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MINIREVIEW

Diagnosis of Q Fever

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INTRODUCTION

Query (Q) fever, due to *Coxiella burnetii*, is a ubiquitous zoonosis. It was first described by Derrick (29) in 1935 in Queensland, Australia, during an outbreak of a febrile illness among abattoir workers. Subsequently, Burnet and Freeman (19) isolated a fastidious intracellular bacterium from guinea pigs that had been injected with blood or urine from Derrick's patients and named it *Rickettsia burnetii*. This bacterium was morphologically and biochemically similar to other gram-negative bacteria. On the basis of cultural and biochemical characteristics, Philip (137) classified *R. burnetii* in a new genus, *Coxiella*, named after Herald R. Cox, who first isolated this microorganism in the United States. This genus contained only one species, *C. burnetii*. Since then, it has been isolated from several mammals and from ticks, and it may persist in the environment. The disease may be acquired by the respiratory or digestive route. The incidence of Q fever is unknown and may be underestimated. Over the last few years, the apparent increase in the incidence of this disease may be due to increased reporting or diagnosis. The clinical presentation of Q fever is polymorphic and nonspecific and may be acute, most often pneumonia or hepatitis, or chronic, most often endocarditis. Inapparent and subclinical infections are common. The diagnosis of Q fever relies mainly upon serology, the most commonly used method being the immunofluorescence assay. Serological testing for Q fever should always be done for a patient with a febrile illness and negative blood cultures.

BACTERIOLOGY

C. burnetii is a short (0.3 to 1.0 μm), pleomorphic rod that possesses a membrane similar to that of gram-negative bacteria (Fig. 1). It shares characteristics with bacteria included in the genus *Rickettsia*, including a small genome (65), staining by the Gimenez method (54), strict intracellular growth in eukaryotic cells, and association with arthropods (196). *C. burnetii* can be cultivated on cell layers from clinical or animal samples and can persist in daughter cells without affecting the viability of these persistently infected cells. On the other hand, *C. burnetii* demonstrates particular characteristics, such as a G+C content of 43% (196), a sporulation-like process conferring resistance to harsh environmental conditions (113), passive entry into host cells by phagocytosis, and a survival in phagolysosomes where a low pH (pH 4.5) is necessary for its metabolism (61, 62, 116). Moreover, contrary to the rickettsia, classified in the α1 subdivision of the family *Proteobacteria*, *C. burnetii* has recently been classified into the γ subdivision of this family, close to *Rickettsiella grylli*, *Legionella* spp., and *Francisella* spp., on the basis of a comparison of the sequences of the 16S rRNA-encoding gene (195). Another major characteristic of *C. burnetii* is its antigenic variation, called phase variation. This phenomenon, similar to the rough-smooth variation in enterobacteria, is due to partial loss of lipopolysaccharide (LPS) (60, 186). The LPS represents a major virulence determinant of *C. burnetii* (59). When isolated from animals or humans, *C. burnetii* expresses phase I antigens and is very infectious (a single bacterium may infect a human). Phase I LPS, with its extended carbohydrate structure, sterically blocks access of antibody to surface proteins (59). This may explain, at least in part, why the bacterium persists at unknown sites after recovery from acute cases of Q fever, accompanied by lifelong seropositivity. After subculture in cells or embryonated eggs, modification of the LPS results in an antigenic shift to the phase II form, which is less infectious. This LPS modification makes surface proteins accessible for antibodies (59, 186). LPS seems to be the only antigen and immunogen differing between phase I and II in *C. burnetii* (3). This antigenic peculiarity is extremely valuable for the serological differentiation between acute and chronic Q fever.

C. burnetii expresses a low degree of genetic heterogeneity among strains. However, variation in the composition of LPS has been demonstrated (60, 185). Moreover, 20 different genotypes have been delineated by pulsed-field gel electrophoresis (65) and/or restriction fragment length polymorphism analysis (178). The 1.6×10^6-bp genome comprises a chromosome and four copies of a plasmid. Four different plasmids have been described for the Nine Mile strain, and they vary in their size (117, 160, 162, 190). Besides host factors which probably play a major role in the clinical form of the disease (143), the roles of LPS type, plasmid type (161, 173, 177, 203), and the route of infection (111) are still controversial.

EPIDEMIOLOGY

Q fever is a worldwide zoonosis. The reservoirs are extensive but only partially known and include mammals, birds, and arthropods, mainly ticks. While an important reservoir seems to be small wild rodents, the most commonly identified sources of human infection are farm animals such as cattle, goats, and sheep. Pets, including cats (67), rabbits, and dogs, have also been demonstrated to be potential sources of urban outbreaks. Cats are suspected as an important reservoir of *C. burnetii* in urban areas and may be the source of urban outbreaks (90, 108, 118). In Canada, 6 to 20% of cats have anti-*C. burnetii* antibodies (67). Wild rats have been suspected as an important

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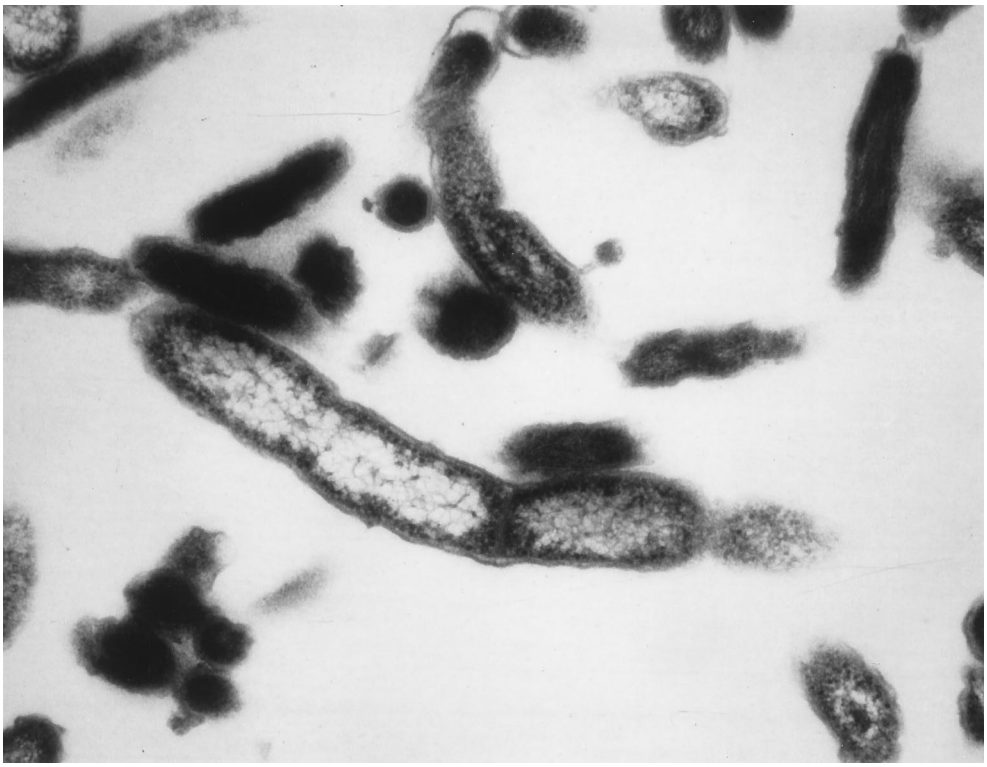

