INFECTION AND IMMUNITY, June 1997, p. 2443–2447 Vol. 65, No. 6
0019-9567/97/$04.00+0

Pathologic Changes during Acute Q Fever: Influence of the Route of Infection and Inoculum Size in Infected Guinea Pigs

BERNARD LA SCOLA, HUBERT LEPIDI, AND DIDIER RAOULT*

Unité des Rickettsies, UPRESA 6020, Faculté de Médecine, Université de la Mediterrannée, 13885 Marseille Cedex 05, France

Received 17 October 1996/Returned for modification 10 December 1996/Accepted 26 February 1997

As assessed by both standard histological staining and immunochemistry, intraperitoneal inoculation of *C. burnetii* in guinea pigs led to pathologic changes mainly in the liver, whereas intranasal inoculation led to pathologic changes mainly in the lungs. Myocarditis and positive blood cultures were observed only in those animals which received the highest inoculum. We therefore conclude that both the route of infection and the size of the inoculum influence clinical expression in acute Q fever.

Q fever is a worldwide zoonosis caused by *Coxiella burnetii*, an obligately intracellular organism which multiplies in phagolysosomes of infected cells. In most cases, infection is asymptomatic. The most common acute clinical manifestations are self-limited febrile illnesses, granulomatous hepatitis, and pneumonia (16). Cases of febrile eruptions, myocarditis, pericarditis, and meningoencephalitis have been reported. Asymptomatic forms and life-threatening forms such as myocarditis can be observed during the same outbreak (3). Most cases of chronic Q fever are manifested as endocarditis. Clinical manifestations of acute Q fever vary from one geographical area to another. For example, in Nova Scotia in southeast Canada (9), Switzerland (3), and northern Spain (18), the main manifestation of acute Q fever is pneumonia, while in France (16) or southern Spain (18), it is granulomatous hepatitis. It has been hypothesized that the route of infection, as demonstrated in a murine model (10), and the size of the inoculum could influence the clinical presentation of acute cases. However, in mice *C. burnetii* infection results in either a self-limiting febrile illness or latent infection, whereas in guinea pigs, as in humans, acute Q fever is a life-threatening disease and infectious foci are cleared of microorganisms faster and more effectively (8). A guinea pig model of acute Q fever is therefore probably more relevant to disease in humans. We herein compared the pathological changes observed with infection via the intraperitoneal (i.p.) route (mimicking digestive contamination) and via the respiratory route, in a guinea pig model. The influence of inoculum size was also assessed.

Forty-two male Hartley guinea pigs were inoculated with the Nine Mile I strain of *C. burnetii*, titrated in shell vials by using an indirect immunofluorescence technique (11). In group A, 16 guinea pigs were inoculated i.p. with 10^5 IU of *C. burnetii*. In group B, 13 guinea pigs were inoculated i.p. with 10^2 IU of *C. burnetii*. In group C, 13 guinea pigs were inoculated intranasally with 10^2 IU of *C. burnetii*. Rectal temperature and weight were recorded every 3 days. Guinea pigs were serially sacrificed during the 1st month and at the end of the 4th month. Blood for serology and culture was obtained at the time of sacrifice. Antibodies to phase I and phase II *C. burnetii* antigens in the sera of guinea pigs were detected by a microimmunofluorescence test as previously described (17). Blood cultures were performed by using the shell vial assay (13). The heart, the lungs, the liver, the spleen, and the left kidney were excised. Sections of paraffin-embedded tissues were stained with hematoxylin-eosin-saffron. The slides were then coded and read by one of us (H.L.), a pathologist, who was unaware of the study hypothesis. For detection of the presence of *C. burnetii* in tissues, immunochemistry was performed on deparaffinized sections of all organs by using an anti-*C. burnetii* rabbit polyclonal antibody with an LSAB K 680 kit (Dako, Trappes, France) according to the manufacturer's instructions.

All animals inoculated i.p. had a febrile response (temperature, ≥40°C) at day 3, whereas none of the animals inoculated intranasally showed a febrile response at ≥40°C (data not shown). Body weight loss, at its highest at day 6 for all guinea pigs, was not significantly different among the three groups (Table 1). The kinetics and titers of antibodies were similar in all groups of guinea pigs (data not shown). Blood cultures were positive only in animals infected with high levels of inoculum and sacrificed at day 3 or 6. Similarly, with human cases of acute Q fever, *C. burnetii* is isolated in blood culture from only 17% of untreated patients, and isolation is in most cases achieved in samples from patients with no detectable antibodies early in the course of the disease (12).

