JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1995, p. 428–431 Vol. 33, No. 2
0095-1137/95/$04.00+0
Copyright © 1995, American Society for Microbiology

Isolation of *Coxiella burnetii* from Heart Valves of Patients Treated for Q Fever Endocarditis

KATHRIN MÜHLEMANN,[1]* LUKAS MATTER,[1] BERNHARD MEYER,[2] AND KURT SCHOPFER[1]

Institute of Medical Microbiology, University of Berne, Berne,[1] and Institute of Clinical Microbiology and Immunology, St. Gallen,[2] Switzerland

Received 12 September 1994/Returned for modification 7 October 1994/Accepted 7 November 1994

***Coxiella burnetii* was isolated from the valve material of two patients who underwent valvectomy because of progressive congestive heart failure due to endocarditis. In each case antibiotic therapy was administered for several months prior to valvectomy. Classical histopathological examination of the valves did not reveal an etiology. However, coxiella-like organisms were demonstrated in valvular material with Köster, Stamp, and Giemsa stains, and the organisms were grown in cell culture. Antibody titers were consistent with the diagnosis of chronic *C. burnetii* infection. This report illustrates the advantage of simple and fast staining techniques and cell culture for the demonstration and isolation of *C. burnetii* in the heart valve tissue of patients with Q fever endocarditis.**

Although *Coxiella burnetii* is found worldwide, few seroprevalence studies have been performed. In Switzerland approximately 10% of the urban population has antibodies to *C. burnetii*, and in selected rural areas antibody prevalence may be as high as 31% (5). Studies from other countries have found similar results (2, 12). In the United States disease due this organism is thought to be unusual. However, public health reporting is not universally required (19).

Acute infection with *C. burnetii* may proceed to a chronic disease, mainly chronic endocarditis or granulomatous hepatitis (19). However, the frequency of this event is not known. Since the first descriptions of Q fever endocarditis in 1959 by Evans et al. (6) and Andrews and Marmion (3), further experience with this disease has been limited to a series of case reports. Q fever endocarditis is characterized by chronic progression, high titers of specific antibodies, and persistently negative blood cultures. However, the proof of the diagnosis by isolation of the agent through the infected tissue is usually not attempted.

The cases that we describe here were characteristic of Q fever with positive serology. Staining techniques were used to demonstrate organisms in valve tissue, and a definitive diagnosis was made by recovery of the organisms from heart valves. The exemplary character of our observations warrants a more detailed description.

MATERIALS AND METHODS

Patient 1 was a 59-year-old male with a heart murmur that was first noted when he was 16 years old. At the age of 54 he noted increasing dyspnea and palpitation during exercise. On hospital admission in November 1987, biventricular congestive heart failure was diagnosed. There were marked proteinuria (5.8 g/24 h) and impaired renal function (serum creatinine, 134 μmol/liter). Supportive therapy was initiated. He was seen again in February 1988, and at this time a purpuric rash on the lower extremities and splenomegaly were noted. Ultrasound and catheterization of the heart revealed mitral valve insufficiency with thickened leaflets and an almost completely detached anterior leaflet. A diagnosis of Q fever endocarditis was suspected; antibody titers to *C. burnetii* were determined by complement fixation against phase I and II antigens (Table 1). Antibiotic therapy with doxycycline and rifampin was initiated in March 1988. One month later, refractory heart failure necessitated mitral valve replacement. During surgery no obvious signs of active endocarditis were observed; therefore, only the severely damaged anterior leaflet was replaced (St. Jude Medical Prosthesis). The excised leaflet was thickened and focally calcified, but no vegetations were observed. The patient did well following surgery but remained on antibiotic therapy with doxycycline and rifampin.

Patient 2 was a 52-year-old brewery worker with a cardiac murmur that was first noted when he was 41. Sonography performed in 1987 revealed aortic insufficiency and stenosis. During the year prior to admission he experienced recurrent purpura of the lower extremities accompanied by fatigue, nausea, and night sweats. He was hospitalized in September 1988, and on admission physical examination revealed clubbing, purpura, and hepatosplenomegaly. Granulomatous hepatitis was diagnosed upon liver biopsy. Abnormal laboratory findings included pancytopenia (hemoglobin, 119 g/liter; leukocyte count, 2,200/mm^3; platelet count, 113,000/mm^3), hypergammaglobulinemia (36 g/liter), rheumatoid factor (1:2,560), and circulating immune complexes (66%). No activity of the classical pathway of complement was detectable by radial hemolysis. Blood cultures were negative. Q fever endocarditis was suspected, and antibody titers to *C. burnetii* antigens were determined (Table 1). Treatment with doxycycline and rifampin was initiated in October 1988; because of nausea, rifampin was discontinued and trimethoprim-sulfamethoxazole was added. Aortic insufficiency and the suspicion of paravalvular abscess formation (sonography) necessitated aortic valve replacement (Carpentier Edwards Bio Prosthesis) in December 1988. At surgery a malformed, probably congenitally bicuspid aortic valve with prominent calcified vegetations as well as an annular abscess beyond the anterior commissure was found. The patient recovered uneventfully but remained on continuous antibiotic therapy with doxycycline and rifampin.