FIG. 1. *C. burnetii*. Transmission electron micrograph showing a gram-negative-like wall cell. Magnification, ×75,000.

reservoir in Great Britain (193). All these mammals, when infected, shed the desiccation-resistant organisms in urine, feces, milk, and, especially, birth products (80, 106). Reactivation of infection occurs in female mammals during pregnancy. Q fever causes abortions in goats and, less frequently, sheep and causes reproductive problems in cattle (7, 192, 204). High concentrations of *C. burnetii* (up to 10^9 bacteria per g of tissue) are found in the placentas of infected animals (4). Due to its resistance to physical agents, probably related to its sporulation process (89, 113), *C. burnetii* survives for long periods in the environment.

In humans, infection results from inhalation of contaminated aerosols from amniotic fluid or placenta or contaminated wool. Therefore, Q fever is an occupational hazard. At greatest risk are persons in contact with farm animals, but also at risk are laboratory personnel who work with infected animals (75). When looking for the source of *C. burnetii* exposure, the investigator should search for contact with a parturient or newborn animal. Mammals also shed *C. burnetii* in milk, and thus, consumption of raw milk could be a source of infection (47, 118, 180). Sexual transmission of Q fever has been demonstrated in the mouse (86) and has been suspected in humans (100). Sporadic cases of human-to-human transmission following contact with an infected parturient woman have been reported and have been suspected to occur by direct aerosol transmission. It has also been proven to occur via transplacental transmission, resulting in congenital infections (98, 153), via intradermal inoculation (14, 39), and via blood transfusion (21, 151). Ticks transmit *C. burnetii* to domestic mammals but not to humans (80). *C. burnetii* may persist asymptomatically in humans throughout life. However, pregnancy, a cardiac valvular abnormality, a vascular aneurysm or prosthesis, hemodialysis (91), and immunodeficiency, including AIDS (64, 77, 97, 143, 147), may promote reactivation of dormant *C. burnetii*.

In Europe, acute Q fever cases are more frequently reported in spring and early summer. They may occur at all ages, but they are more frequent in men than in women. Q fever is usually benign, but mortality occurs in 1 to 11% of patients with chronic Q fever (143). *C. burnetii* is endemic in every part of the world except New Zealand (68, 78). Since the clinical presentation is very pleomorphic and nonspecific, the incidence of Q fever among humans is probably underestimated, and diagnosis particularly relies upon the physician's awareness of the symptoms of Q fever and the presence of a reliable diagnostic laboratory. In southern France, 5 to 8% of cases of endocarditis are due to *C. burnetii*, and the prevalence of acute Q fever is 50 cases per 100,000 inhabitants (180). Seroepidemiological surveys have shown that 18.3% of blood donors in Morocco, 26% in Tunisia (125), 37% in Zimbabwe (82), 44% in Nigeria (11), 10 to 37% in northeast Africa, and 14.6 to 36.6% in different areas of Canada (109, 112) had anti-*C. burnetii* antibodies. Large outbreaks of Q fever have also been reported in the Basque country in Spain (1), in Switzerland (37), in Great Britain (57), in Berlin, Germany (164), and more recently, in southern France (unpublished data).

CLINICAL MANIFESTATIONS

Clinical signs of Q fever are often subclinical or extremely mild. For example, during a Q fever outbreak in Switzerland (37), of the 415 patients diagnosed with Q fever, 224 were seropositive but asymptomatic (54%) and only 2% of those affected were hospitalized. *C. burnetii* infections may be acute or chronic.

Acute infection. The incubation period has been estimated to be approximately 20 days (range, 14 to 39 days) (37). There is no typical form of acute Q fever. The clinical signs vary greatly from patient to patient. The most important diagnostic clue is the epidemiological circumstance. Typically, three major presentations are described. These are as follows.

(i) Self-limited flu-like syndrome. A self-limited flu-like syndrome is the most common manifestation of Q fever. In Spain, this form of Q fever has been demonstrated as the cause of 21% of episodes of fever lasting for more than 1 week and less than 3 weeks. The most frequent symptoms, usually following a sudden onset, are high-grade fever (104°F or 40°C), fatigue, headache, and myalgias. In 1973 Derrick (30) described the course of infection in 173 patients infected with *C. burnetii*. The duration of fever was 5 to 57 days, with a median of 10 days. He noted that the duration of fever increased with increasing age and in 28% of cases fever relapsed (30).

(ii) Pneumonia. Atypical pneumonia is one of the most commonly recognized forms of acute Q fever. This is the major manifestation of acute Q fever in Nova Scotia, Canada (103), in the Basque country in Spain (1), and in Switzerland (37), while in France (180), Ontario, California (23), and Australia (168), hepatitis is the predominant form of acute Q fever. Marrie et al. (107) demonstrated that 3.7% of all patients with community-acquired pneumonia admitted to a tertiary-care teaching hospital in Nova Scotia over a 5-year period were due to *C. burnetii*, which is similar to the findings of Lieberman et al. (93) in Israel (5.8%). Most cases are clinically asymptomatic or mild, characterized by a nonproductive cough, fever, and minimal auscultatory abnormalities, but some patients present with acute respiratory distress (105). Pleural effusion can also be present. Findings on the chest radiograph are nonspecific.