Except for the kidneys, all organs studied developed specific pathomorphological changes. Hepatic and splenic damage appeared as multifocal granulomas consisting of mononuclear aggregates and composed mainly of macrophages and lymphocytes with a few polymorphonuclear leukocytes (Fig. 1). These granulomas were focal, variable in diameter, and scattered throughout the liver lobules or the portobiliary spaces and the splenic red pulp. Residues of necrotic foci of hepatocytes were found around and/or within some of the inflammatory aggregates. These granulomas were apparent by day 3 and disappeared at day 15 in the spleen but persisted up to 30 days in the liver. In the lungs, the dominant feature consisted of mononuclear cell infiltration in the widening alveolar septa, appearing at day 6. Numerous mononuclear cells and some polymorphonuclear leukocytes were seen in the alveoli. Cellular infiltrates in the lungs were the only pathologic changes observed in guinea pigs sacrificed at 14 weeks. Several foci of myocarditis, consisting mainly of mononuclear cell infiltration interstitially, were found only among animals inoculated with the highest level of inoculum. Interestingly, myocarditis was devoid of valvular inflammation in infected guinea pigs, as has been observed among human acute Q fever sufferers. This tropism of *C. burnetii* for the myocardium has also been observed with

* Corresponding author. Mailing address: Unité des Rickettsies, UPRESA 6020, Faculté de Médecine, Université de la Méditerrannée, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 05, France. Phone: 33 91 38 55 17. Fax: 33 91 83 03 90. E-mail: RAOULT@pacwan.mm-soft.fr.

