



## Emergence of Q fever

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### Abstract

Q fever is a worldwide zoonosis with many acute and chronic manifestations caused by the pathogen *Coxiella burnetii*. Farm animals and pets are the main reservoirs of infection, and transmission to human beings is mainly accomplished through inhalation of contaminated aerosols. Persons at greatest risk are those in contact with farm animals and include farmers, abattoir workers, and veterinarians. The organs most commonly affected during Q fever are the heart, the arteries, the bones and the liver. The most common clinical presentation is an influenza-like illness with varying degrees of pneumonia and hepatitis. Although acute disease is usually self-limiting, people do occasionally die from this condition. Endocarditis is the most serious and most frequent clinical presentation of chronic Q fever. Vascular infection is the second most frequent presentation of Q fever. The diagnosis of Q fever is based on a significant increase in serum antibody titers. The treatment is effective and well tolerated, but must be adapted to the acute or chronic pattern with the tetracyclines to be considered the mainstay of antibiotic therapy. For the treatment of Q fever during pregnancy the use of long-term cotrimoxazole therapy is proposed.

**Keywords:** *Coxiella burnetii*, Q fever, Epidemiology

### Introduction

*Coxiella burnetii* is a strictly intracellular Gram-negative bacterium that live and multiply in the phagolysosomes of infected cells, such as monocytes and macrophages, in an acidic environment (pH 4.8) (1). *C. burnetii* is extremely resistant to heat, pressure, and chemical stress and can survive for months in stressful environments (2). The resistance to stressful environments, together with the high rate of infectivity and ease of transmission between humans classifies *C. burnetii* as a “Category B” warfare agent. Current epidemiological studies indicate that Q fever is a public health problem in many countries, especially where people are in direct contact with domestic animals, such as cattle, sheep, or goats. Moreover, the potential impact of Q fever on public health has been demonstrated by an ongoing outbreak in the Netherlands (3).

*C. burnetii* displays antigenic variation that is similar to the smooth-rough variation present in other Gram-negative bacteria; this antigenic variation is related to changes in the bacterial lipopolysaccharide (LPS) layer (4). When isolated from animals or humans, *C. burnetii* expresses a phase I antigen and is extremely infectious (a single bacterium can infect a human). After the bacterium is subcultured in cells or embryonated eggs, the LPS layer becomes modified and the bacterium transitions to the non-infectious phase II form. Patients with acute Q fever have serum antibodies that are directed against the phase II antigens, whereas patients with chronic Q fever have a serum antibodies against the phase I and phase II antigens (5); thus, antigenic shift is a valuable marker for differentiating acute from chronic Q fever. Although it was previously classified in the order Rickettsiales, 16s rDNA sequence analysis places *C. burnetii* with *Legionella* and *Francisella*

in the gamma subdivision of the Proteobacteria (6, 7). The genome of the first American isolate of *C. burnetii*, which was isolated from a tick and termed the Nine Mile strain, is 1,995,275 bp (8). Genomic analysis showed that *C. burnetii* possesses more genes involved in metabolic processes than other intracellular bacteria, such as chlamydia, rickettsia, or anaplasma (8). *C. burnetii* has a circular chromosome and a facultative plasmid. Four plasmid types have been identified, although not all of the strains have plasmids (7, 9). In a murine model, there appeared to be a correlation between the LPS phase, the plasmid type and clinical manifestations of acute fever (10, 11).

### **Epidemiology**

*C. burnetii* can infect and persist in a wide range of domestic and free-living ungulates. Horses, pigs, dogs, cats, camels and buffaloes, as well as wild and domestic birds such as chickens, pigeons, ducks, geese and turkeys can be infected without showing any clinical signs (12). *C. burnetii* has also been isolated from rabbits, squirrels, mice, deer and other free-living animals (2). However, cattle, sheep and goats are the most important reservoirs for human infection (12). *C. burnetii* is readily excreted in the milk, urine, feces and uterine discharge of infected cattle, sheep, goats and other ungulates, although the period of shedding varies between species. Moreover, the bacterium is present in high numbers in the amniotic fluid, placenta and fetal membranes of parturient ewes, goats and cattle (13-15). Over  $10^9$  bacteria per gram of placenta are released from infected animals at the time of delivery (12), and the animals may continue to shed infectious particles long after abortion (16). Experimental and epidemiological evidence clearly show that contaminated aerosols are the source of *C. burnetii* contamination in humans (17). *C. burnetii* is very stable in the natural environment and may survive for several weeks in areas where animals were present. Infection occurs after the inhalation of aerosols generated from infected placenta and body fluids, or as contaminated dust produced by desiccation of the primary source. Al-

though infected milk or contaminated food can be a risk factor for acquiring Q fever (18), the data from experiments where contaminated milk was administered is contradictory (19,20). Contaminated hides and wool may also be a source of infection if there is direct contact with the bacterium, or after infected feces has dried and been inhaled as airborne dust particles. Tick species can be naturally infected with *C. burnetii*, and large numbers of the bacteria are shed in tick feces (21). Although ticks are important vectors for maintaining bacterial survival in nature, they are not essential vectors for animal or human infection. Sexual transmission of Q fever has been demonstrated in mice (22), and viable *C. burnetii* has been isolated in bull semen (23). Moreover, Milazzo *et al.* described sexual transmission of occupationally acquired Q fever from a man to his wife (24).

### **Risk factors**

The people with the greatest risk of infection are in contact with farm animals, such as farmers, slaughterhouse workers, and veterinarians (Table 1). In a group of patients with acute fever, Raoult *et al.* found that 8% were farmers or veterinarians; moreover, 37.9% of the patients lived in a rural environment, 23.2% had consumed farm goat cheese, and 35.4% were in contact with newborn or pregnant animals (25). Immunosuppression was also seen in 4.7% of the patients (25). A study in France from 1982 to 1990 with 323 patients hospitalized with acute Q fever showed that infection was more frequently reported in men than in women (sex ratio, 2.5:1) (25). However, the data for infection in children indicates that boys and girls are almost equally represented (26). Patients with a previous valvulopathy, immunocompromised patients (e.g., HIV infection), and pregnant women have a higher risk of contracting chronic Q fever (25,27,28). Patients with endocarditis are mostly male, and the mean age of affected patients is greater than patients with acute Q fever. Moreover, valvular insufficiency, valvular stenosis, mechanical prosthetic valves and bioprosthetic valves are associated with endocarditis (25).

### Geographic Distribution

Because *C. burnetii* infection does not appear to affect the health and reproduction of domestic animals, the geographic distribution of animal coxiellosis is not well established. Consequently, in most countries Q fever is not included on the list of nationally notifiable diseases. Epidemiological data have been mostly obtained from investigations of defined outbreaks, from serosurveys of in humans and animals in specific areas, or from data obtained from public health laboratories. However, after *C. burnetii* was classified as a Category B potential bioweapon, there was an increased interest in Q fever. Q fever has been described worldwide, except in New Zealand.

### Europe

In a French survey of 942 serum samples collected from blood donors in Marseille, Tissot Dupont et al. (1992) detected specific anti-*C. burnetii* antibodies in 38 samples, which corresponds to a seroprevalence of 4.03 per 100 inhabitants (29). Moreover, they found significant seasonal increases in infection in April and June that were not correlated with the sheep birthing season, which occurs in October (29). Similarly, Frankel et al. reported periodic variations in the monthly distribution between January 2000 and December 2009, with peaks between April and September. They also observed that the percentage of positive cases increased from 1% in 1989 to 3-4% in 2005-2009 (30). Additionally, they reported that the annual incidences of acute Q fever and endocarditis were 2.5 per 100,000 inhabitants and 0.1 per 100,000 inhabitants, respectively (30). In France, Q fever outbreaks have occurred in Banon (1987), with 40 cases of people working on a farm with goats; Briançon (1996), with 29 confirmed cases; Chamonix (2002), with 101 confirmed cases with the acute form of the disease; and Cholet (2009), with 50 confirmed cases (18, 31, 32).

The infection prevalence in the Netherlands over the last three years (182, 1,000 and 2,361,206 cases in 2007, 2008 and 2009, respectively) has demonstrated the importance of detecting Q fe-

ver infection in livestock, especially goats (33). Investigations have shown that outbreaks in 2007, 2008 and 2009 in the Netherlands started spreading around 2005 (33). Subsequently, measures were taken to vaccinate a large number of goats to prevent disease transmission (33). Van der Hoek et al. described the distribution of human Q fever in 2009 in the Netherlands, where 59% of the cases lived within a 5-km radius of an infected dairy sheep or dairy goat farm (34). Furthermore, the incidence was 69 per 100,000 in populations that lived within 5 km of infected livestock and 6 per 100,000 outside the 5-km radius (34).