The duration of symptoms varies from 10 to 90 days. The mortality rate ranges from 0.5 to 1.5%, depending upon the series (30, 180).

(iii) Hepatitis. Three major forms of hepatitis may be encountered: an infectious hepatitis-like form of hepatitis with hepatomegaly but seldom with jaundice, clinically asymptomatic hepatitis, and prolonged fever of unknown origin with characteristic granulomas on liver biopsy (35, 131, 140, 157, 194).

(iv) Other manifestations. Many other clinical manifestations of acute Q fever are possible: maculopapular or purpuric exanthema in 10% of patients (180), pericarditis and/or myocarditis (which is frequently fatal), and severe headache. Aseptic meningitis and/or encephalitis, which occur in 0.2 to 1.3% of patients with Q fever (15, 28, 34, 55, 63, 102, 110, 156), are rarely accompanied by seizures and coma (34, 156). Polyradiculoneuritis (12), optic neuritis (165), hemophagocytosis (43), hemolytic anemia (22), transient hypoplastic anemia (69), thyroiditis, gastroenteritis (95), pancreatitis, lymphadenopathy mimicking lymphoma (142), erythema nodosum (24), bone marrow necrosis (13, 58), inappropriate secretion of antidiuretic hormone (9), mesangioproliferative glomerulonephritis related to antiphospholipid antibodies (184), and splenic rupture (6) are uncommon manifestations of acute Q fever.

Chronic infection. Chronic Q fever was initially described as lasting for more than 6 months after the onset (151). It occurs in approximately 5% of patients infected with *C. burnetii* and may develop insidiously months to years after the acute disease. In the chronic form of Q fever, *C. burnetii* multiplies in macrophages, and a permanent rickettsemia results in very high levels of persistent antibodies. Typically, the heart is the most commonly involved organ, followed by arteries, bones, and liver (18). Endocarditis usually occurs in patients with previous valvular damage or those who are immunocompromised (143, 147, 149, 182). Chronic Q fever represents 3% of all cases of endocarditis in England and Lyon, France (129), and 15% in Marseille, France (48), and its annual incidence is 0.75 cases per 1 million population in Israel (167). Clinically, the disease usually presents as a subacute or acute blood culture-negative endocarditis (18, 150, 152, 187). Symptoms are not specific. Arterial embolism occurs in about 20% of patients (174). Vegetations are only rarely seen by transthoracic cardiac ultrasonography. They are usually smooth and nodular (76). Because of the lack of specificity of symptoms, the diagnosis is often delayed 12 to 24 months, resulting in an increased mortality rate. Other manifestations of chronic Q fever include infections of aneurysms or vascular grafts (49), isolated hepatitis possibly complicated by hepatic fibrosis and cirrhosis (18, 187), and osteoarthritis and osteomyelitis (13, 40, 146). Rare cases of pericardial effusion (189), pulmonary interstitial fibrosis (2), pseudotumor of the lung (72, 96), lymphoma-like presentation (17), amyloidosis (79), and mixed cryoglobulinemia (42) have been reported in the literature.

Q fever during pregnancy. Both acute and chronic Q fever have been described during pregnancy. In mammals, *C. burnetii* undergoes reactivation during pregnancy and thus is responsible for higher rates of abortion, prematurity, and low birth weight (27, 141, 192, 204). In humans, it has been isolated from the placenta of a woman who became pregnant 2 years after an episode of acute Q fever (139, 175), but few cases have been reported (8, 31, 98, 104, 153, 155, 175). Clinically, although most cases seem to be asymptomatic (98), complications may complicate the course of the disease, such as in utero fetal death (153), placentitis (8), or thrombocytopenia (155). Although intrauterine transmission of *C. burnetii* has been documented, the consequences of congenital Q fever remain to be determined.

NONSPECIFIC LABORATORY DIAGNOSIS

Acute Q fever. The leukocyte count in patients with acute Q fever is usually normal (18). However, 25% of patients have an elevated leukocyte count, ranging from 14×10^9 to 21×10^9/liter. The erythrocyte sedimentation rate may be elevated. Thrombocytopenia is noted in 25% of patients. Liver enzyme levels are elevated in as many as 85% of patients. The increase in transaminase levels is usually moderate, ranging from 2 to 10 times normal values. During an episode of prolonged fever, the association of a normal leukocyte count, thrombocytopenia, and elevated hepatic enzyme levels are evocative of Q fever. However, thrombocytosis ($>400 \times 10^9$ liter) may be encountered during convalescence. Twenty percent of patients have an elevated creatine phosphokinase level. In Q fever meningoencephalitis, a mild lymphocytic pleiocytosis is frequently noted in the spinal fluid.

Autoantibodies are commonly found during the course of Q fever, but their real significance is still unknown. They are probably an epiphenomenon of the disease but can be responsible for severe complications. A variety of these autoantibodies have been described in acute Q fever, including antimitochondrial antibodies (41, 92) and anti-smooth muscle antibodies (92). A moderate level of anti-smooth muscle antibodies is frequent at the time of seroconversion (151). Other antibodies, such as antibodies to phospholipids, especially anticardiolipin antibodies (74, 138, 159, 166, 184) or lupus anticoagulant (126), should be detected prior to an hepatic biopsy. Although they had been suspected to be identical, anti-phase II antibodies to *C. burnetii* and anticardiolipin antibodies have been demonstrated to be different (126). It is possible that the antibodies to phospholipids could be triggered by an increased

level of exposure of anionic phospholipids from endothelial cells damaged by *C. burnetii* (126). These antibodies are usually transient and disappear during convalescence (184). Poux et al. (138) have detected both immunoglobulin G (IgG) and IgM anticardiolipin antibodies in a patient with acute Q fever and observed an early disappearance of IgM in the first 2 months of convalescence. An acquired inhibitor of blood coagulation factor IX (antihemophilia B) has also been reported (5).