Demonstration of *C. burnetii*. Parts of the valve material from both patients were used for standard histopathological examination and electron microscopy. Additional pieces were frozen in liquid nitrogen and stored at −80°C. Frozen sections of the stored tissue were later prepared. The sections were air dried, heat fixed, and stained with Giemsa stain and according to the methods of Hansen and Köster (11) and Stamp et al. (21).

Isolation of *C. burnetii*. Valve material from patient 1 was kept at −80°C until inoculation, whereas the material from patient 2 was processed within few hours after surgery. Pieces of tissue (5 by 2 by 3 mm) were minced finely with a blade and suspended in 10 ml of Eagle's modified minimum essential medium containing 2% fetal calf serum and no antibiotics. Then 0.2 to 0.4 ml of this suspension was inoculated onto human fetal lung fibroblasts (MCR-5) grown on 0.80-mm-diameter coverslips in 8-ml plastic tubes. Cell cultures were centrifuged (500 × *g*, 10 min, room temperature), 1 ml of minimum essential medium–2% fetal calf serum was added, and cultures were incubated at 35°C in an atmosphere containing 5% CO_2. After 2 and 7 days of incubation, passages were made from one culture. Medium was aspirated, and cells were harvested by scraping them with a needle from one culture. Harvested medium and cells were inoculated onto fresh fibroblast monolayers and handled as described for primary cultures.

Indirect immunofluorescence staining. Cell monolayers were examined for organisms by indirect immunofluorescence staining after 5, 7, and 10 days of incubation. The staining procedure was as follows: (i) fixation with methanol-acetone (1:1) for 15 min at +4°C, (ii) incubation in human serum with high antibody titers to *C. burnetii* (1:40,000) at a dilution of 1:80 for 30 min at 35°C,

* Corresponding author. Mailing address: Institute of Medical Microbiology, University of Berne, Friedbühlstr. 51, 3010 Berne, Switzerland. Phone: 41 31 632 32 56. Fax: 41 31 382 38 09. Electronic mail address: muehlemann@imm.unibe.ch.

TABLE 1. Antibody patterns to *C. burnetii* in two patients with chronic Q fever endocarditis at different times after diagnosis

Test and antibody	Antigen (phase)	Titer in:					
		Patient 1 at mo:			Patient 2 at mo:		
		0	1	3	0	4	14
Immunofluorescence							
Ig (polyvalent)		30,000		40,000	200,000	>10,000	160,000
IgM		80		80	5,120	640	80
IgA		8,000		1,280	50,000	8,000	5,120
IgG	I		2,621,440		655,360		
	II		40,960		10,240		
IgM	I		160		10,240		
	II		160		5,120		
IgA	I		10,240		81,920		
	II		320		20,480		
Complement fixation	I		2,560		2,560		
	II		1,280		640		

(iii) incubation with sheep anti-human immunoglobulin (Ig) (Wellcome; 1:500) for 30 min at 35°C, and (iv) counterstaining with Evans blue and mounting on a glass slide. Between steps cells were washed with phosphate-buffered saline (pH 7.0) three times for 5 min each time at room temperature. Specificity was evaluated by simultaneous staining of uninoculated cell monolayers and inoculated cultures with human serum negative for antibodies to *C. burnetii.*

RESULTS

On routine histopathological examination valve tissue from both patients showed vascularization, focal calcifications, and sparse mononuclear cell infiltrates. No microorganisms were noted. A diagnosis of degenerative alterations, probably of rheumatic origin, was made in patient 1. In patient 2 a congenitally bicuspid aortic valve was diagnosed.

However, smears and frozen sections stained by Köster, Stamp, and Giemsa stains showed focally abundant microorganisms highly suggestive of *C. burnetii* (Fig. 1 and 2). Organisms consistent with *C. burnetii* were also seen by electron microscopy in valve tissue from patient 1.