In the UK, approximately 70 cases of Q fever are identified each year because of routine surveillance and seroprevalence studies; the data indicate that approximately 27% of farmers and 10% of the general population have antibodies against the bacterium (35,36). The rate of prevalence, which ranges from 0.15 to 0.35 cases per 100,000 people per year, does not appear to have changed substantially during the last 45 yr (36). A majority of the cases have been reported in northern Ireland and southwestern England, and Q fever accounts for about 3% of all endocarditis cases in England and Wales (37). Between 1980 and 1996, eight outbreaks in the United Kingdom were reported in the literature (2). An outbreak of Q fever occurred in South Wales from July through September 2002 with 95 cases of acute Q fever (38), and in Cheltenham in June 2007 with 30 confirmed or probable cases of Q fever (39). Q fever has been also reported in soldiers returning from Afghanistan and Belize (40). In Germany, 100-500 human infections indicative of *C. burnetii* are reported annually. However, the rate is increasing, most likely as a consequence of recent outbreaks. Infected sheep have been implicated as the source of infection in 24 out of 40 documented outbreaks reported in Germany between 1947 and 1999 (41). In 2003, a large outbreak with 299 Q fever cases was associated with a farmers market in Soest (42). In June 2005, a large Q fever outbreak with 331 cases was reported in Winzerla in East Germany (43). The probable cause was cited as the close

proximity between a sheep meadow and the nearby residential area (43).

In Spain, from 1981 to 1985, 249 Q fever cases from various regions were serologically diagnosed at the Centro Nacional de Microbiología, Virología e Inmunología Sanitarias (44). The highest seroprevalence data were from northern Spain (Cantabria, 48.6% and Basque country, 38.5%), Salamanca (50.2%), and Leon (40.6%); this increased seroprevalence may be because there is a greater prevalence of cattle rising in this region (45, 46). In contrast, Q fever seems to be less prevalent in central Spain (Madrid, 12.7%) and southern Spain (Huelva, 4-6%) (46). In Gipuzkoa (Basque country), 1,261 cases of Q fever were diagnosed (955 in men) between 1984 and 2004 (47). The most frequent clinical manifestation was pneumonia (79%); half of the patients were hospitalized and 10 were admitted to the intensive care unit (47). In Italy, a survey performed throughout the Campania area indicated a Q fever seroprevalence of 11.8% in sheep, 6.3% in goats, 14% in cattle and 7% in dogs (48). In Vicenza (northeastern Italy), a total of 58 cases were identified in a 5-month period (49). The male to female ratio was 2.8:1, and the mean age of the patients was 42 yr (range: 20-65 yr); hospitalization was required for 48% of the cases (49). In 2003, an outbreak of Q fever was reported in 65 prisoners in Como, most likely due to exposure of dust contaminated by a passing flock of sheep (50).

In northern Greece, approximately 4.7% of 3,686 patients with “atypical pneumonia” had antibodies against *C. burnetii* antigens (51); a retrospective study in Crete revealed that the prevalence of *C. burnetii* antibodies in 1,298 patients was 7.6% (52). A seasonal occurrence was noted, with the majority of cases being diagnosed between January and June. Approximately 35.4% of the patients had contact with animals or consumed unpasteurized milk or fresh cheese.

Q fever has also reported in Eastern Poland, where antibodies to the *C. burnetii* phase II antigen were found in about 18% of farmers; however, no patients living in an urban environment tested positive (53). In the Slovak republic, *C.*

*burnetii* phase I and phase II antibodies were detected in 38% and 63% of Veterinary University employees, respectively (54). In Sweden, a seroprevalence of 24–30% in sheep farmers and 12% in veterinarians was reported, compared to 5-17% prevalence in non-risk groups (55). In Switzerland, only 30 to 90 Q fever cases are reported annually to the Federal Office of Public Health (2).

### *United States*

Although Q fever is considered enzootic in ruminants in the United States, the epidemiology of human Q fever infection is poorly understood. A national study that assessed the number of cases by state reported 1,168 cases from 1948 to 1977 (average= 39 per year) (56). From 1978 through 1999, 436 cases (average= 20 per year) of human Q fever were reported (57). After Q fever became nationally reportable in 1999, 255 human Q fever cases (average= 51 per year) were reported that had an onset during 2000 through 2004 (57). The median age of the cases was 51 years, and most cases were male (77%) (57). Estimates have suggested that the average annual incidence of Q fever is 0.28 cases per million people and is highest in people 50-59 yr of age (0.39 cases per million) (57). The infection prevalence varies with seasons, with 39% of cases occurring from April to June; however, cases have been reported throughout the year, which suggests that human Q fever infection may also have non-seasonal influences (57). The eastern states had the lowest incidence of Q fever, which ranged from 0.0 to 0.10 per million people. The states with the highest annual incidence (> 0.50 cases per million) included Idaho, Kentucky, North Dakota, Nebraska, Nevada, Oregon, Tennessee, and Wyoming (57).

After *C. burnetii* was classified as a Category B potential bioweapon, increased interest in Q fever and subsequent commercialization of related serological assays have aided in the identification of infected US soldiers. In the 1990s, there were only a few detected cases of Q fever in Iraq during the first Gulf War (58). Therefore, Q fever was considered a minor threat at the begin-



ning of the current war in Iraq. However, Q fever is currently endemic in Iraq and several US military personnel have been diagnosed (59). In fact, Q fever is currently one of the most common infectious diseases acquired by US soldiers in Iraq; since 2005, 5 reports describing Q fever cases in US soldiers have been published (59).

### **Asia**

Q fever is endemic in Israel, and between 1981 and 1990, 758 Q fever cases were reported to the Ministry of Health (60). A group of 34 patients with Q fever and endocarditis was reported more recently (61). The national incidence of Q fever endocarditis in Israel was estimated to be 3.5 cases per year or approximately 0.75 cases per 1 million people per year (2). Recently, a large outbreak of Q fever in an urban school in central Israel was reported, and it was suggested that the bacterium was spread through a contaminated air conditioning system (62). Serological evidence for *C. burnetii* infection was evident in 144 (88%) of the 164 tested individuals (62). In Samsun Tekkeköy (north Turkey), 33 (8.1%) of 407 subjects were identified as having evidence of a past infection (63). In human serum samples collected from 1978 to 1991 in Japan, Htwe *et al.* found that the average seroprevalence of Q fever was 16.5% for the general population and 22.5% in veterinarians (n=275); furthermore, the rate was 11.2% in meat-processing workers (n= 107) and 15.2% in respiratory disorder patients (n=184), but only 1.6% in healthy controls (n= 60) (64).

### **Australia**

Between 1977 and 1994, 202 to 860 cases were reported annually in Australia. Most cases were recorded in Queensland and New South Wales, and no cases were recorded in northern Tasmania (65). The national notification rates ranged from 3.11 to 4.99 per 100,000 people in the study period. Notification was 2.4-fold greater in Queensland than in New South Wales and 8-fold greater in Queensland than in southern Australia (65). Q fever is uncommon in Australia's Northern Territory, as there were no reported cases until 2002

(66). From 2002 to 2007 only 12 cases of Q fever were reported, which represents a much lower notification rate compared to the surrounding Australian states (66). In North Queensland from July 1994 to January 2006, 27 patients were diagnosed with Q fever (67). In this study, 33% of patients had pneumonia, 41% had deranged liver function tests and 25% had hepatomegaly (67).

### **New Zealand**

A 1993 seroepidemiological study in New Zealand that tested 2,181 cattle and 12,556 sheep found that all of the samples were negative (68).

### **Clinical manifestations**

#### **Acute Q fever**

Primary infection with *C. burnetii* is asymptomatic in approximately 60% of cases (Figure 1). During an outbreak of 415 cases of Q fever in Switzerland, 54% of patients were asymptomatic and only 2% were hospitalized (69). Symptomatic infection is more prevalent in adults than children and more likely in men than women (32). The incubation period for acute infection is approximately 20 d; the common clinical manifestations include a self-limited febrile illness, atypical pneumonia, or a granulomatous hepatitis (25). The most frequent clinical presentation of acute Q fever may vary from one area to another; for example, pneumonia is the major clinical presentation in Nova Scotia and in Switzerland, whereas hepatitis is observed more frequently than pneumonia in France, Ontario, and California (2).