Chronic Q fever. In Q fever endocarditis, clinical and biological symptoms are related to the predominantly cell-mediated inflammatory response to the microorganism (18, 150, 182). Conventional blood cultures remain negative. The laboratory manifestations of an inflammatory syndrome are usual, including anemia, elevated erythrocyte sedimentation rate, and polyclonal hypergammaglobulinemia. The leukocyte count may be normal, increased, or decreased. Thrombocytopenia and elevated hepatic enzyme levels are commonly found. Renal involvement is common, characterized by an elevated creatinine level and microhematuria. Monoclonal immunoglobulins are rarely observed (42), whereas cryoglobulins are frequently found. Autoantibodies are also frequent in chronic Q fever, particularly rheumatoid factor, anti-smooth muscle (92), or antinuclear antibodies. Antimitochondrial antibodies (41, 92), circulating anticoagulant antibodies, and a positive Coombs' test may also be observed (92).

SPECIFIC LABORATORY DIAGNOSIS

Collection and storage of specimens. *C. burnetii* is a very infectious disease. Thus, only biosafety level 3 laboratories and experienced personnel should be allowed to manipulate contaminated specimens and cultivate this microorganism from clinical samples. Several human specimens are suitable for the detection of *C. burnetii*, but their availability depends on the clinical presentation. DNA amplification may be performed from blood, cerebrospinal fluid, bone marrow, cardiac valve biopsy, vascular aneurysm or graft, bone biopsy, or liver biopsy specimens; milk; placenta; fetal specimens in case of an abortion; and cell culture supernatants. Blood should be collected on EDTA or sodium citrate, and the leukocyte layer should be saved for the amplification. Solid specimens should be kept frozen at −80°C before testing. *C. burnetii* may be cultivated from the leukocyte layer of a heparinized blood, whole blood, plasma, bone marrow, cerebrospinal fluid, cardiac valve biopsy, vascular aneurysm or graft, bone biopsy, or liver biopsy specimen; milk; placenta; and fetal specimens in case of an abortion but not from blood collected on EDTA or sodium citrate. All specimens, excluding whole blood, should be stored at −80°C and should be forwarded on dry ice to the diagnostic laboratory. Whole blood should be kept at 4°C. Furthermore, erythrocytes should not be inoculated onto shell vials because they lead to a high background at the time of the examination with UV light.

Pathological examination. The immune response during Q fever is associated with an inflammatory reaction, which results in the formation of granulomatous lesions most commonly involving the lungs, liver, and bone marrow. La Scola et al. (87) have demonstrated that the route of infection may influence the pathological changes. The histology of Q fever pneumonia in humans has rarely been studied (72, 73, 94, 132). Macroscopically, red or gray hepatization may be present. Microscopically, interstitial edema and infiltration by lymphocytes and macrophages occur. Alveolar spaces are filled with histiocytes, and intra-alveolar focal necrosis and hemorrhage have been described. A necrotizing bronchitis and bronchiolitis may be encountered. Microorganisms are usually lacking in these lesions. Giant cells and plasma cells were seen in a pulmonary pseudotumor due to *C. burnetii* (72). The hepatic lesions are different in acute and chronic Q fever. In acute cases, the characteristic findings are granulomatous lesions containing the so-called doughnut granuloma, which consist of dense fibrin rings surrounding a central lipid vacuole (35, 56, 131, 140, 169) (Fig. 2A). Granulomatous changes and necrosis were noted in bone marrow (25, 124, 169). In chronic cases, pathological findings are nonspecific: lymphocytic infiltration and foci of spotty necrosis (72). The vegetations in Q fever endocarditis are often smooth and nodular. The valve is often infiltrated with foamy macrophages, which are filled with *C. burnetii* cells.

Immunodetection of *C. burnetii* in tissues. The detection of *C. burnetii* in tissues is especially informative in patients who are undergoing treatment for chronic Q fever. Samples can be tested fresh or after formalin fixation and paraffin embedding. Valvular or vascular samples are most valuable. Several techniques are available, including immunoperoxidase staining (16) (Fig. 2B), a capture enzyme-linked immunosorbent assay system (ELISA) or enzyme-linked immunosorbent fluorescence assay system (176), or tests with monoclonal antibodies (114, 119, 176). The last technique can also be used for detection of antigen in paraffin-embedded tissues (148). Brouqui et al. (16) examined the valves of 17 patients with Q fever endocarditis using immunohistochemical methods. The organisms were clustered as a single intracytoplasmic mass within mononuclear cells and usually occupied the entire cytoplasm. Kocianova and Lukacova (83) could detect *C. burnetii* in the hemolymph of ticks using an immunodot blot test.

DNA amplification. PCR has successfully been used to detect *C. burnetii* DNA in cell cultures and clinical samples (171) (Table 1). Initially, methods used specific hybridization of labelled DNA probes to nucleic acid amplified from clinical samples (50, 51, 99, 199). These methods were very sensitive and specific but were available only in specialized research laboratories. The availability of primers derived from genes specific to *C. burnetii* has allowed a simple and reliable method for the detection of this bacterium, even in paraffin-embedded tissues (171, 172). Moreover, PCR has proven to be more sensitive than standard culture techniques for retrospective diagnosis with frozen samples and for the follow-up of patients treated for chronic Q fever (171). In our experience, specimens kept frozen at −80°C are suitable for PCR for several years. Fritz et al. (52) have used PCR to quantify the amount of *C. burnetii* in tissue. In our laboratory, we routinely use primers derived from the *htpAB*-associated repetitive element (70, 197). This element exists in at least 19 copies in the *C. burnetii* Nine Mile I genome, and PCR based upon this gene is very sensitive (197).

