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Review

Q fever

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ABSTRACT

Q fever is a zoonotic disease caused by the ubiquitous pathogen *Coxiella burnetii* responsible for acute and chronic clinical manifestations. Farm animals and pets are the main reservoirs of infection, and transmission to human beings is mainly accomplished through inhalation of contaminated aerosols. This illness is associated with a wide clinical spectrum, from asymptomatic or mildly symptomatic seroconversion to fatal disease. In humans Q fever can manifest as an acute disease (mainly as a self-limited febrile illness, pneumonia, or hepatitis) or as a chronic disease (mainly endocarditis), especially in patients with previous valvulopathy and to a lesser extent in immunocompromised hosts and in pregnant women. In contrast in animals, Q fever is in most cases, strikingly asymptomatic. The definite diagnosis of Q fever is made based on a significant increase in serum antibody titers, the determination of which often requires considerable time, and therefore patients must be monitored for a certain period. 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. Several actions have been proposed to prevent and reduce the animal and environmental contamination. Vaccination of animals in infected flocks, as well as in uninfected ones close to them, with an efficient vaccine can prevent abortions and shedding of the bacteria.

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1. Introduction

Q fever is a zoonosis caused by *Coxiella burnetii*, a small obligate intracellular gram-negative bacterium that is prevalent throughout the world (Maurin and Raoult, 1999). Farm animals and pets are the main reservoirs of infection, and transmission to human beings is mainly accomplished through inhalation of contaminated aerosols. This illness is associated with a wide clinical spectrum, from asymptomatic or mildly symptomatic seroconversion to fatal disease.

Q fever was described in 1935 as an outbreak of febrile illness in abattoir workers in Brisbane, Australia (Derrick, 1937). Derrick examined all those who were affected and could not arrive at a diagnosis from the patients' history, physical examination, and a few investigations. As a result, he termed the illness "Q" for query fever. Later, some workers suggested that the Q stood of Queensland, the state in which the disease was first described (McDade, 1990). However, once the epidemiology of the disease became known and its status as a zoonosis established, this investigation lost favor. Subsequently, Burnet and Freeman (1937) 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 (1948) 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.

During the last decade our knowledge on Q fever has greatly expanded, mainly due to the identification of new clinical manifestations, the recognition of the role of host factors in the expression of acute Q fever and evolution to chronic infection, and the adoption of prolonged combination antibiotic regimens for Q fever endocarditis.

2. Bacteriology

C. burnetii is a small, obligate intracellular Gram-negative bacterium that cannot be grown in axenic medium. It is a small pleomorphic rod (0.2–0.4 μm wide, 0.4–1.0 μm long) with a membrane similar to that of a Gram-negative bacterium (Maurin and Raoult, 1999). It replicates to high numbers within a parasitophorous vacuole of eukaryotic host cells, with an estimated doubling time of 20–45 h (Mertens and Samuel, 2007). The organism may occur as a small-cell variant or large-cell variant. The small-cell variant is a compact, small rod with a very electron-dense center of condensed nucleoid filaments. The large-cell variant is larger and less electron-dense and is the metabolically active intracellular form of *C. burnetii*. It undergoes sporogenic differentiation to produce resistant, spore-like forms, the small-cell variants. These are released when the cells lyse and can survive for long periods in the environment.

C. burnetii was classified in the *Rickettsiales* order, the *Rickettsiaceae* family, and the *Rickettsiae* tribe together

with the genera *Rickettsia* and *Rochalimaea*. To date based on 16S rRNA sequence analysis; the bacterium was reclassified from the order *Rickettsiales* to *Legionellales*, and falls in the gamma group of Proteobacteria. Within this proteobacteria group, the bacterium's phylogenic neighbours include *Legionellae* spp, *Francisella tularensis*, and *Rickettsiella* spp. (Raoult et al., 2005).

C. burnetii possesses a small circular chromosome of approximately 5 Mbp. Most isolates harbor additionally one of four previously described plasmids of 32–51 kb in size, which carry about 2% of the genome information. Strains without a resident plasmid carry instead a 16 kb plasmid-like sequence integrated in the chromosome (Mallavia, 1991). The genome has a G + C content of 43 mol% and 2134 coding sequences are predicted, of which 719 (33.7%) are hypothetical, with no significant similarity to other genes in the database (Seshadri et al., 2003; Hoover et al., 1992). Moreover, many of the 83 pseudogenes that have been identified in *C. burnetii* contain single frameshifts, point mutations, or truncations which imply a recent origin and indicate that genome reduction is a relatively early outgoing process (Seshadri et al., 2003). Three degenerate transponsors and 20 IS elements are also identified, with 21 copies of a unique IS110-related isotype IS*1111*, five IS30 and three ISAs1 family elements (Seshadri et al., 2003).

3. Epidemiology

Q fever has been described worldwide except in New Zealand. From 1999 to 2004, there were 18 reported outbreaks of Q fever from 12 different countries involving two to 289 people. Six outbreaks involved sheep; three involved goats; one resulted from exposure to goat manure; one from exposure to ovine manure; one involved exposure to wild animals; one involved exposure to cats and dogs; and in two outbreaks the source was unknown (Arricau-Bouvery and Rodolakis, 2005).