A flu-like symptom is the most common manifestation of Q fever (Table 2). The onset is typically abrupt, and the fever ranges 39 to 40° C and typically remains elevated all day. The fever typically plateaus within 2 to 4 d, and after 5 to 14 d the body temperature rapidly returns to normal. Fever is usually accompanied by severe headaches, fatigue and myalgias. Pulmonary involvement is a major clinical presentation. Q fever is usually mild and produces a non-productive cough, fever, and minimal auscultatory abnormalities. Radiographic analyses often lead to a diagnosis of atypical pneumonia. Complications associated with

acute Q fever pneumonia are rare and may include encephalitis, renal failure, congestive heart failure, respiratory failure, and myocarditis. These symptoms can last from 10 to 90 d, and the mortality ranges from 0.5% to 1.5% (2, 25). During acute Q fever hepatitis, the alkaline phosphatase AST and ALT levels are usually 2 to 3-fold higher than the normal values. Patients with hepatitis exhibit autoantibodies, including antibodies directed to smooth muscle, anticardiolipin, antiphospholipids, circulating anticoagulant and antinuclear antibodies (70). Patients that develop hepatitis are typically younger, are not immunocompromised, are more febrile, have an increased number of headaches, myalgia, thrombocytopenia and display more frequently elevated erythrocyte sedimentation rates (25). On the other hand, patients with pulmonary involvement are typically older, immunocompromised, less frequently febrile, have less headaches and myalgias, have more electrocardiographic abnormalities and less frequent thrombocytopenia (25). Myocarditis has only occasionally been reported as a manifestation of *C. burnetii* infection. In a series of 1, 276 acute Q fever cases, Raoult *et al.* reported myocarditis in 8 patients, which represents 0.6% of all acute Q fever cases (25). Myocarditis appears to be the most severe manifestation of acute Q fever and is associated with a worse prognosis than most other forms of the disease (71). Moreover, patients who die from myocarditis are significantly younger compared to patients who die from noncardiac causes (71). However, *C. burnetii* has rarely been described as a cause of pericarditis. Moreover, it is difficult to establish a specific diagnosis, which means that the etiology of most cases is not determined. As a result, Q fever pericarditis may be under-diagnosed (2). Skin lesions have been found in 5 to 21% of Q fever patients in different groups (29, 72, 73). Q fever also produces a rash that is nonspecific and may consist of pink macular lesions or purpuric red papules of the trunk (74, 75). Central nervous system involvement has been also reported, such as an extrapyramidal neurologic syndrome similar to Parkinson disease (76), Millard-Gubler syndrome (77), a cerebellar syndrome and

a pyramidal syndrome (78). Cases of encephalitis, meningoencephalitis, and encephalomyelitis have been also reported to occur late during acute Q fever (25).

### **Chronic Q fever Endocarditis**

Endocarditis is the most serious and most frequent clinical presentation of chronic Q fever; 1-5% of patients with acute Q fever and 60-80% of patients with chronic Q fever develop endocarditis (2, 79) (Table 3). *C. burnetii* may cause as much as 5% of all endocarditis cases in some regions (80) and more than half of the documented cases of endocarditis with negative blood cultures; these data suggest that *C. burnetii* more commonly causes endocarditis than *Bartonella* spp., *Brucella* spp., *Tropheryma whipplei*, *Mycoplasma* spp., or *Legionella* spp. (81). More than 800 cases of endocarditis were reported in various studies between 1949 and 2005 (82). The major studies were conducted in the United Kingdom and Ireland (227 cases), France (264 cases), Spain (62 cases), Israel (35 cases), Switzerland (21 cases), Australia (18 cases) and in Canada (10 cases) (82). Although Q fever endocarditis can be spontaneously fatal if untreated, the mortality is less than 10% when the appropriate antibiotic is administered (83). A previous study showed that endocarditis-related mortality increased with time from 4% at 3 yr to 10% at 5 yr and 19% at 10 yr (81). Q fever endocarditis occurs almost exclusively in patients with previous cardiac valve defects and/or in immunocompromised patients. Some patients who experience Q fever endocarditis have clinically silent and previously undiagnosed valve diseases (84). As a result, echocardiography screening should be performed in Q fever patients (84). An underlying heart disease, which commonly involves the aortic and mitral valves, may be congenital, rheumatic, or degenerative. Cardiac symptoms are related to heart failure in 67% of infected patients with previous valvulopathy, dyspnea, acute pulmonary edema, angina, and palpitations. Other constitutional symptoms include fever, malaise, weakness, weight loss, fatigue, chills, anorexia and

night sweats (85). Although diagnosis of Q fever endocarditis may be missed in the early stage of the disease, the symptoms progressively complement one another after several months and evolve into a more evident clinical presentation, including considerable hepatomegaly, renal insufficiency, purpuric rash, and embolic manifestations, which may result in death.

### Other

The parts of the body most commonly affected by Q fever are the heart, arteries, bones and the liver. Vascular infection is the second most frequent presentation of Q fever. An aortic aneurism can be infected by *C. burnetii*, which can lead to an intestinal fistula or a spondylitis, as well as a vascular graft. The prognosis is poor in the absence of treatment (86). Most patients with aneurysms and vascular grafts have previous aortic abnormalities (mainly infrarenal aneurysm) (25). Bone infections are mainly reported in adults that are immunocompromised or have a joint prosthesis. Two types of osteoarticular infections have been reported during Q fever: osteomyelitis (87, 88) and aortic graft infection with contiguous spinal osteomyelitis (87,89). Chronic Q fever involvement of the liver is frequently associated with endocarditis, although few cases of isolated chronic hepatitis have been described (90). Rare cases of pericardial effusion, pulmonary interstitial fibrosis, a pseudotumor of the lung, a lymphoma-like presentation, amyloidosis and mixed cryoglobulinemia have been reported in the literature (2).

### Pregnant women

Relatively little data are available regarding the impact of Q fever on pregnancy. Q fever during pregnancy can result in spontaneous abortion (26%), intrauterine fetal death (5.3%), premature delivery (44.7%), intrauterine growth retardation (5.3%) (2), or no adverse events (15.8%) (2). Fetal death may be caused by direct fetal infection, because *C. burnetii* has been found in the placenta and the fetal viscera (27). Carcopino et al. illustrate a link between placentitis and obstetric complications and the abortifacient potential of *C. bur-*

*netii* infection in humans (91). Because pregnant women with placentitis have a higher rate of intrauterine fetal death, it was hypothesized that placentitis corresponded to a higher bacterial load in the placenta and/or to a systemic infection (91). Transplacental infection of the fetus *in utero* is possible (27, 92), although the consequences remain unknown and the association with obstetric complications remains hypothetical. To date, teratogenicity has never been associated with *C. burnetii* infection; for the two cases of teratogenicity that were observed by Carcopino *et al.*, there was no evidence of a direct association with *C. burnetii* infection (91).

Q fever contraction during pregnancy also affects the mother and is associated with an increased risk of contracting the chronic form (2, 91, 92) and spontaneous abortions of future pregnancies (93). In a study by Carcopino *et al.*, 80% of the pregnant patients that were infected during their first trimester developed chronic Q fever, which is almost double the rate observed for patients infected during the second and third trimesters (91). It is likely that the main risk factor for the development of chronic Q fever is the duration of infection during pregnancy, because patients more often develop a "chronic" serologic profile when infected during their first trimester of pregnancy (91, 92). Finally, because Q fever can be reactivated during another pregnancy, these patients should have regular serological follow-ups (91, 92).

### Diagnosis

The laboratory diagnostic parameters for acute Q fever are nonspecific, thus, diagnosis is usually made by serology. Ninety percent of infected patients with acute Q fever present a normal leukocyte count (Table 4). Thrombocytopenia is the most frequent abnormality and may occur in up to 25% of patients at initial presentation (94). Elevated transaminase levels have been reported in 38.3 to 60% of patients for AST and 33.3 to 80% for ALT (94, 95). Autoantibodies are commonly present, including smooth muscle antibodies and, less frequently, cold agglutinins, anti-prothrom-

binase, anti-hemophilic B antibodies, and a positive Coombs test (94).

*C. burnetii* virulence is particularly high, and only Biosafety level 3 laboratories and experienced personnel should be allowed to interact with contaminated specimens and cultivate this microorganism from clinical samples. Blood, cerebrospinal fluid, bone marrow, cardiac valves, a vascular aneurysm or graft, bone biopsy, liver biopsy, milk, placenta, and fetal specimens after abortion can all be cultured (96-98). Human embryonic lung fibroblasts (HEL cells) grown in shell vials are routinely used to isolate *C. burnetii* (99). A Light-Cycler Nested PCR (LCN-PCR) that amplifies a multicopy 20-copy htpAB-associated element sequence has been adapted to diagnose both acute and chronic Q fever (100), and this method may help in early diagnosis of chronic Q fever (101). Due to its high sensitivity and specificity, the repetitive IS 11-11 element is the best gene for detection of *C. burnetii* in patients with active Q fever (101, 102). Molecular assays can be used to test patients for acute infection before the appearance of antibodies and can be used to test clinical samples for Q fever endocarditis (101). Recently, the complete *C. burnetii* genome sequence became available, which allows researchers to choose a diagnosis target from a larger number of DNA sequences. The histopathology of heart valves of patients with Q fever endocarditis have significant fibrosis and calcification but not inflammation of large vegetations and immunohistochemical analysis allows the confirmation of the diagnosis (103). Recently, Lepidi *et al.* proposed a new method named autoimmunohistochemistry for the detection of the *C. burnetii* endocarditis (104).