Culture of *C. burnetii*. The isolation of *C. burnetii* must be done only in biosafety level 3 laboratories due to its extreme infectivity. This microorganism can be isolated by inoculation of specimens onto conventional cell cultures (monkey kidney cells, Vero cells) or into embryonated hen yolk sacs (127) or laboratory animals, such as mice or guinea pigs (200). Embryonated eggs die 7 to 9 days after inoculation. Guinea pigs develop fever 5 to 8 days after intraperitoneal inoculation. The spleen is the most valuable organ for the recovery of *C. burnetii*. Ground spleen extracts should subsequently be inoculated into embryonated eggs. Although they are now used less often, these methods remain helpful in cases requiring isolation from tissues contaminated with multiple bacteria or in order to obtain phase I *Coxiella* antigens from phase II cells. The recent development of a cell microculture system from a commercially available method for virus culture, the shell vial

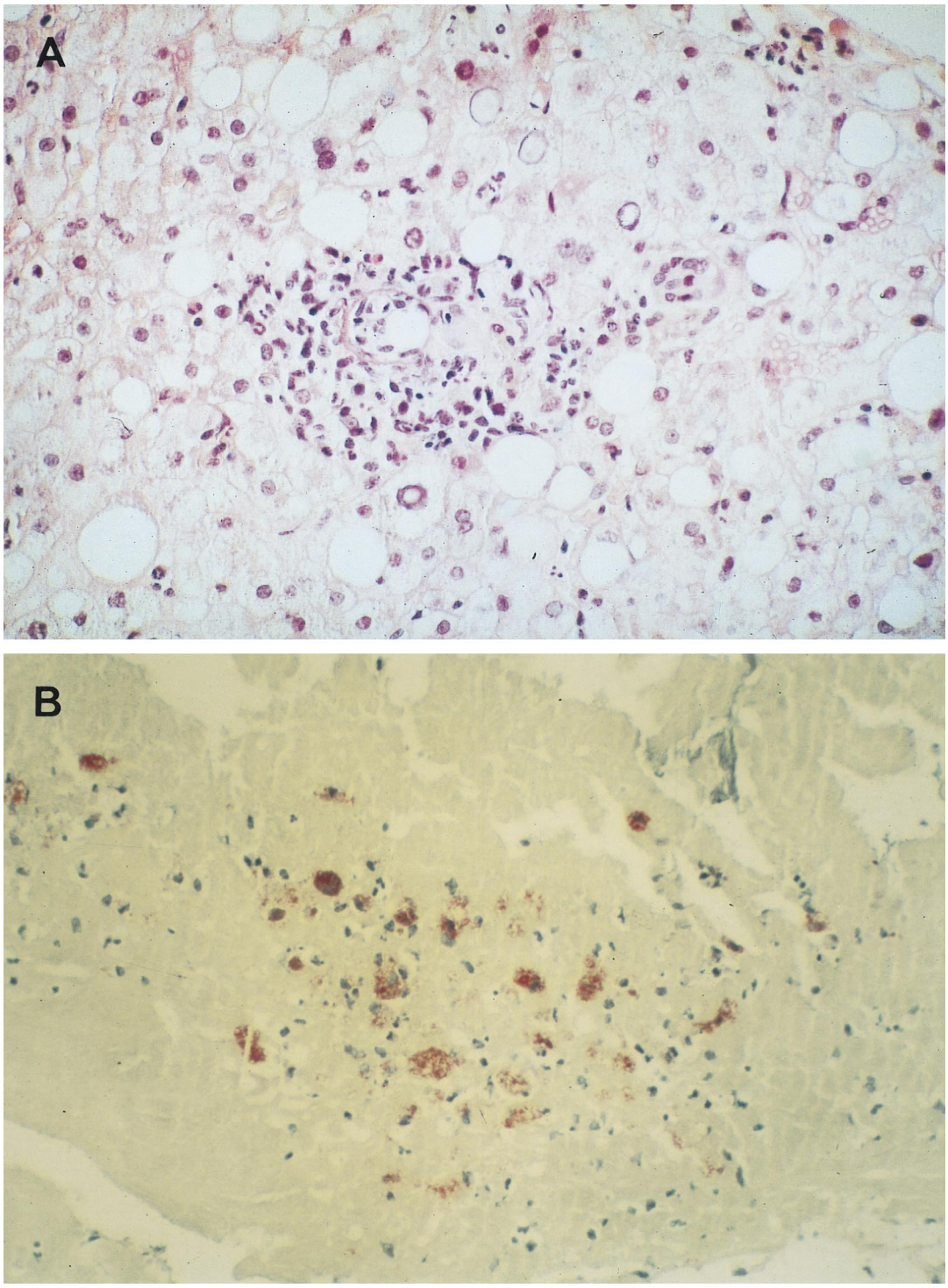

FIG. 2. (A) Liver in acute Q fever. A liver biopsy specimen was stained with hematoxylin-phloxin-saffron. One granuloma is seen within the fatty liver parenchyma. The lesions consist of inflammatory infiltrates made of epithelioid cells, polymorphonuclear leukocytes, and histiocytes. Magnification, ×400. (B) Immunoperoxidase staining of a cardiac valve biopsy specimen showing fibrous valvular tissue comprising inflammatory infiltrates made of histiocytes. Magnification, ×40.

cell culture system, has allowed for improvement in the isolation of intracellular bacteria, especially *C. burnetii* (101, 154). Specimens are inoculated onto human embryonic lung fibroblasts grown on a 1-cm^2 coverslip within a shell vial. A 1-h centrifugation step enhances the attachment and penetration of bacteria into the cells. After an incubation period of 6 days, detection of *C. burnetii* within the cells is achieved by microscopic examination after staining. The organism appears as a short rod which is not stained by Gram staining but which is visible after Giemsa or Gimenez staining (54). The identification of *C. burnetii* within the cells is performed by a direct immunofluorescence assay with polyclonal or monoclonal an-

TABLE 1. Genes and derived primers available for PCR amplification of *C. burnetii*