Indirect immunofluorescence staining revealed abundant positively staining cellular inclusions in cultures from both patients after 5 days of incubation (Fig. 3). No staining was seen in the negative controls.

DISCUSSION

C. burnetii was isolated in fibroblast cultures from removed aortic valve material from two patients with chronic Q fever infections. In both patients a diagnosis of chronic *C. burnetii* infection was suggested by persisting high antibody titers. However, the histopathological and electron microscopic examinations of the valve material were not indicative of an ongoing infectious disease process. Although both patients were given appropriate antibiotic treatment for a prolonged period of time, viable organisms could be recovered from the excised valve material.

This report demonstrates that chronic Q fever infections may be missed because acute infection is often asymptomatic or presents as flu-like illness. Unpredictable progression to chronic disease may be slow and characterized by protean clinical manifestations. In patients with preexisting valve disease, symptoms may be misinterpreted as the spontaneous course of the primary valvular affection, as suggested in patient 1 and described in other reports (7, 15).

High titers of antibodies to phase I antigen of *C. burnetii*, especially IgA antibodies, are characteristic of chronic infection (17), but in some cases antibody levels may be low (23)

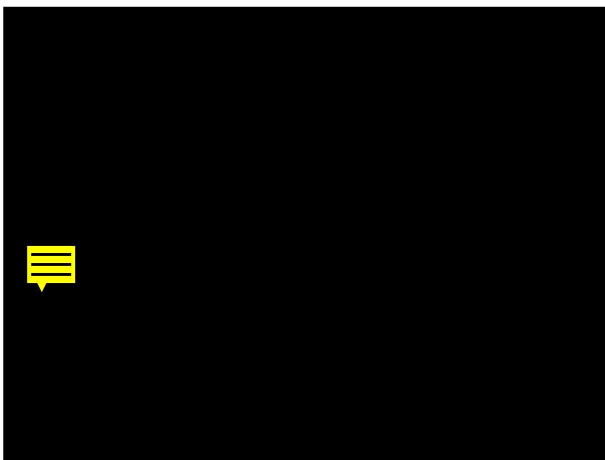

FIG. 1. Köster staining of an aortic valve smear preparation. The group of red-stained pleomorphic particles (arrowhead) is highly suggestive of *C. burnetii*. Cellular elements appear blue (open circles). (Magnification, ×1,000.) Stamp stain gives an identical picture.

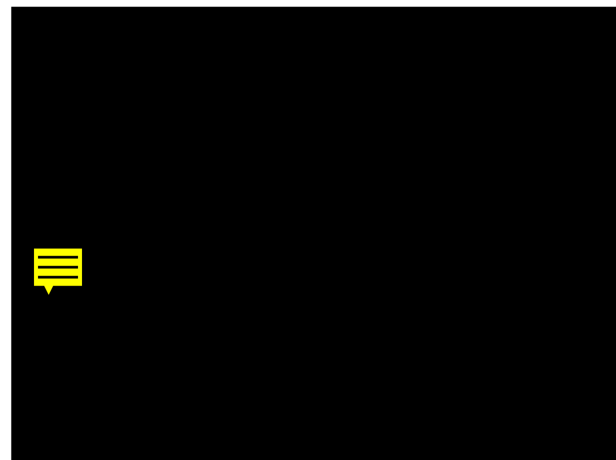

FIG. 2. There are abundant purple-staining microorganisms in this Giemsa stain of a frozen section (3 to 4 μm) from the excised aortic valve of patient 2. The agents are pleomorphic, with rods and coccoid forms. Their localization appears to be mainly extracellular. (Magnification, ×1,000.)

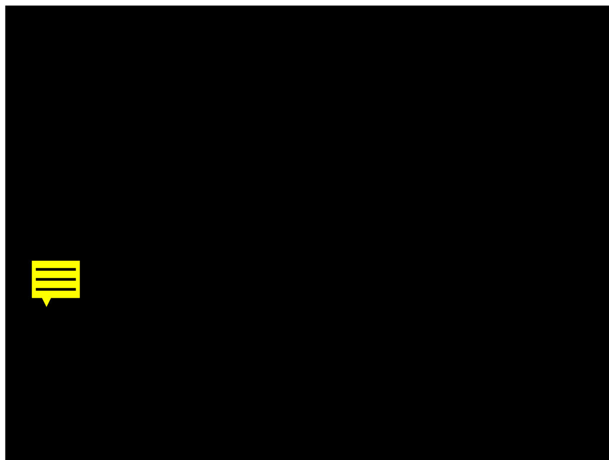

FIG. 3. Immunofluorescence staining of a cell culture inoculated with mitral valve suspension from patient 1 shows an infected fibroblast after incubation for 10 days. (Magnification, ×500.)

and they do not prove endocardial infection. Therefore, the ability to demonstrate the organisms in valve tissue is important in establishing a definitive diagnosis of Q fever endocarditis, as previously shown by Turck et al. (23).