Microimmunofluorescence (IFA) remains the reference technique for diagnosing Q fever (Fig. 2). Screening is performed with anti-phase II anti-immunoglobulins at a serum dilution of 1: 50. The positive sera are serially diluted and tested for the presence of anti-phase I and anti-phase II IgG, IgM and IgA antibodies (105). Seroconversion can be detected 7 to 15 d after the onset of clinical symptoms, and most patients have detectable

antibodies by the third week (105). A titer of 200 for the IgG anti-phase II antibody and 50 for the IgM anti-phase II antibody are usually associated with acute Q fever (106). For cases where acute Q fever is suspected, serology should be repeated 14 d after the first serum sample is tested. For cases with confirmed acute Q fever, echocardiography should be performed to examine possible cardiac valve defects. The presence of a cardiac valve defect would indicate that the patient should receive a doxycycline and hydroxychloroquine prophylactic regimen for at least 12 mo. If the patient does not have a cardiac valve defect, serology should be repeated at 3 and 6 mo; if the IgG anti-phase I antibody titers are less than 800, a previous infection should be considered. In acute Q fever, antibody titers reach their maximum levels 4 to 8 wks after the onset of disease and decrease gradually over the following 12 mo (107). An IgG anti-phase I antibody titer higher than 800 is usually associated with chronic Q fever, and infective endocarditis should be assessed with transesophageal echocardiography and PCR (106). Anti-phase I IgA titers do not impact chronic Q fever diagnosis. Duke's criteria are used worldwide to calculate the diagnostic score for infective endocarditis. Duke's criteria have been modified to include one positive blood culture for *C. burnetii* and a phase I IgG titer of  $\geq 1:800$  as the major criteria for Q fever endocarditis diagnosis (80). Antibody titers can be used to monitor the course of treatment, where the IgM titers first decrease, followed by a decrease in the IgA titers. The IgG, IgM and IgA titers for phase I antigens should decrease two-fold in response to successful treatment (108).

### **Treatment**

Acute Q fever is most often a mild disease and the treatment prescribed depends on the clinical presentation; pneumonia usually resolves without treatment within 15 d (Fig. 3). Because diagnosis confirmation via serology is typically only available for convalescent patients, after 2 to 3 wk of the disease, empirical therapy is recommended in severely ill patients because of the potential for



delay in diagnosis. Tetracycline compounds, especially doxycycline, are the drugs currently recommended to treat acute Q fever illness; typically, a doxycycline regimen of 200 mg for 15 to 21 d is prescribed. In a nonrandomized comparison of two regimens of acute Q fever treatment, the average duration of fever was 3.3 d in untreated patients, 2 d in patients that received tetracycline (500 mg four times a day), and 1.7 d in patients treated with doxycycline (100 mg four times a day) (73). Ofloxacin (600 mg/d) and pefloxacin (800 mg/d) were reported to be effective for Q fever-induced pneumonia, although treatment can be prolonged to 16 d with ofloxacin and 21 d with pefloxacin (109). Alternatively, the combination of pefloxacin (800 mg/day) and rifampin (1, 200 mg/d) for 21 d successfully treated patients with prolonged Q fever (110). Erythromycin (500 mg q.i.d.) has been used successfully to treat Q fever pneumonia cases (111, 112), although it was ineffective at a daily intravenous dosage of 4 g in severe cases (110). Macrolide compounds, such as moxifloxacin, do not demonstrate bactericidal activity against *C. burnetii in vitro* (113).

Q fever endocarditis is the most frequent clinical presentation of chronic Q fever; most patients with Q fever endocarditis die without antibiotic treatment, although disease evolution is slow and may last for years. *C. burnetii* is difficult to eradicate because it resides within the host cells. Relapse and death was common among patients that were infected in the 1960s and 1970s, and as many as two-thirds of the patients died in some studies (114, 115). Since then, the mortality has been reduced with prolonged treatment with antibiotic combinations (116); additionally, substances that raise the pH of phagolysosomes have also been effective *in vitro* (117, 118) and *in vivo* (83). Antibiotic combinations including co-trimoxazole may not be the best treatment for Q fever endocarditis, because the beta-lactams used either alone or in combination with aminoglycosides are not effective (110). Rifampin combined with either doxycycline or co-trimoxazole has also been used, although in most cases rifampin treatment was stopped after few months because of its interac-

tion with anticoagulants that are frequently simultaneously prescribed (110). The recommended treatment is a combination of doxycycline and hydroxychloroquine for 18 mo to 3 yr, although no data are available that describe the optimum treatment duration (81). Hydroxychloroquine may have some deleterious effects, such as increasing the risk of retinopathy, or necessitating a regular ophthalmologic examination; thus, the serum hydroxychloroquine level should be monitored to ensure that it stays near the normal value of 160.2 mg/liter. Until recently, the duration of treatment depended upon a defined serological cure, where the anti-phase I IgG titer is less than 800. However, Million *et al.* proposed that a phase I IgG titer of 800 should no longer be the sole criterion to determine treatment duration; they propose that the best approach would be to determine the time needed to obtain a *C. burnetii* negative state by available diagnostic methods to prevent serological relapse (81). Studies have suggested that combination treatment for 18 mo should be the recommended treatment duration for Q fever endocarditis, except for patients with prosthetic valves or for special cases with bad clinical or serological evolution (81). This duration should be extended only in the absence of favorable serological outcomes, defined by a four-fold decrease in the phase I IgG and IgA titers and the complete disappearance of phase II IgM at 1 yr. Serological monitoring for at least 5 yr is appropriate, given the risk of relapse. Patients with prosthetic valves often have a poor prognosis, which may be explained by the presence of a foreign surface that promotes antibiotic-resistant infections. However, there are no data to advise systematic surgery in such patients, and Million *et al.* reported that the 32% of patients with prosthetic valves presented serological cure without or before surgery (81). Patients with at least a four-fold decrease in the phase I antigen-specific IgG and IgA titer at 1 yr have better outcomes, which confirms the prognostic value of serological monitoring to assess the treatment efficacy. Moreover, the presence of phase II antigen-specific IgM at 1 yr, which was previously identified as an unexpectedly poor prognos-

tic factor associated with mortality, seems to be related directly to *C. burnetii* infection and may represent the humoral immune response to prolonged active infection (81).

Treatment of Q fever during pregnancy is a challenge. The data regarding the effectiveness of typical treatments, such as doxycycline and quinolone, are contradictory. Cotrimoxazole can be administered, although the compound has a theoretical risk of neonatal bilirubinemia when used just prior to delivery (119) and has only a bacteriostatic effect. Because a 3 wk cotrimoxazole treatment regimen did not prevent intrauterine fetal death (27), Raoult *et al.* proposed the use of long-term cotrimoxazole therapy (92). Carcopino *et al.* showed that long-term cotrimoxazole treatment protected against maternal chronic Q fever ( $P= 0.001$ ), placental infection ( $P= 0.038$ ), obstetric complications ( $P= 0.009$ ), and intrauterine fetal death ( $P= 0.018$ ), which was found to be related to placental infection ( $P= 0.008$ ) (91). Obstetric complications, such as intrauterine growth and premature delivery, were observed in 43.8% of pregnant women that received long-term cotrimoxazole therapy. No cases of spontaneous abortion, oligoamnios, or intrauterine fetal death were observed. Cotrimoxazole therapy, when administered for a minimum of 5 wk, also prevented the need for postpartum treatment with a combination of doxycycline and hydroxychloroquine ( $P= 0.033$ ) (91).

**Table 1:** Risk factors associated with Q fever

<b>Acute Q fever:</b>
Male
Rural life
Occupational exposure
Contact with animals
Raw cheese consumption
Immunosuppression
<b>Chronic Q fever</b>
Valvulopathy
Immunosuppression
Pregnancy