Gene	Reference(s)	Primers (sequences)
16S rRNA	199	16S1 (5′-CTC CTG GCG GCG AGA GTG GC-3′) 16S2N (5′-GTT AGC TTC GCT ACT AAG AAG GGA ACT TCC C-3′)
23S rRNA	71	976F (5′-AGG TCC TGG TGG AAA GGA ACG-3′) 1446R (5′-TCT CAT CTG CCG AAC CCA TTG C-3′)
16S-23S rRNA internal transcribed spacer	170, 179	16SF (5′-TTG TAC ACA CCG CCC GTC A-3′) 23SR (5′-GGG TT (CGT) CCC CAT TCG G-3′) 16SS (5′-GAA GTC GTA ACA AGG TA-3′) 23SS (5′-TCT CGA TGC CAA GGC ATC CAC C-3′)
Superoxide dismutase	171	CB1 (5′-ACT CAA CGC ACT GGA ACG GC-3′) CB2 (5′-TAG CTG AAG CCA ATT CGC C-3′)
Plasmid QpRS	199	QpRS01 (5′-CTC GTA CCC AAA GAC TAT GAA TAT ATC-3′) QpRS02 (5′-CAC ATT GGG TAT CGT ACT GTC CCT-3′)
Plasmid QpH1	199	QpH11 (5′-TGA CAA ATA GAA TTT CTT CAT TTT GAT-3′) QpH12 (5′-GCT TAT TTT CTT CCT CGA ATC TAT GAA T-3′)
cbbE	199	G4131 (5′-CTG ATG TGT CAA GTA ATG TCG G-3′) G4132 (5′-CTT CAT GGT TAT GAT TCT GCG-3′)
htpAB	197	Trans1 (5′-TAT GTA TCC ACC GTA GCC AGT C-3′) Trans2 (5′-CCC AAC AAC ACC TCC TTA TTC-3′)

ti-*C. burnetii* antibodies conjugated to fluorescein isothiocyanate (154) (Fig. 3). In our laboratory, we systematically amplify shell vial supernatants by PCR. Although Mühlemann et al. (119) could isolate *C. burnetii* from the heart valves of patients treated for Q fever endocarditis, better results are obtained if clinical specimens are collected prior to the initiation of antibiotic therapy (121). Fifteen percent of untreated patients with Q fever pneumonia have positive blood cultures by this method, as do 53% of patients with endocarditis (53, 121).

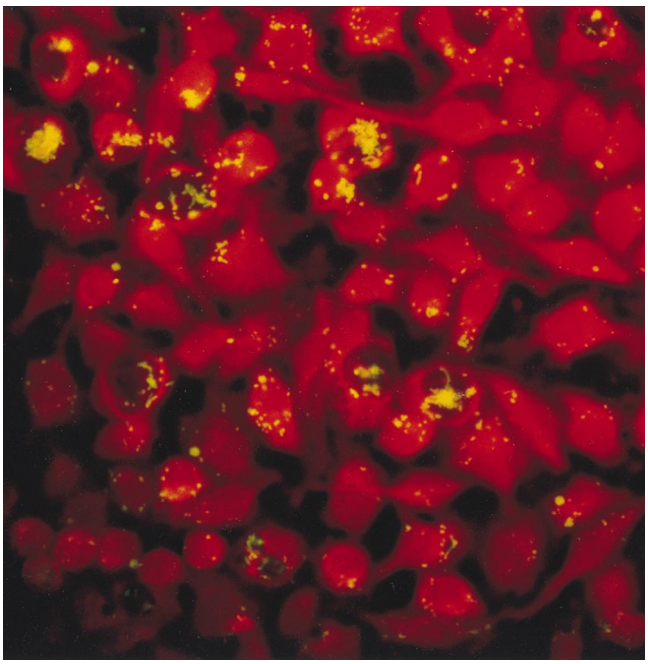

FIG. 3. Shell vial cell culture system: direct immunofluorescence assay incorporating monoclonal anti-*C. burnetii* antibodies conjugated to fluorescein isothiocyanate. The *C. burnetii* isolates appear as short rods. Magnification, ×870.

Serological diagnosis of Q fever. Since the clinical diagnosis is difficult, in most instances, the diagnosis of Q fever relies upon serology. Several methods have been described: microagglutination (46, 81, 123), complement fixation (66, 120, 134), radioimmunoassay (33), indirect immunofluorescence antibody tests (immunofluorescence assay) (44, 134), indirect haemolysis test (183), ELISA (84, 133, 188, 191, 201), enzyme-linked immunosorbent fluorescence assay (163), dot immunoblotting, and Western immunoblotting (10, 198). Criteria to be taken into account in choosing a diagnostic test include its specificity, sensitivity, positive predictive value, cost, and the amount of antigen required. The most reliable and commonly used methods are indirect immunofluorescence, complement fixation, ELISA, and microagglutination. Only the first two are commercially available. The sensitivity, specificity, and positive predictive value of immunofluorescence assay, complement fixation, and ELISA are presented in Table 2.

(i) Indirect immunofluorescence. Currently, the immunofluorescence assay is the reference method for the serodiagnosis of Q fever (130). It is the simplest and one of the most accurate serological techniques. In order to prepare antigens for this test, phase II *C. burnetii* Nine Mile reference strain is grown in confluent layers of L929 mouse fibroblasts, while phase I antigens are obtained from the spleens of mice inoculated with phase II organisms (181). This method of preparation has been demonstrated to yield antigens with the highest sensitivity for *C. burnetii* antibody detection (85). In our laboratory, we use a microimmunofluorescence technique, which requires very small amounts of antigens. Sera are diluted in phosphate-buffered saline with 3% nonfat powdered milk to avoid the nonspecific fixation of antibodies. This method can be used to determine antibodies to phases I and II in the IgG, IgM, and IgA fractions. However, test results can be confounded by the presence of a rheumatoid factor. Thus, a rheumatoid factor absorbant is used in order to remove IgG before the determi-

TABLE 2. Sensitivity, specificity, and positive and negative predictive values of different serological tests for diagnosis of Q fever

Test	Form of disease	Titer	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Reference
Microimmunofluorescence	Acute	Anti-phase II IgG, ≥1:200; anti-phase II IgM, ≥1:50	58.4	92.2	NA[a]	88–96.6	181
	Chronic	Anti-phase I IgG, ≥1:800	100	NA	98.6	NA	181
		Anti-phase I IgG, ≥1:1,600	NA	NA	100	NA	181
ELISA	Acute	Anti-phase II IgG, ≥1:1,024	80	>99	NA	NA	191
		Anti-phase II IgM, ≥1:512	84				
	Acute (screening)	Anti-phase II IgG, ≥1:128; anti-phase II IgM, ≥1:128	NA	97.3–98.7	NA	NA	191
Microagglutination	Acute	1:64	81.6	98.6	NA	NA	123

[a] NA, not available.

nation of IgM and IgA (181). The choice of a negative cutoff titer depends upon the source and purity of the antigen and the amount of background antigen stimulation in the population to be studied. We use a 1:50 dilution as our first positive dilution (181). Screening is performed with anti-phase II anti-immunoglobulins with a 1:50 dilution for the tested sera. Positive sera are then serially diluted and tested for the presence of anti-phase I and II IgG, IgM, and IgA. Seroconversion is usually detected 7 to 15 days after the onset of clinical symptoms. About 90% of patients have detectable antibodies by the third week.