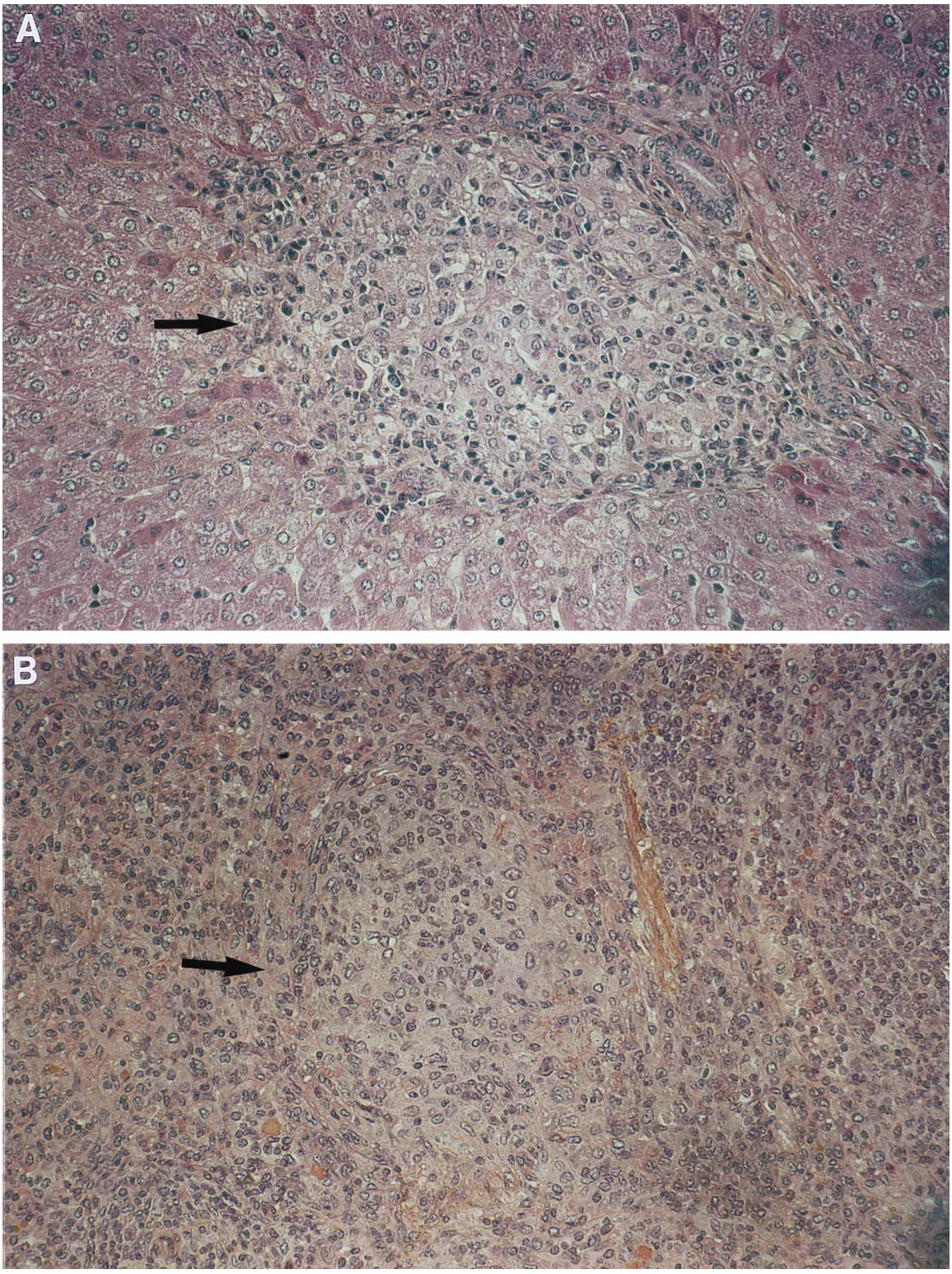

FIG. 1. (A) Liver from a guinea pig sacrificed at day 20. An area of granulomatous inflammation is indicated (arrow) at the periphery of a portobiliary space; it is composed largely of macrophages and has lesser numbers of lymphocytes and polymorphonuclear leukocytes. (B) Spleen from a guinea pig sacrificed at day 9. Typical granuloma in the red pulp (arrow) is composed primarily of macrophages. For both panels, hematoxylin-phloxine-saffron stain was used; magnification, ×250.