Others have also noted the limitations of classical histopathology for etiologic diagnosis of Q fever endocarditis (14, 15). The histopathological presentation may be indistinguishable from rheumatic heart disease and degenerative involution in malformed valves. Machiavello, Gimenez, and Giemsa stains as well as immunological techniques have been advocated to demonstrate *C. burnetii* in tissue sections (4, 10, 13). In our hands two stains widely used in veterinary laboratories to demonstrate *Coxiella* and *Brucella* infections, Köster and Stamp stains, were very useful. The application of these staining techniques to smears and frozen sections from valve tissue was a fast, easy-to-perform, and cost-saving method. Also, the bright red color of stained organisms was easier to differentiate against the background than the purple Giemsa stain. We recommend use of Köster and Stamp stains for the histopathological evaluation of cases of suspected Q fever endocarditis or endocarditis of unknown origin. Molecular methods such as PCR have been used for the detection of *C. burnetii* (9). However, such techniques are not widely available in routine pathology and clinical microbiology laboratories. Also, they do not provide any information about the viability of the organisms. Inoculation into guinea pigs, mice, and embryonated eggs is a well-established method for the recovery of *C. burnetii* (1). It is, however, laborious and biohazardous. Fernàndez et al. (8) and Raoult et al. (18, 22) described the use of cell culture as an alternative method, and in our hands it proved both easy and reproducible with fresh as well as frozen tissue.

The patients described in this report were treated for 1 and 2 months prior to isolation of the organisms. More recently we have been able to recover *C. burnetii* from the aortic valve of an additional patient, who has been treated for 12 months with doxycycline and trimethoprim-sulfamethoxazole, when the aortic valve had to be replaced because of heart failure (unpublished observation). These cases demonstrate that the currently available chemotherapy is inefficient in eliminating viable coxiellas from endocardial tissue, as reported by others (18). Valve tissue from such patients should be searched for viable organisms if they undergo heart surgery.

Q fever endocarditis is thought to be a rare disease. However, the true incidence of cardiac involvement following *C. burnetii* infection is unknown and may be higher than suspected regarding the uncharacteristic clinical manifestations of this disease. It is generally accepted that valvular abnormalities favor the development of this complication. It seems reasonable to believe that Q fever endocarditis may be more common than is presently appreciated, since bicuspid anomalies occur with a prevalence of 0.9 to 2% (20) and, for instance, in Switzerland the seroprevalence of *C. burnetii* infection ranges from 10 to 31% (5). This hypothesis has already been suggested by Palmer and Young (16) and seems particularly appropriate for a disease with such nonspecific and protean manifestations.

The etiologic diagnosis is important for the management of patients with endocarditis. Simple serologic, histopathologic, and culture methods are now available and should be applied in all cases of suspected Q fever endocarditis or endocarditis of unknown origin.

ACKNOWLEDGMENTS

We are grateful to our clinical colleagues from the Medical Departments of the Kantonspital St. Gallen for providing the clinical data. The support of the heart surgeons, M. Turina, University Hospital of Zürich, and L. Egloff, Heart Centre Hirslanden Zürich, is especially appreciated. We acknowledge as well the friendly and fruitful collaboration with J. Schneider and M. Spycher from the Institute of Pathology, University Hospital of Zürich, who have been particularly helpful in discussing the histopathological and electron microscopic preparations. We thank G. Dupuis, Valais Central Institute Sion, for additional serologic investigations. M. A. Menegus, Clinical Microbiology Laboratories, Rochester, N.Y., is acknowledged for critical and encouraging comments on the manuscript.