**Table 2:** Clinical syndromes associated with Q fever (25)

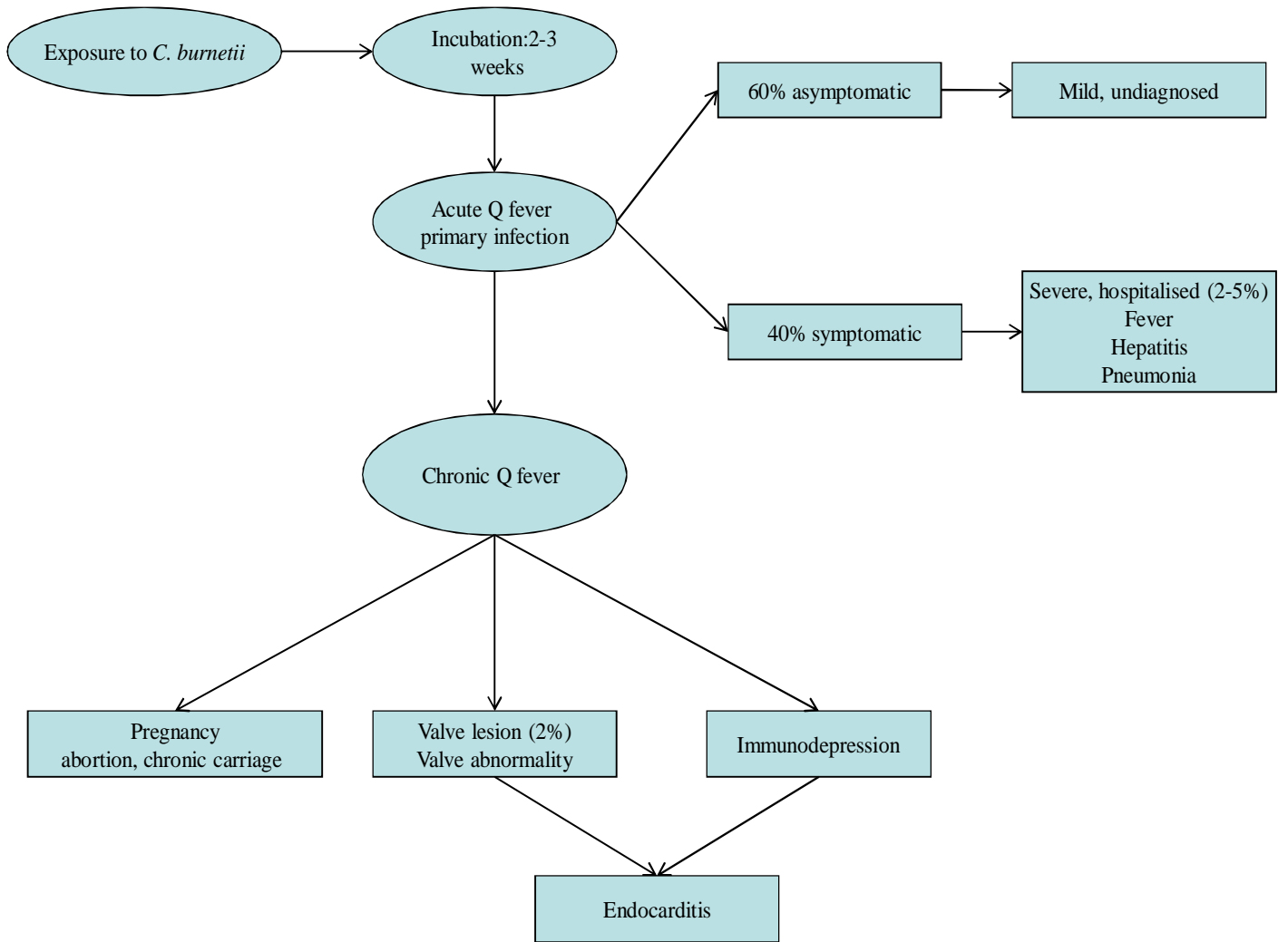
<b>Clinical manifestation</b>	<b>Frequency</b>
Hepatitis	40%
Pneumonia and Hepatitis	20%
Pneumonia	17%
Fever	14%
Undetermined	3%
Meningoencephalitis	1%
Pericarditis	1%
Myocarditis	1%
Meningitis	0.5%

**Table 3:** The prevalence of various forms of chronic Q fever (25)

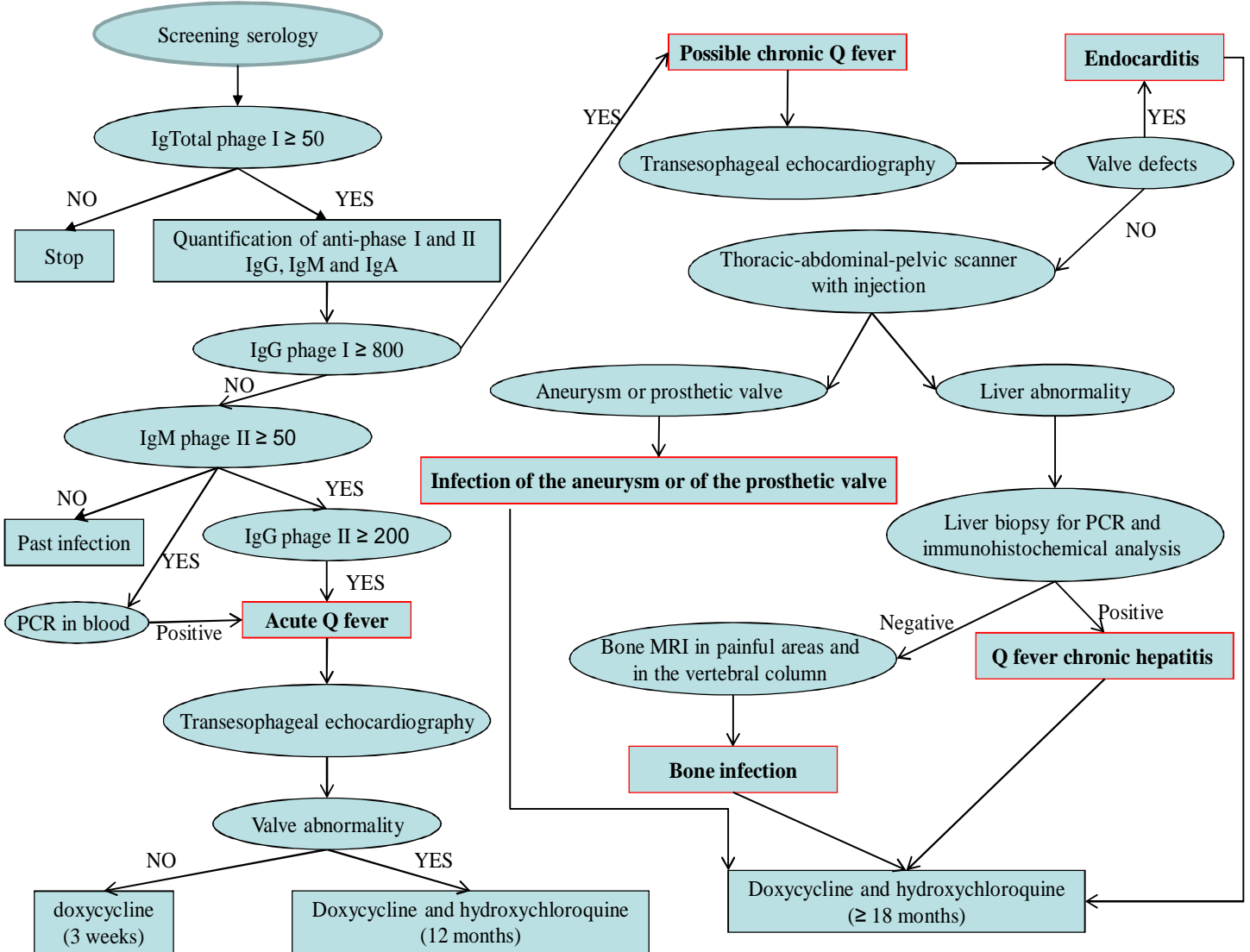
<b>Clinical manifestation</b>	<b>%</b>
Endocarditis	73
Vascular infection	8
Pregnancy	6
Chronic hepatitis	3
Osteoarticular infection	2
Chronic pericarditis	1
Adenopathies	<1
Splenic pseudotumor	<1
Lung pseudotumor	<1
Chronic neuropathy	<1
Non-identified foci	2

**Table 4:** Biochemical and serological parameters for patients with acute Q fever

<b>Parameters</b>	<b>%</b>
Normal white blood cell count	90
Elevated transaminase level	70
Smooth muscle autoantibodies	65
Anti-phospholipase antibodies	50
Thrombocytopenia	25