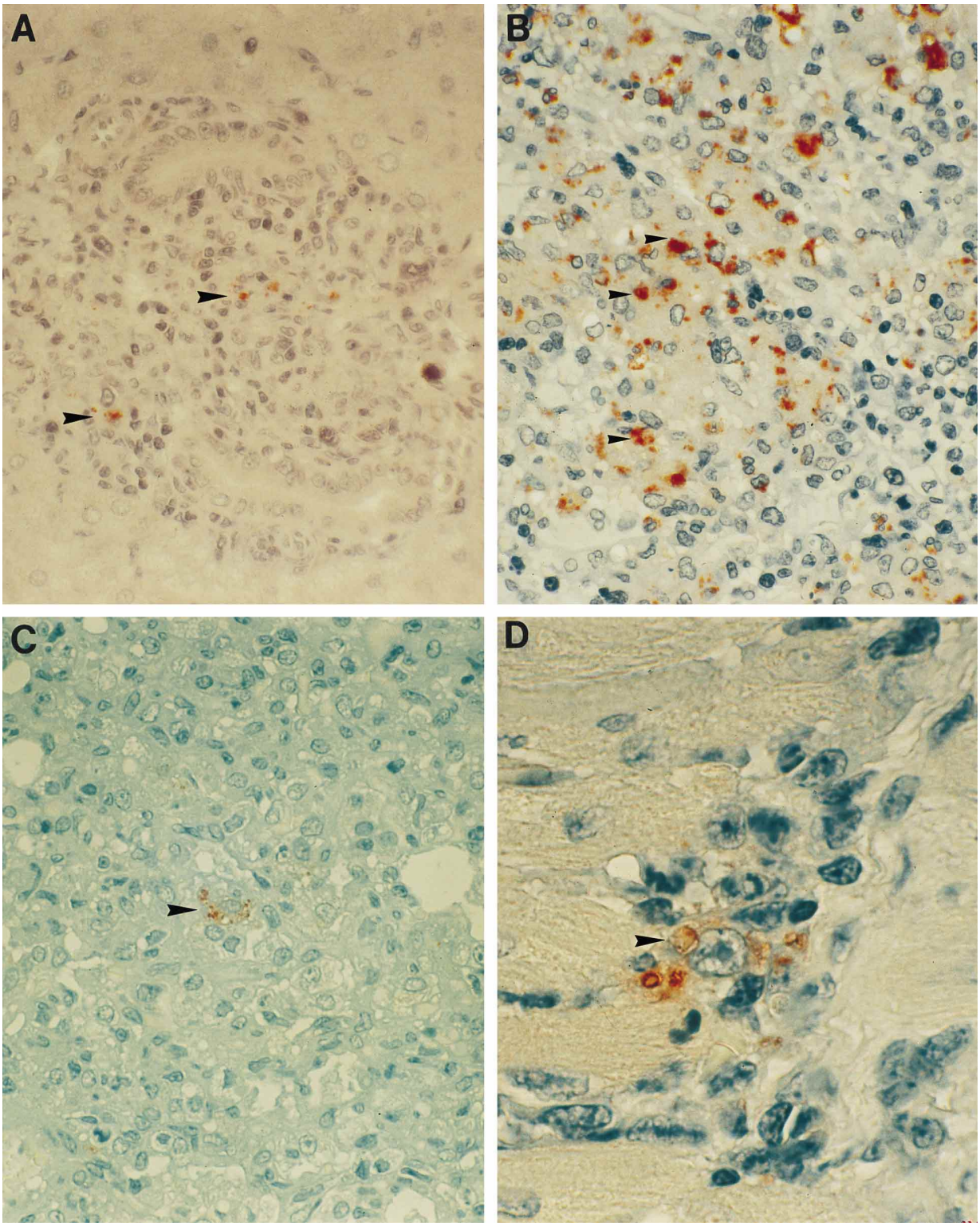

FIG. 2. Demonstration of *C. burnetii* antigen by immunochemistry in liver (A), spleen (B), lung (C), and myocardium (D). The liver and spleen were from guinea pigs sacrificed at day 6, and the lung and myocardium were from animals sacrificed at day 9. Cytoplasmic vacuoles of macrophages are packed with coarse granular immunopositive material (arrows), especially in the spleen. Magnification, ×400 (A, B, and C) and ×1,000 (D).

TABLE 1. Histological changes and percentage of body weight loss in guinea pigs infected with *C. burnetii*

Sacrifice time and group[a] (*n*)	Heart	Score[b] (mean ± SD)			% Body wt loss[c]
		Spleen	Liver	Lung	
Early					
A1 (16)	0.5 ± 0.73[d]	0.94 ± 1.18	0.88 ± 0.81	0.44 ± 0.89	
B1 (8)	0	1 ± 0.93	0.88 ± 0.64	0.25 ± 0.71	
C1 (8)	0	0.5 ± 0.76	0.13 ± 0.35[e]	1.38 ± 1.19[f]	
Late					
B2 (5)	0	0	0	0.4 ± 0.55	
C2 (5)	0	0	0	1.4 ± 0.55[g]	
Total					
A (14)					9.78 ± 4.07
B (12)					8.95 ± 4.11
C (12)					7.14 ± 3.63

[a] Groups were infected with the following numbers of *C. burnetii* organisms: 10^5 i.p. (A), 10^2 i.p. (B), and 10^2 intranasally (C). In the early sacrifice group (days 3, 6, 9, 12, 15, 20, 25, and 30), four guinea pigs were sacrificed at each time point: two in group A1 and one each in groups B1 and C1. In the late sacrifice group, five guinea pigs from group B2 and five from group C2 were sacrificed at the end of the 14th week.

[b] Criteria assessed were cellular infiltrates, appearing as granulomas or not and with or without cell necrosis. These were scored in each organ as follows: 0, not present; 1, present but hard to find; 2, easy to detect but limited in amount and distribution; 3, diffusely present. The analysis of nonparametric variance was performed by using the Kruskal-Wallis test. Groups were compared by the multiple range test.

[c] From the day of inoculation to day 6.

[d] Significantly different from values obtained with groups B1 and C1 ($P = 0.028$).

[e] Significantly different from values obtained with groups A1 and B1 ($P = 0.034$).

[f] Significantly different from values obtained with groups A1 and B1 ($P = 0.023$).

[g] Significantly different from values obtained with group B2 ($P = 0.031$).

human Q fever (3, 16) and is probably underestimated. A dose-response effect in clinical findings has been described for human volunteers (15), for monkeys (6), and from indirect evidence in outbreaks (9) in that the incubation period for the disease ranged from 7 to 30 days, according to the intensity of the exposure. Granulomas observed in most organs were comparable in constitution to those reported in humans. Most immunopositive cells, morphologically identified as macrophages (Fig. 2), were seen at days 3 and 6, and no immunoreactive cells were visualized after 12 days. *C. burnetii* is usually not detected in such granulomas in humans by immunochemistry (7, 20); however, in most cases, biopsies are performed several days or weeks after the onset of fever and therefore always more than 2 weeks after inoculation. Changes in the airways were more pronounced among animals inoculated intranasally, and the i.p. route led to significantly greater changes in the liver. This observation could explain the variation in manifestations of acute Q fever among different countries. Several epidemiological studies have suggested that ingestion of raw, presumably contaminated milk is a risk factor for acquisition of Q fever in humans (1, 4, 16), whereas in other cases infection is due to the inhalation of contaminated aerosols. Although it is likely that the route of infection determines the predominant manifestation of Q fever, both the oral and the aerosol routes can cause pneumonia and hepatitis, as can an i.p. route of infection. Interestingly, in humans experimentally inoculated aerogenously with *C. burnetii* (2) or with a naturally acquired infection (5), histologic changes in the liver are more striking than blood chemical findings. Most patients with acute Q fever probably have histological hepatitis, with or without chemical abnormalities or clinical signs of hepatitis or pneumonia.