REFERENCES

1. **Aitken, I. D., K. Bögel, E. Cracea, E. Edlinger, D. Houwers, H. Krauss, M. Rady, J. Rehacek, H. G. Schiefer, N. Schmeer, I. V. Tarasevich, and G. Tringali.** 1987. Q-fever in Europe: current aspects of aetiology, epidemiology, human infection, diagnosis and therapy. Infection **15:**323–327.
2. **Akesson, A., A. Macellaro, P. Tull, J. C. Williams, and L. Norlander.** 1991. Epidemiology of Q fever in Sweden. Scand. J. Infect. Dis. **23:**153–157.
3. **Andrews, P. S., and B. P. Marmion.** 1959. Chronic Q-fever: morbid anatomical and bacteriological findings in a patient with endocarditis. Br. Med. J. **14:**983–988.
4. **Baumgärtner, W., H. Dettinger, N. Schmeer, and E. Hofmeister.** 1988. Evaluation of different fixatives and treatments for immunohistochemical demonstration of *Coxiella burnetii* in paraffin-embedded tissues. J. Clin. Microbiol. **26:**2044–2047.
5. **Dupuis, G., O. Péter, M. C. Mottiez, and M. Vouilloz.** 1986. Séro-prévalence de la fièvre Q humaine en Suisse. Schweiz. Med. Wochenschr. **116:**494–498.
6. **Evans, A. D., D. E. B. Powel, and C. D. Burrell.** 1959. Fatal endocarditis associated with Q-fever. Lancet **i:**864–865.
7. **Fergusson, R. J., T. R. D. Shaw, A. H. Kitchin, M. B. Matthews, J. M. Inglis, and J. F. Peutherer.** 1985. Subclinical chronic Q-fever. Q. J. Med. **57:**669–676.
8. **Fernàndez-Guerrero, M. L., J. M. Muelas, J. M. Aguado, G. Renedo, J. Fraile, F. Soriano, and E. De Villalobos.** 1988. Q-fever endocarditis on porcine bioprosthetic valves. Ann. Intern. Med. **108:**209–213.
9. **Frazier, M. J., L. P. Mallavia, J. E. Samuel, and O. G. Baca.** 1990. DNA probes for the identification of Coxiella burnetii strains. Ann. N. Y. Acad. Sci. **590:**445–458.
10. **Giménez, D. F.** 1964. Staining rickettsiae in yolk-sac cultures. Stain Technol. **39:**135–140.
11. **Hansen, K., and H. Köster.** 1936. Nachweis von Brucella abortus (Bang) auf Grund der Alkalifestigkeit. Dtsch. Tierärztl. Wochenschr. **44:**739–742.
12. **Kelly, P. J., L. A. Matthewman, P. R. Mason, and D. Raoult.** 1993. Q fever in Zimbabwe. S. Afr. Med. J. **83:**21–25.
13. **Kimbrough, R. C., R. A. Ormsbee, M. Peacock, W. R. Rogers, R. W. Bennetts, J. Raaf, A. Krause, and C. Gardner.** 1979. Q-fever endocarditis in the United States. Ann. Intern. Med. **91:**400–402.
14. **Kristinsson, A., and H. H. Bentall.** 1967. Medical and surgical treatment of Q-fever endocarditis. Lancet **ii:**693–697.
15. **Lev, B. I., A. Shachar, S. Segev, P. Weiss, and E. Rubinstein.** 1988. Quiescent Q-fever endocarditis exacerbated by cardiac surgery and corticosteroid therapy. Arch. Intern. Med. **148:**1531–1532.

16. **Palmer, S. R., and S. E. J. Young.** 1982. Q-fever endocarditis in England and Wales, 1975–81. Lancet **ii:**1448–1449.
17. **Peacock, M. G., R. N. Philip, J. C. Williams, and R. S. Faulkner.** 1983. Serological evaluation of Q fever in humans: enhanced phase I titers of immunoglobulins G and A are diagnostic for Q fever endocarditis. Infect. Immun. **41:**1089–1098.
18. **Raoult, D., P. Y. Levy, J. R. Harle, J. Etienne, P. Massip, F. Goldstein, M. Micoud, J. Beytout, H. Gallais, and G. Remy.** 1990. Chronic Q fever: diagnosis and follow-up. Ann. N. Y. Acad. Sci. **590:**51–60.
19. **Reimer, G. L.** 1993. Q fever. Clin. Microbiol. Rev. **6:**193–198.
20. **Roberts, W. C.** 1970. The congenitally bicuspid aortic valve. Am. J. Cardiol. **26:**72–83.
21. **Stamp, J. T., A. D. McEwen, J. A. A. Watt, and D. I. Nisbet.** 1950. Enzootic abortion of ewes. I. Transmission of the disease. Vet. Rec. **62:**251–254.
22. **Subramanya, N. I., J. S. Wright, and M. A. R. Khan.** 1982. Failure of rifampicin and cotrimoxazole in Q-fever endocarditis. Br. Med. J. **285:**343–344.
23. **Turck, W. P. G., G. Howitt, L. A. Turnberg, H. Fox, M. Longson, M. B. Matthews, and R. Das Gupta.** 1976. Chronic Q-fever. Q. J. Med. **45:**193–217.