**Fig. 1:** Pathophysiology of Q fever



**Fig. 2:** Serological strategy for Q fever diagnosis



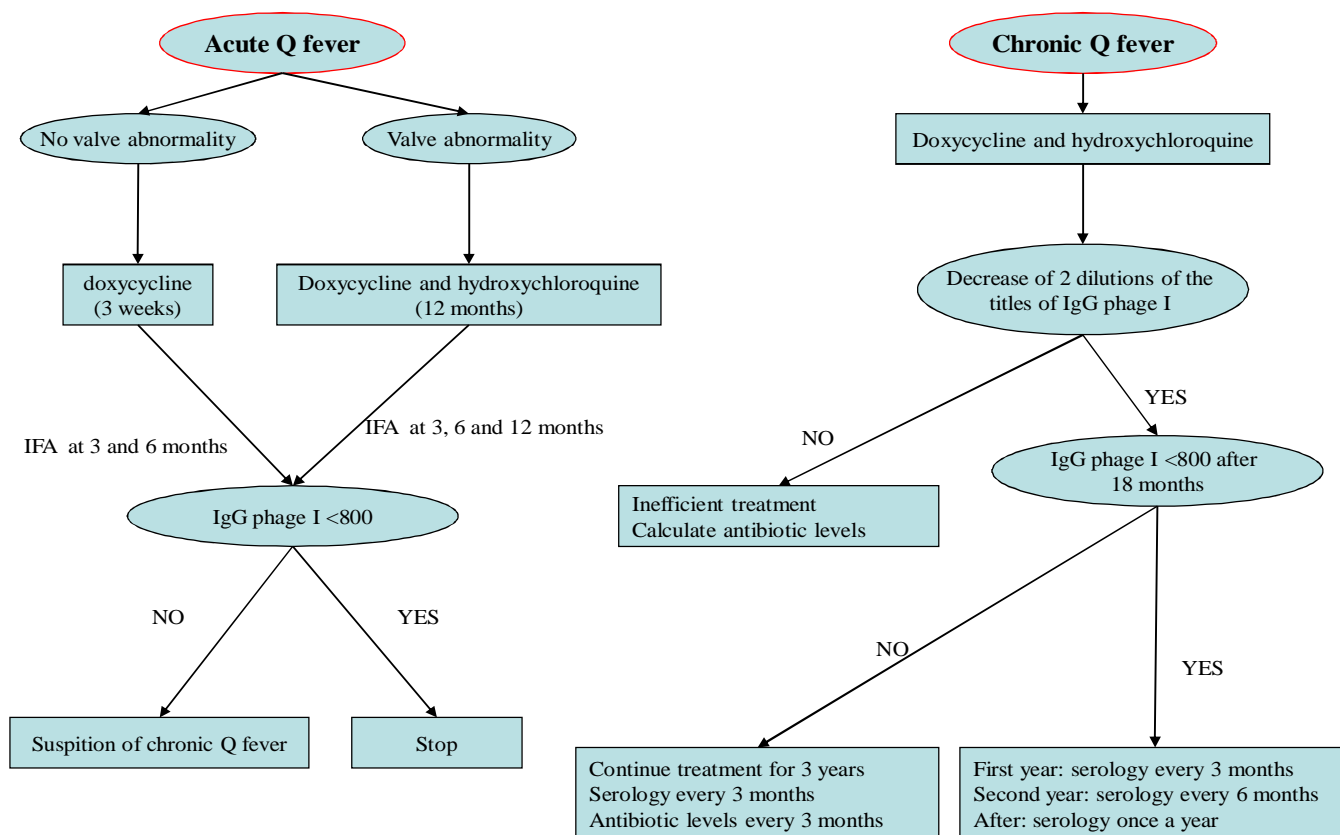


Fig. 3: Monitoring strategy for Q fever

## Ethical Considerations

Ethical issues including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc. have been completely observed by the authors.

## References

- Vogel JP (2004). Turning a tiger into a house cat: using *Legionella pneumophila* to study *Coxiella burnetii*. *Trends Microbiol*, 12(3): 103-105.
- Maurin M, Raoult D (1999). Q fever. *Clin Microbiol Rev*, 12(4): 518-53.
- Roest HI, Tilburg JJ, Van Der Hoek W, Vellema P, Van Zijderveld FG, Klaassen CH, Raoult D (2011). The Q fever epidemic in The Netherlands: history, onset, response and reflection. *Epidemiol Infect*, 139(1): 1-12.
- Stoker MGP, Fiset P (1956). Phase variation of the Nine Mile and other strains of *Rickettsia burnetii*. *Can J Microbiol*, 2: 310-21.
- Raoult D, Levy PY, Harle JR, Etienne J, Massip P, Goldstein F, et al. (1990). Chronic Q fever: Diagnosis and follow up. *Ann N Y Acad Sci*, 590: 51-60.
- Stein A, Saunders NA, Taylor AG, Raoult D (1993). Phylogenic homogeneity of *Coxiella burnetii* strains as determined by 16S ribosomal RNA sequencing. *FEMS Microbiol Lett*; 113: 339-44.
- Raoult D, Marrie T, Mege J (2005). Natural history and pathophysiology of Q fever. *Lancet Infect Dis*, 5(4): 219-26.
- Seshadri R, Paulsen IT, Eisen JA, Read TD, Nelson KE, Nelson WC, et al. (2003). Complete genome sequence of the Q-fever pathogen *Coxiella burnetii*. *Proc Natl Acad Sci U S A*, 100(9): 5455-460.

9. Samuel JE, Frazier ME, Mallavia LP (1990). Comparison of *Coxiella burnetii* plasmids to homologous sequences present in a plasmidless endocarditis causing isolate. *Ann N Y Acad Sci*, 590: 33-38.
10. Stein A, Louveau C, Lepidi H, Ricci F, Baylac P, Davoust B, Raoult D (2005). Q fever pneumonia: virulence of *Coxiella burnetii* pathovars in a murine model of aerosol infection. *Infect Immun*, 73(4): 2469-77.
11. Beare PA, Samuel JE, Howe D, Virtaneva K, Porcella SF, Heinzen RA (2006). Genetic diversity of the Q fever agent, *Coxiella burnetii*, assessed by microarray-based whole-genome comparisons. *J Bacteriol*, 188(7): 2309-24.
12. Babudieri B (1959). Q fever: A zoonosis. *Adv Vet Sci*, 5: 82-182.
13. Abinanti FR, Welsh HH, Lennette EH, Brunetti O (1953). Q fever studies XVI. Some aspects of experimental infection induced in sheep by the intratracheal route of inoculation. *Am J Hyg*, 57: 170-184.
14. Luoto L (1955). A capillary agglutination test for bovine Q fever animals may be asymptomatic. *J Immunol*, 71: 226-31.
15. Marmion BP, Stoker MGP (1950). Q fever in Great Britain- Epidemiology of an outbreak. *Lancet*, 2:611-616.
16. Berri M, Souriau A, Crosby M, Crochet D, Lechopier P, Rodolakis A (2001). Relationships between the shedding of *Coxiella burnetii*, clinical signs and serological responses of 34 sheep. *Vet Rec*, 148(16): 502-505.
17. Angelakis E, Raoult D (2010). Q Fever. *Vet Microbiol*, 140(3-4):297-309.
18. (Fishbein DB, Raoult D (1992). A cluster of *Coxiella burnetii* infections associated with exposure to vaccinated goats and their unpasteurized dairy products. *Am J Trop Med Hyg*, 47(1): 35-40.
19. Editorial (1950). Experimental Q fever in man. *Br Med J*, 1: 1000.
20. Krumbiegel ER, Wisniewski HJ (1970). Q fever in Milwaukee. II. Consumption of infected raw milk by human volunteers. *Arch Environ Hlth*, 21: 63-65.
21. Rolain JM, Gouriet F, Brouqui P, Larrey D, Janbon F, Vene S, Jarnestrom V, Raoult D (2005). Concomitant or consecutive infection with *Coxiella burnetii* and tickborne diseases. *Clin Infect Dis*, 40(1): 82-88.
22. Kruszezwska D, Tylewska-Wierzbansowska ST (1993). *Coxiella burnetii* penetration into the reproductive system of male mice, promoting sexual transmission of infection. *Infect Immun*, 61(10): 4188-95.
23. Kruszezwska D, Tylewska-Wierzbansowska S (1997). Isolation of *Coxiella burnetii* from bull semen. *Res Vet Sci*, 62: 299-300.
24. Milazzo A, Hall R, Storm PA, Harris RJ, Win-slow W, Marmion BP (2001). Sexually transmitted Q fever. *Clin Infect Dis*, 33(3):399-402.
25. Raoult D, Tissot-Dupont H, Foucault C, Gouvernet J, Fournier PE, Bernit E, Stein A, Nesri M, Harle JR, Weiller PJ (2000). Q fever 1985-1998- Clinical and epidemiologic features of 1,383 infections. *Medicine*, 79(2): 109-23.
26. Maltezou HC, Raoult D (2002). Q fever in children. *Lancet Infect Dis*, 2(11):686-691.
27. Raoult D, Stein D (1994). Q fever during pregnancy- a risk for women, fetuses, and obstetricians. *N Engl J Med*, 330: 371.
28. Raoult D, Levy P, Tissot-Dupont H, Chiche Portiche, Tamalet C, Gastaut JA, Salducci J (1993). Q fever and HIV infection. *AIDS*, 7: 81-86.
29. Tissot-Dupont H, Raoult D, Brouqui P, Janbon F, Peyramond D, Weiller PJ, Chiche-portiche C, Nezri M, Poirier R. (1992). Epidemiologic features and clinical presentation of acute Q fever in hospitalized patients: 323 French cases. *Am J Med*, 93: 427-34.
30. Frankel D, Herve R, Renvoise O, Raoult D (2011). Q fever in France, 1985-2009. *Emerg Infect Dis*, 17(3): 350-56.
31. Carrieri MP, Tissot-Dupont H, Rey D, Brousse P, Renard H, Obadia Y, Raoult D (2002). Investigation of a slaughterhouse-related outbreak of Q fever in the French Alps. *Eur J Clin Microbiol Infect Dis*, 21(1): 17-21.
32. Tissot-Dupont H, Vaillant V, Rey S, Raoult D (2007). Role of sex, age, previous valve lesion, and pregnancy in the clinical expression and outcome of Q fever after a large outbreak. *Clin Infect Dis*, 44(2): 232-37.
33. Enserink M (2010). Infectious diseases. Questions abound in Q-fever explosion in the Netherlands. *Science*, 327(5963): 266-67.