The inference of the model used in this study is that the route of infection and the size of the inoculum are factors which determine the main manifestations of disease. In the future, this study could be repeated with clinical strains responsible for hepatitis or pneumonia, in order to explore a possible importance of strain differences in the clinical presentation of acute Q fever.

This work was supported by convention CNAMTS/INSERM 4-API 10. We are grateful to Richard Birtles for reviewing the manuscript.

REFERENCES

1. **Benson, W. W., D. W. Brock, and J. Mather.** 1963. Serologic analysis of a penitentiary group using raw milk from a Q fever infected herd. Public Health Rep. **78:**707–710.
2. **Dupont, H., R. B. Hornick, H. S. Levin, M. I. Rapoport, and T. E. Woodward.** 1971. Q fever hepatitis. Ann. Med. Interne **74:**198–206.
3. **Dupuis, G., O. Peter, D. Pedroni, and J. Petite.** 1985. Aspects cliniques observés lors d'une épidémie de 415 cas de fièvre Q. Schweiz. Med. Wochenschr. **115:**814–818.
4. **Fishbein, D. B., and D. Raoult.** 1992. A cluster of *Coxiella burnetii* infections associated with exposure to vaccinated goats and their unpasteurized dairy products. Am. J. Trop. Med. Hyg. **47:**35–40.
5. **Gerstl, B., E. R. Movitt, and J. R. Shaken.** 1956. Liver function and morphology in Q fever. Gastroenterology **30:**813–819.
6. **Gonder, J. C., R. A. Kishimoto, M. D. Kastello, C. E. Pedersen, Jr., and E. W. Larson.** 1979. Cynomolgus monkey model for experimental Q fever infection. J. Infect. Dis. **139:**191–196.
7. **Hoffmann, C. E., and S. W. Heaton, Jr.** 1982. Q fever hepatitis. Clinical manifestations and pathological findings. Gastroenterology **83:**474–479.
8. **Khavkin, T.** 1991. Experimental studies of the infectious process in Q fever, p. 71–106. *In* T. J. Marrie (ed.), Q fever, vol. 1. The disease. CRC Press, Boca Raton, Fla.
9. **Marrie, T. J., H. Durant, J. C. Williams, E. Mintz, and D. M. Waag.** 1988. Exposure to parturient cats: a risk factor for acquisition of Q fever in Maritime Canada. J. Infect. Dis. **158:**101–108.
10. **Marrie, T. J., A. Stein, D. Janigan, and D. Raoult.** 1996. Route of infection determines the clinical manifestations of acute Q fever. J. Infect. Dis. **173:** 484–487.
11. **Maurin, M., A. M. Benoniel, P. Bongrand, and D. Raoult.** 1991. Phagolysosomal alkalinization and the bactericidal effect of antibiotics: the Coxiella burnetii paradigm. J. Infect. Dis. **166:**1097–1102.
12. **Musso, D., and D. Raoult.** 1995. *Coxiella burnetii* blood cultures from acute and chronic Q fever patients. J. Clin. Microbiol. **33:**3129–3132.
13. **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 strains in HEL cells. J. Clin. Microbiol. **28:**2482–2484.
14. **Srigley, J. R., H. Vellend, N. Palmer, M. J. Phillips, W. R. Geddie, A. W. Van Nostrand, and V. D. Edwards.** 1985. Q-fever. The liver and bone marrow pathology. Am. J. Surg. Pathol. **9:**752–758.

15. **Tiggert, W. D., and A. S. Benenson.** 1956. Studies on Q fever in man. Trans. Assoc. Am. Physicians **69:**98–104.
16. **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.
17. **Tissot-Dupont, H., X. Thirion, and D. Raoult.** 1994. Q fever serology: cutoff determination for microimmunofluorescence. Clin. Diagn. Lab. Immunol. **1:**189–196.
18. **Velasco, F. P.** 1996. Fiebre Q. Junta de Castilla y Leon, Spain.
19. **Walker, D. H.** 1988. Pathology of Q fever, p. 17–28. *In* D. H. Walker (ed.), Biology of rickettsial diseases. CRC Press, Boca Raton, Fla.
20. **Westlake, P., M. Russell, and J. K. Kelly.** 1987. The pathology of Q fever hepatitis. A case diagnosed by liver biopsy. J. Clin. Gastroenterol. **9:**357–363.

Editor: S. H. E. Kaufmann