(ii) Complement fixation. Complement fixation is very specific, although it is less specific than the immunofluorescence assay, but it lacks sensitivity (134). Sera are heat inactivated before testing against phase II antigens (66). This method detects both anti-phase I and II antibodies. However, a prozone phenomenon may be present with serum specimens from patients with chronic Q fever, and this phenomenon could result in a false-negative test. It is also more time-consuming than the immunofluorescence assay (136). Moreover, cross-reaction with hen egg antigens may result in false-positive results. The interpretation of results requires acute- and convalescent-phase serum samples. Seroconversion is detected later by the complement fixation test than by the immunofluorescence assay or ELISA (between 10 and 20 days after the onset of symptoms) (57, 134).

(iii) ELISA. First described by Field et al. (44), ELISA was described as more specific and sensitive than complement fixation for the diagnosis of Q fever. It was then proposed as a good method for seroepidemiological surveys (135). Peter et al. (133) and Cowley et al. (26) have demonstrated that this technique was even more sensitive than the immunofluorescence assay and could serve for the serodiagnosis of Q fever. However, it is a more laborious technique than the immunofluorescence assay and it requires a considerable experience in interpreting the results. Therefore, its application to Q fever diagnosis is still limited. Technically, microtiter plates are coated with purified *C. burnetii* antigens. Serially diluted sera in phosphate-buffered saline containing 0.1% Tween 20 are then incubated with the antigens, and antibodies are detected with alkaline phosphatase-conjugated rabbit anti-human IgG, IgM, and IgA. Both anti-phase I and II antibodies are detected.

(iv) Western blotting. Western immunoblotting has been reported as both a specific and a sensitive method for the diagnosis of Q fever (10). However, in our experience (several thousands of tests), the results were not reproducible and the test lacked specificity (unpublished data). Furthermore, it was time-consuming and was not suitable for screening. It is based on the entire spectrum of antigens of *C. burnetii* and allows a differentiation of the humoral response to a number of different antigenic components of this microorganism. The molecular masses of the different antigens ranges from 10 to 100 kDa.

(v) Dot immunoblotting. Cowley et al. (26) demonstrated that dot immunoblotting was as sensitive and as specific as ELISA and immunofluorescence assay but more sensitive than complement fixation. They also proposed this method as a screening test.

(vi) Microagglutination. Microagglutination is simple and sensitive and can detect an early antibody response to *C. burnetii* (81). It has a major disadvantage, especially when compared to the immunofluorescence assay, in that it requires large amounts of antigens. More recently, high-density particle agglutination has been demonstrated to be highly specific and much more sensitive than microagglutination (123).

(vii) Indirect hemolysis test. Tokarevich et al. (183) proposed an indirect hemolysis test as early as 1990. They suggested that this test could be highly sensitive and specific and could serve for the follow-up of chronic Q fever.

(viii) Radioimmunoassay. Doller et al. (33) proposed a radioimmunoassay for the diagnosis of Q fever. This technique, based on the IgM antibody-capture principle, uses ^{125}I and therefore must be used in radioactivity-equipped laboratories.

Cross-adsorption. Cross-adsorption is used for the detection of antibodies cross-reacting with other bacteria. This cross-reactivity will vary depending on the serological technique used and the host animal from which the antiserum is obtained. Cross-reactions against *Legionella pneumophila* (38, 45), *Legionella micdadei* (32, 122), and *Bartonella quintana* and *Bartonella henselae* (88) have been reported. Confirmation of antigenic cross-reactivity is made by cross-adsorption and Western immunoblotting. A cross-adsorption study is carried out by mixing separately the serum to be tested with the bacteria involved in the cross-reaction. Cross-adsorption of the

TABLE 3. Interpretation of *C. burnetii* serology results[a]

Form of disease	Microimmunofluorescence assay inverse titer						Complement fixation assay titer	
	Anti-phase II			Anti-phase I			Anti-phase II	Anti-phase I
	IgG	IgM	IgA	IgG	IgM	IgA		
Acute	400	200	50	50	200	0	≥1:40	0
Acute	400	100	0	0	0	0		
Treated	400	0	0	200	0	0	1:1	0
Treated	100	0	0	100	0	0		
Chronic	3,200	0	800	3,200	0	800	≥1:200	≥1:200
Chronic	6,400	800	400	6,400	800	400		

[a] The titers reported here are characteristic examples of the antibody responses observed during Q fever.

serum sample being studied results in the disappearance of homologous antibodies when adsorption is performed with the bacterium responsible for the disease, whereas disappearance of only homologous antibodies is observed when adsorption is performed with antigens of the bacteria responsible for the cross-reaction. One major limitation of this method is that it requires large amounts of antigens.