34. Van Der Hoek W, Dijkstra F, Schimmer B, Schneeberger PM, Vellema P, et al. (2010). Q fever in the Netherlands: an update on the epidemiology and control measures. *Euro Surveill*, 15(12).
35. Marmion BP, Stoker MGP (1958). The epidemiology of Q fever in Great Britain. *Br Med J*, 2: 809-16.
36. Thomas DR, Treweek L, Salmon RL, Kench SM, Coleman TJ, Meadows D, Morgan-Capner P, Caul EO (1995). The risk of acquiring Q fever on farms: A seroepidemiological study. *Occup Environ Medicine*, 52: 644-47.
37. Palmer SR, Young SE (1982). Q-fever endocarditis in England and Wales, 1975-81. *Lancet*, 2(8313):1448-49.
38. Van Woerden HC, Mason BW, Nehaul LK, Smith R, Salmon RL, Healy B, et al. (2004). Q fever outbreak in industrial setting. *Emerg Infect Dis*, 10(7): 1282-89.
39. Wallensten A, Moore P, Webster H, Johnson C, van der Burgt G, Pritchard G, Ellis-Iversen J, Oliver I (2010). Q fever outbreak in Cheltenham, United Kingdom, in 2007 and the use of dispersion modelling to investigate the possibility of airborne spread. *Euro Surveill*, 15(12).
40. Glennie JS, Bailey MS (2010). UK Role 4 military infectious diseases at Birmingham Heartlands Hospital in 2005-9. *J R Army Med Corps*, 156(3): 162-64.
41. Hellenbrand W, Breuer T, Petersen L (2001). Changing epidemiology of Q fever in Germany, 1947-1999. *Emerg Infect Dis*, 7(5): 789-96.
42. Porten K, Rissland J, Tigges A, Broll S, Hopp W, Lunemann M, et al. (2006). A super-spreading ewe infects hundreds with Q fever at a farmers' market in Germany. *BMC Infect Dis*, 6: 147.
43. Gilsdorf A, Kroh C, Grimm S, Jensen E, Wagner-Wiening C, Alpers K (2008). Large Q fever outbreak due to sheep farming near residential areas, Germany, 2005. *Epidemiol Infect*, 136(8): 1084-87.
44. Tellez A, Sainz C, Echevarria C, de Carlos S, Fernandez MV, Leon P, Brezina R (1988). Q fever in Spain: acute and chronic cases, 1981-1985. *Rev Infect Dis*; 10:198-202.
45. Pascual-Velasco F, Montes M, Marimon JM, Cilla G (1998). High seroprevalence of *C. burnetii* in eastern Cantabria (Spain). *Int J Parasitol*, 27(1):142-45.
46. Cardenosa N, Sanfeliu I, Font B, Munoz T, Nogueras MM, Segura F (2006). Short report: seroprevalence of human infection by *Coxiella burnetii* in Barcelona (northeast of Spain). *Am J Trop Med Hyg*, 75(1): 33-35.
47. Montes M, Cilla G, Vicente D, Nieto V, Ercibengoa M, Perez-trallero E. Gipuzkoa (2006). Basque Country, Spain (1984-2004): a hyperendemic area of Q fever. *Ann NY Acad Sci*, 1078: 129-32.
48. Capuano F, Parisi A, Cafiero MA, Pitaro L, Fenizia D (2004). *Coxiella burnetii*: what is the reality? *Parassitologia*, 46(1-2):131-34.
49. Manfredi Selvaggi T, Rezza G, Scagnelli M, Rigoli R, Rasso M, de Lalla F, et al. (1996). Investigation of a Q-fever outbreak in Northern Italy. *Eur J Epidemiol*, 12: 403-408.
50. Starnini G, Caccamo F, Farchi F, Babudieri S, Brunetti B, Rezza G (2005). An outbreak of Q fever in a prison in Italy. *Epidemiol Infect*, 133(2): 377-80.
51. Alexiou-Daniel S, Antiniadis A, Pappas K, Malisiovas N, Papapanagiotou I (1990). Incidence of *Coxiella burnetii* infections in Greece. *Hell Iatr*, 56, 251-55.
52. Tselentis Y, Gikas A, Kofteridis D, Kyriakakis E, Lydataki N, Bouros D, Tsaparas N (1995). Q fever in the greek Island of Crete: Epidemiologic, Clinical, and Therapeutic data from 98 cases. *Clin Infect Dis*, 20:1311-16.
53. Cisak E, Chmielewska-Badora J, Mackiewicz B, Dutkiewicz J (2003). Prevalence of antibodies to *Coxiella burnetii* among farming population in eastern Poland. *Ann Agric Environ Med*, 10(2): 265-67.
54. Dorko E, Kalinova Z, Weissova T, Pilipcinec E (2008). Seroprevalence of antibodies to *Coxiella burnetii* among employees of the Veterinary University in Kosice, eastern Slovakia. *Ann Agric Environ Med*, 15(1):119-24.
55. Macellaro A, Akesson A, Norlander L (1993). A survey of Q-Fever in Sweden. *Eur J Epidemiol*, 213-16.
56. D'Angelo LJ, Baker EF, Jr, Schlosser W (1979). Q fever in the United States 1948- 1977. *J Infect Dis*, 139: 613-15.
57. McQuiston JH, Holman RC, McCall CL, Childs JE, Swerdlow DL, Thompson HA (2006). National surveillance and the epidemiology of human Q fever in the United States, 1978-2004. *Am J Trop Med Hyg*, 75(1): 36-40.

58. Leung-Shea C, Danaher PJ (2006). Q fever in members of the United States armed forces returning from Iraq. *Clin Infect Dis*, 43(8): e77-e82.
59. Raoult D (2009). Reemergence of Q fever after 11 September 2001. *Clin Infect Dis*, 48(5): 558-59.
60. Yarrow A, Slater PE, Costin C (1990). Q fever in Israel. *Public Health Rev*, 18(2): 129-37.
61. Siegman-Igra Y, Kaufman O, Keysary A, Rzotkiewicz S, Shalit I (1997). Q fever endocarditis in Israel and a worldwide review. *Scand J Infect Dis*, 29(1):41-49.
62. Amitai Z, Bromberg M, Bernstein M, Raveh D, Keysary A, David D, et al. (2010). A large Q fever outbreak in an urban school in central Israel. *Clin Infect Dis*, 50(11): 1433-38.
63. Gozalan A, Rolain JM, Ertek M, Angelakis E, Coplu N, Basbulut EA, Korhasan BB, Esen B (2010). Seroprevalence of Q fever in a district located in the west Black Sea region of Turkey. *Eur J Clin Microbiol Infect Dis*, 29(4): 465-69.
64. Htwe KK, Yoshida T, Hayashi S, Miyake T, Amano KI, Morita C (1993). Prevalence of antibodies to *Coxiella burnetii* in Japan. *J Clin Microbiol*, (3):722-23.
65. Garner MG, Longbottom HM, Cannon RM, Plant AJ (1997). A review of Q fever in Australia 1991-1994. *Aust NZ J Pub Health*, 21(7): 722-30.
66. Ralph A, Markey P, Schultz R (2007). Q fever cases in the Northern Territory of Australia from 1991 to 2006. *Commun Dis Intell*, 31(2): 222-27.
67. Gale M, Ketheesan N, Govan B, Kennedy RL, Norton R (2007). Q fever cases at a North Queensland centre during 1994-2006. *Intern Med J*, 37(9): 644-46.
68. Hilbink F, Penrose M, Kovacova E, Kazar J (1993). Q fever is absent from New Zealand. *Intern J Epidemiol*, 22(5): 945-49.
69. Dupuis G, Petite J, Peter O, Vouilloz M (1987). An important outbreak of human Q fever in a Swiss Alpine valley. *Int J Epidemiol*, 16: 282-87.
70. Levy PY, Raoult D, Razongles JJ (1989). Q-fever and autoimmunity. *Eur J Epidemiol*, 5(4): 447-53.
71. Fournier PE, Etienne J, Harle JR, Habib G, Raoult D (2001). Myocarditis, a rare but severe manifestation of Q fever: report of 8 cases and review of the literature. *Clin Infect Dis*, 32(10):1440-47.
72. Dupuis G, Peter O, Luthy R, Nicolet J, Peacock M, Burgdorfer W (1986). Serological diagnosis of Q fever endocarditis. *Eur Heart J*, 7: 1062-66.
73. Spelman DW (1982). Q fever: a study of 111 consecutive cases. *Med J Australia*, 1: 547-53.
74. Jadin J (1963). Les rickettsioses en Afrique centrale. *Bull Soc Pathol Exot Filial*, 56: 571-86.
75. Jadin J, Giroud P (1950). La fièvre Q au Ruanda Urundi. *Bull Soc Pathol Exot Filial*, 43: 667-72.
76. Gallagher WH (1961). Q fever. *J am Med Assoc*, 177: 187-89.
77. Haegy JM, Laplatte G (1983). La fièvre Q, une pathologie méconnue. A propos de 2 observations de formes graves de fièvre Q. *J Med Strasbourg*; 14: 21-4.
78. Ladurner G, Stunzner D, Lechner H, Sixl W (1976). Q-fever meningoencephalitis. Case report. *Neurvenarzt*, 46:274-275.
79. Brouqui P, Tissot-Dupont H, Drancourt M, Berland Y, Etienne J, Lepoint C, et al. (1993). Chronic Q Fever. Ninety-two cases from France, including 27 cases without endocarditis. *Arch Intern Med*, 153: 642-48.
80. Fournier PE, Casalta JP, Habib G, Messana T, Raoult D (1996). Modification of the diagnostic criteria proposed by the Duke Endocarditis Service to permit improved diagnosis of Q fever endocarditis. *Am J Med*, 100(6): 629-33.
81. Million M, Thuny F, Richet H, Raoult D (2010). Long-term outcome of Q fever endocarditis: a 26-year personal survey. *Lancet Infect Dis*, 10(8): 527-35.
82. Tissot-Dupont H, Raoult D (2007). Clinical Aspects, Diagnosis and Treatment of Q fever. *Rickettsial Diseases*, 291-301.
83. Raoult D, Houpikian P, Tissot Dupont H, RISS JM, Arditi-Djiane J, Brouqui P (1999). Treatment of Q fever endocarditis: comparison of two regimens containing doxycycline and ofloxacin or hydroxychloroquine. *Arch Int Med*, 159: 167-73.
84. Fenollar F, Thuny F, Xeridat B, Lepidi H, Raoult D (2006). Endocarditis after acute Q fever in patients with previously undiagnosed valvulopathies. *Clin Infect Dis*, 42(6): 818-21.



