, M. T., F. Espersen, N. Høiby, S.-N. Cho, A. Friis-Møller, and J. S. Reif.** 1983. Cross-reactions between *Legionella pneumophila* (serogroup 1) and twenty-eight other bacterial species, including other members of the family *Legionellaceae*. Infect. Immun. **39:**1441–1456.
4. **Cooper, M. D., M. R. Hollingdale, J. W. Vinson, and J. Costa.** 1976. A passive hemagglutination test for diagnosis of trench fever due to *Rochalimaea quintana*. J. Infect. Dis. **134:**605–609.
5. **Dobija-Domaradzki, M., J.-L. Hausser, and F. Gosselin.** 1984. Coexistence chez un même patient de la maladie des légionnaires et de la fièvre Q. Can. Med. Assoc. J. **130:**1022–1023.
6. **Dwyer, D. E., V. L. Gibbons, L. M. Brady, and A. L. Cunningham.** 1988. Serological reaction to *Legionella pneumophila* Group 4 in a patient with Q fever. J. Infect. Dis. **158:**499–500.
7. **Edelstein, P. H.** 1992. Detection of antibodies to *Legionella*, p. 459–466. *In* N. R. Rose, E. Conway de Macario, J. L. Fahey, H. Friedman, and G. M. Penn (ed.), Manual of clinical laboratory immunology, 4th ed. American Society for Microbiology, Washington, D.C.
8. **Finidori, J. P., D. Raoult, N. Bornstein, and J. Fleurette.** 1992. Study of cross-reaction between *Coxiella burnetii* and *Legionella pneumophila* using indirect immunofluorescence assay and immunoblotting. Acta Virol. **36:**459–465.
9. **Gimenez, D. F.** 1964. Staining rickettsiae in yolk-sac cultures. Stain Technol. **39:**135–140.
10. **Halberstam, M., H. D. Isenberg, and E. Hilton.** 1992. Abscess and empyema caused by *Legionella micdadei*. J. Clin. Microbiol. **30:**512–513.
11. **Harrison, T. G., and N. A. Saunders.** 1994. Taxonomy and typing of *Legionellae*. Rev. Med. Microbiol. **5:**79–90.
12. **Kilborn, J. A., L. A. Manz, M. O'Brien, M. C. Douglass, H. M. Horst, W. Kupin, and E. J. Fisher.** 1992. Necrotizing cellulitis caused by *Legionella micdadei*. Am. J. Med. **92:**104–106.
13. **La Scola, B., and D. Raoult.** 1996. Serological cross-reactions between *Bartonella quintana*, *Bartonella henselae*, and *Coxiella burnetii*. J. Clin. Microbiol. **34:**2270–2274.
14. **Park, D., A. Pugliese, and B. A. Cunha.** 1994. *Legionella micdadei* prosthetic valve endocarditis. Infection **22:**213–215.
15. **Pasculle, A. W., R. L. Myerowitz, and C. R. Rinaldo.** 1979. New bacterial

agent of pneumonia isolated from renal-transplant recipients. Lancet **ii:**58–61.
16. **Peter, O., G. Dupuis, and M. Peacock.** 1985. Evaluation of the complement fixation and indirect immunofluorescence test in the early diagnosis of primary Q fever. Eur. J. Clin. Microbiol. **4:**394–396.
17. **Raoult, D.** 1990. Host factors in the severity of Q fever. Ann. N. Y. Acad. Sci. **590:**33–38.
18. **Raoult, D., G. Vestris, and M. Enea.** 1990. Isolation of 16 strains of *Coxiella burnetii* from patients by using a sensitive centrifugation cell culture system and establishment of the strains in HEL cells. J. Clin. Microbiol. **28:**2482–2484.
19. **Schwebke, J. R., R. Hackman, and R. Bowden.** 1990. Pneumonia due to *Legionella micdadei* in bone marrow transplant recipients. Rev. Infect. Dis. **12:**824–828.
20. **Stein, A., N. A. Saunders, A. G. Taylor, and D. Raoult.** 1993. Phylogenetic homogeneity of *Coxiella burnetii* strains as determined by 16S ribosomal RNA sequencing. FEMS Microbiol. Lett. **113:**339–344.
21. **Steinmetz, I., C. Rheinheimer, I. Hübner, and D. Bitter-Suermann.** 1991. Genus-specific epitope on the 60-kilodalton *Legionella* heat shock protein recognized by a monoclonal antibody. J. Clin. Microbiol. **29:**346–354.
22. **Tissot Dupont, H., D. Raoult, P. Brouqui, F. Janbon, D. Peyramond, P. J. Weiller, C. Chicheportiche, M. Nezri, and R. Poirier.** 1992. Epidemiologic features and clinical presentation of acute Q fever in hospitalized patients: 323 French cases. Am. J. Med. **93:**427–434.
23. **Tissot Dupont, H., X. Thirion, and D. Raoult.** 1994. Q fever serology: cutoff determination for microimmunofluorescence. Clin. Diagn. Lab. Immunol. **1:**189–196.
24. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
25. **Tzianabos, T., C. W. Moss, and J. E. McDade.** 1981. Fatty acid composition of rickettsiae. J. Clin. Microbiol. **13:**603–605.
26. **Weiss, E., J. C. Williams, and H. A. Thompson.** 1991. The place of *Coxiella burnetii* in the microbial world, p. 1–19. *In* J. C. Williams and H. A. Thompson (ed.), Q fever: the biology of *Coxiella burnetii*. CRC Press, Inc., Boca Raton, Fla.
27. **Wilkinson, H. W., A. L. Reingold, B. J. Brake, D. L. McGiboney, G. W. Gorman, and C. V. Broome.** 1983. Reactivity of serum from patients with suspected legionellosis against 27 antigens of *Legionellaceae* and *Legionella*-like organisms by indirect immunofluorescence assay. J. Infect. Dis. **147:**23–31.