Interpretation of results of serological testing. The predictive value of positive and negative results is affected by the prevalence of the disease, creating a certain degree of background antigen stimulation in the studied population (115). Therefore, the determination of cutoff values is very important for the interpretation of the serological results (Table 3). For example, in Marseille we use a 1:50 serum dilution for immunofluorescence assay screening, whereas in Halifax we use a 1:8 dilution (109). While a diagnostic test needs to be very sensitive, a seroepidemiology test needs to be very specific to prevent false-positive results due to cross-reacting antibodies. The antigenic variation of *C. burnetii* is extremely useful in differentiating acute and chronic illness. In acute Q fever, antibodies to phase II antigens predominate, and their titer is higher than the phase I antibody titer. As with many other infectious diseases, IgM antibodies are the first to appear. On the other hand, in chronic forms of the disease, such as endocarditis, elevated anti-phase I antibodies are uniformly detected. As cutoff values in the immunofluorescence assay, Tissot-Dupont et al. (181) recommend titers of anti-phase II IgG of ≥200 and titers of anti-phase II IgM of ≥50 for the diagnosis of acute Q fever and titers of anti-phase I IgG ≥800 for the diagnosis of chronic Q fever. They have also demonstrated that anti-phase I IgA titers did not contribute to the diagnosis of chronic Q fever. Recently, we showed that an anti-phase I IgG *C. burnetii* titer of ≥1:800 was diagnostic of Q fever in case of an endocarditis, and thus, we have modified the Duke criteria for the diagnosis of Q fever endocarditis to include one positive blood culture for *C. burnetii* and a phase I IgG titer of ≥1:800 as major criteria for the diagnosis of Q fever endocarditis (48). A complement fixation titer of 1:40 is diagnostic for acute Q fever (57), while a 1:200 titer of antibody to phase I is diagnostic for chronic Q fever (136). With the ELISA, a fourfold or greater increase in the humoral immune response to *C. burnetii* antigens is highly predictive of Q fever. Waag et al. (191) proposed cutoff values of ≥1,024 for anti-phase II IgG, ≥512 for anti-phase II IgM, and ≥128 for anti-phase I IgG and IgM antibodies. With Western immunoblotting, chronic Q fever results in the detection of a greater number of protein antigens. The presence of antibodies reacting with 50-, 80-, and 160-kDa antigens is indicative of a chronic form of the disease (10).

Prognosis. In acute Q fever, immunofluorescence assay titers reach their maximum levels 4 to 8 weeks after the onset of disease and then decrease gradually over the following 12 months (57). Dupuis et al. (36) have shown that IgM titers declined to undetectable levels after 10 to 12 weeks, while it was 17 weeks in the study by Field et al. (44). The ELISA technique could even detect specific antibodies for more than 5 years after an acute episode (191). Guigno et al. (57) have tried to correlate the evolution of acute Q fever and the IgG/IgM ratio based on a single serum, but these data were too imprecise to be useful. Outschoorn et al. (128) have developed an ELISA for the detection of IgG subclass antibodies and have suggested that IgG2 could play a protective role or limit chronic disease. Complement fixation and immunofluorescence assay can be combined for the follow-up. A drop in the complement fixation titer often implies resolution and will occur before an immunofluorescence assay titer drop. The persistence of high levels of anti-phase I antibodies, despite appropriate treatment, or the reappearance of such antibodies should raise suspicion of possible chronic Q fever. Patients with valvular or vascular abnormalities, those who are immunodeficient, and pregnant women should have repeated *C. burnetii* serology tests if they have a medical history of acute Q fever or a prolonged and unexplained febrile episode. In case of acute Q fever in such a patient, an immunofluorescence assay should be done monthly for at least 6 months. The follow-up of patients treated for chronic Q fever should also be done serologically. During therapy, serological testing should be carried out once monthly for 6 months and every 3 months thereafter. The levels of antibodies decrease very slowly. IgM antibodies, when present, disappear first, and then the IgA antibodies disappear, but the IgG titers remain positive for years. Antimicrobial treatment can be stopped after 18 months to 3 years if the anti-phase I IgG titer by immunofluorescence assay is below 1:400 and anti-phase I IgA is undetectable (144, 151). Other attempts to predict the course of chronic Q fever have been made. Camacho et al. (20) used radial diffusion to detect the different IgG subclasses. They demonstrated that high IgG1 and IgG3 levels were observed in patients with chronic Q fever, and they suggested that the determination of IgG subclasses could be used to differentiate acute from chronic infection and that during long-term follow-up such testing could be used to detect a relapse. The same investigators (20), using an ELISA, noted that IgA2 antibodies were mostly found in acute Q fever, while IgG1 seemed to be specific for chronic forms of the disease. Sabatier et al. (158) suggested that the determination of lymphocyte subclasses could be predictive of the course of Q fever, with a CD4/CD8 ratio <1 being predictive of a possible relapse.

CONCLUSION

Although described 60 years ago, Q fever is still a poorly understood disease (116, 145). Its reservoirs seem to be related

to any mammal, but ticks may also be reservoirs. The clinical presentation is very pleomorphic and includes severe forms with a poor prognosis. Most often, acute cases present as asymptomatic infections, as a flu-like syndrome, as a pneumonia, or as hepatitis. Host factors probably play an important role in the development of chronic disease, which may present as a blood culture-negative endocarditis or as an infected aneurysm. Although its exact prevalence is unknown, it is likely that the number of cases of Q fever is underestimated. Therefore, the diagnosis must be considered in the case of an unexplained fever, especially if the fever recurred following contact with possibly contaminated mammals. The best tests for diagnosis are those which permit the direct detection of bacteria. They include shell vial cell culture, PCR amplification, and immunodetection with tissue biopsy specimens. All these techniques require a level 3 biosafety laboratory and trained personnel due to the extreme infectivity of *C. burnetii*. In chronic cases, the techniques that allow the direct detection of *C. burnetii* in blood or tissues should be used before the beginning of therapy. As for indirect specific diagnosis, the technique to be used should be very sensitive and should detect antibodies early in the course of the disease. Although many techniques have been described, immunofluorescence assay is the reference method. It is both very specific and sensitive. In case of acute Q fever, diagnosis would be confirmed by an immunofluorescence assay titer greater than or equal to the cutoff value (which must be determined for each geographical area) or by a fourfold increase in the antibody titer detected by immunofluorescence assay, complement fixation, ELISA, or microagglutination. The presence of cross-reacting antibodies should be investigated by cross-adsorption followed by Western immunoblotting.

We recommend that all patients with blood culture-negative endocarditis, febrile patients with aortic aneurysms, and those with prolonged fever, granulomatous hepatitis, or atypical pneumonia in areas where Q fever is endemic should at the very least undergo serological testing for Q fever.

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