85. Raoult D, Abbara S, Jassal DS, Kradin RL (2007). Case records of the Massachusetts General Hospital. Case 5-2007. A 53-year-old man with a prosthetic aortic valve and recent onset of fatigue, dyspnea, weight loss, and sweats. *N Engl J Med*, 356(7):715-25.
86. Botelho-Nevers E, Fournier PE, Richet H, Fenollar F, Lepidi H, Foucault C, et al. (2007). *Coxiella burnetii* infection of aortic aneurysms or vascular grafts: report of 30 new cases and evaluation of outcome. *Eur J Clin Microbiol Infect Dis*, 26(9): 635-40.
87. Ellis ME, Smith CC, Moffat MA (1983). Chronic or fatal Q-fever infection: a review of 16 patients seen in North-East Scotland (1967-80). *Q J Med*, 52: 54-66.
88. Cottalorda J, Jouve JL, Bollini G, Touzet P, Poujol A, Kelberine F, Raoult D (1995). Osteoarticular infection due to *coxiella burnetii* in children. *J Pediat Orthoped*; part B: 219-21.
89. Piquet P, Raoult D, Tranier P, Mercier C (1994). *Coxiella burnetii* infection of pseudoaneurysm of an aortic bypass graft with contiguous vertebral osteomyelitis. *Journal of Vascular Surgery*, 19: 165-68.
90. Turck WPG, Howitt G, Turnberg LA, Fox M, Longson M, Matthews MG, Das Gupta R (1976). Chronic Q fever. *Q J Med*, 45:193-217.
91. Carcopino X, Raoult D, Bretelle F, Boubli L, Stein A (2007). Managing Q fever during pregnancy: the benefits of long-term cotrimoxazole therapy. *Clin Infect Dis*, 45(5): 548-55.
92. Raoult D, Fenollar F, Stein A (2002). Q fever during pregnancy: diagnosis, treatment, and follow-up. *Arch Intern Med*, 162(6): 701-704.
93. Stein A, Raoult D (1998). Q fever during pregnancy: a public health problem in southern France. *Clin Infect Dis*, 27: 592-96.
94. Raoult D (1991). Diagnostic biologique de la fièvre Q. *Revue Française des Laboratoires*, 227: 68-70.
95. Domingo P, Oorbitg J, Colomina J, Alvarez E, Cadafalch J (1988). Liver involvement in acute Q fever. *Chest*, 94:895-896.
96. Gil-Grande R, Aguado JM, Pastor C, Garcia-Bravo M, Gomez-Pellico C, Soriano F, Noriega AR (1995). Conventional viral cultures and shell vial assay for diagnosis of apparently culture-negative *coxiella burnetii* endocarditis. *Eur J Clin Microbiol Infect Dis*, 14: 64-67.
97. Musso D, Raoult D (1995). *Coxiella burnetii* blood cultures from acute and chronic Q-fever patients. *J Clin Microbiol*, 33(12): 3129-32.
98. Raoult D, Vestris G, Enea M (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: 2482-84.
99. Marrero M, Raoult D (1989). Centrifugation-shell vial technique for rapid detection of Mediterranean spotted fever rickettsia in blood culture. *Am J Trop Med Hyg*, 40: 197-99.
100. Fenollar F, Raoult D (2007). Molecular diagnosis of bloodstream infections caused by non-cultivable bacteria. *Int J Antimicrob Agents*, 30 Suppl 1:S7-15.
101. Fenollar F, Fournier PE, Raoult D (2004). Molecular detection of *Coxiella burnetii* in the sera of patients with Q fever endocarditis or vascular infection. *J Clin Microbiol*, 42(11): 4919-24.
102. Fenollar F, Raoult D (2004). Molecular genetic methods for the diagnosis of fastidious microorganisms. *APMIS*, 112(11-12): 785-807.
103. Lepidi H, Houpijian P, Liang Z, Raoult D (2003). Cardiac valves in patients with Q fever endocarditis: microbiological, molecular, and histologic studies. *J Infect Dis*, 187(7): 1097-106.
104. Lepidi H, Coulibaly B, Casalta JP, Raoult D (2006). Autoimmunohistochemistry: a new method for the histologic diagnosis of infective endocarditis. *J Infect Dis*, 193(12): 1711-17.
105. Fournier PE, Marrie TJ, Raoult D (1998). Diagnosis of Q fever. *J Clin Microbiol*, 36(7): 1823-34.
106. Landais C, Fenollar F, Thuny F, Raoult D (2007). From acute Q fever to endocarditis: serological follow-up strategy. *Clin Infect Dis*, 44(10): 1337-40.
107. Guigno D, Coupland B, Smith EG, Farrell ID, Desselberger U, Caul EO (1992). Primary humoral antibody response to *Coxiella burnetii*, the causative agent of Q Fever. *J Clin Microbiol*, 30: 1958-67.
108. Rolain JM, Boulou A, Mallet MN, Raoult D (2005). Correlation between ratio of serum doxycycline concentration to MIC and rapid decline of antibody levels during treatment of Q fever endocarditis. *Antimicrob Agents Chemother*, 49(7): 2673-76.

109. Bertrand A, Janbon F, Jonquet O, Reynes J (1988). Infections par les rickettsiales et fluoroquinolones. *Pathol Biol*, 36: 493-95.
110. Raoult D (1993). Treatment of Q fever. *Antimicrob Agents Chemother*, 37: 1733-36.
111. D'Angelo LJ, Hetherington R (1979). Q fever treated with erythromycin. *Br Med J*, 2: 305-306.
112. Perez-del-Molino A, Aguado JM, Riancho JA, Sampredo I, Matorras P, Gonzales-Macias J (1991). Erythromycin and the treatment of *Coxiella burnetii* pneumonia. *J Antimicrob Chemother*, 28: 455-59.
113. Rolain JM, Maurin M, Raoult D (2001). Bacteriostatic and bactericidal activities of moxifloxacin against *Coxiella burnetii*. *Antimicrob Agents Chemother*, 45(1): 301-302.
114. Raoult D, Etienne J, Massip P, Iacono S, Prince MA, Beaurain P, et al. (1987). Q fever endocarditis in the south of France. *J Infect Dis*, 155: 570-73.
115. Varma MPS, Adgey AAJ, Connolly JH (1980). Chronic Q fever endocarditis. *Br Heart J*, 43: 695-99.
116. Levy PY, Drancourt M, Etienne J, Auvergnat JC, Beytout J, Sainty JM, Goldstein F, Raoult D (1991). Comparison of different antibiotic regimens for therapy of 32 cases of Q fever endocarditis. *Antimicrob Agents Chemother*; 35: 533-37.
117. Maurin M, Benoliel AM, Bongrand P, Raoult D (1992). Phagolysosomal alkalization and the bactericidal effect of antibiotics: the *Coxiella burnetii* paradigm. *J Infect Dis*; 166: 1097-1102.
118. Raoult D, Drancourt M, Vestris G (1990). Bactericidal effect of Doxycycline associated with lysosomotropic agents on *Coxiella burnetii* in P388D1 cells. *Antimicrob Agents Chemother*, 34: 1512-14.
119. Lim WS, Macfarlane JT, Colthorpe CL (2003). Treatment of community-acquired lower respiratory tract infections during pregnancy. *Am J Respir Med*, 2(3): 221-33.