The reservoirs are extensive but only partially known and include mammals, birds, and arthropods, mainly ticks. Although over 40 tick species can be naturally infected with *C. burnetii*, they appear to not be important in the maintenance of infections in livestock or humans (Maurin and Raoult, 1999). The organism does, however, multiply in the gut cells of ticks and large numbers of *C. burnetii* are shed in tick feces. Contaminated hides and wool may be a source of infection for people either by direct contact or after the feces have dried and been inhaled as airborne dust particles. The most commonly identified sources of human infection are farm animals such as cattle, goats, and sheep. *C. burnetii* localizes to the uterus and mammary glands of infected animals (Babudieri, 1959). Pets, including cats, rabbits, and dogs, have also been demonstrated to be potential sources of urban outbreaks. In North America, outbreaks of Q fever have resulted from direct and indirect contact with parturient cats (Marrie and Raoult, 2002). Outbreaks have also been reported following exposure to infected pigeon feces (Stein and Raoult, 1999).

4. Routes of transmission to humans

4.1. Aerosols

From experimental and epidemiological evidence, there is no doubt that contaminated aerosols are the major mechanism whereby *C. burnetii* is transmitted to humans (Tiggert and Benenson, 1956; Gonder et al., 1979; Marrie et al., 1989). Persons in contact with farm animals can be infected by inhalation of contaminated aerosols from amniotic fluid or placenta or contaminated wool but also at risk are laboratory personnel who work with infected animals (Johnson and Kadull, 1966).

4.2. Oral route

Mammals also shed *C. burnetii* in milk, and thus, consumption of raw milk could be a source of infection (Maurin and Raoult, 1999). Although contaminated milk can be a risk factor for Q fever infection (Marmion et al., 1956; Fishbein and Raoult, 1992), the evidence from experiments in which contaminated milk was fed to volunteers were contradictory (Benson et al., 1963; Editorial, 1950; Krumbiegel and Wisniewski, 1970). Ingestion of pasteurized cheese and tobacco smoking can be also risk factors for acquisition Q fever (Hatchette et al., 2000).

4.3. Percutaneous route

Ticks transmit *C. burnetii* to domestic mammals but not to humans (Kazar, 1996).

4.4. Person-to-person transmission

Person-to-person transmission of *C. burnetii* is rare. Transmission of Q fever to attendants during autopsies (Harman, 1949; Gerth et al., 1982) or infection from a patient to the hospital staff (Deutch and Peterson, 1950) can occur.

4.5. Sexual transmission

A recent report describes sexual transmission (Milazzo et al., 2001). Sexual transmission of Q fever has been demonstrated in mice (Kruszewska and Tylewska-Wierzbanowska, 1993) and viable *C. burnetii* has been found in bull semen (Kruszewska and Tylewska-Wierzbanowska, 1997).

4.6. Age and gender

There are several studies in which young age seems to be protective against *C. burnetii*. In a large outbreak of Q fever in Switzerland, symptomatic infection was five times more likely to occur in those over 15 years of age compared with those younger than 15 (Dupuis et al., 1985). In children the sex ratio of clinical cases as well as that of infections is 1:1. The change in sex ratio at puberty can be explained by the protective role of 17-β-estradiol in clinical expression, which has been demonstrated in mice (Leone et al., 2004).

5. Transmission in animals

Infected ticks are probably most important in maintaining the whole cycle of *C. burnetii* (Stoker and Marmion, 1995). Ticks may play a significant role in the transmission of *C. burnetii* among the wild vertebrates, especially in rodents, lagomorphs, and wild birds (Babudieri, 1959; Lang, 1990; Marrie et al., 1989). Dogs can also be infected by tick bite (Mantovani and Benazzi, 1953). Although experimental transmission of *C. burnetii* from infected to uninfected guinea pigs via tick bite has been performed with *Ixodes holocyclus*, *Haemaphysalis bispinosa*, and *Rhipicephalus sanguineus* (Maurin and Raoult, 1999), ticks are not considered essential in the natural cycle of *C. burnetii* infection in livestock (Babudieri, 1959). Ticks expel heavy loads of *C. burnetii* with their feces onto the skin of the animal host at the time of feeding.

Animals which live in close contact can become infected with *C. burnetii*. Sanford et al. described abortions that occurred in goat herds that were exposed to three goats from another herd that kidded prematurely during a fair (Sanford et al., 1994). Twenty-one days after exposure abortions began and affected 20–46% of the pregnant animals in each herd. Moreover, when cows were imported into an area of endemic infection, 40% of uninfected cows became *C. burnetii* infected within 6 months (Huebner and Bell, 1951).

Dogs may be infected by consumption of placentas or milk from infected ruminants, and by the aerosol route. Anti-phase II antibody seroprevalence was found ranging from 7 to 53% among wild brown rat populations in the United Kingdom and the authors hypothesized that wild rats may represent a major reservoir of *C. burnetii* from which domestic animals, especially cats, which are natural predators of these animals, may become contaminated (Webster et al., 1995).

6. Pathogenicity

A major characteristic of *C. burnetii* is its antigenic variation, called phase variation. Organisms isolated from acutely infected animals, arthropods, or humans express a wild virulent form, with smooth full length LPS named Phase I. After several passages in embryonated hen eggs or cell culture, the bacterium shifts from Phase I to an avirulent phase (Phase II), similar to the smooth to rough variation described for many *Enterobacteriaceae* (Hotta et al., 2002). Phase variation is probably not a single step process, as intermediate-phase or semi-rough LPS types have been described (Vodkin and Williams, 1986; Amano et al., 1987). Virulent Phase II bacteria express a truncated, rough LPS molecule and many differ in surface protein composition, surface charge and cell density (Mertens and Samuel, 2007).

The target cells of *C. burnetii* are monocytes/macrophages. Capo et al. showed that virulent *C. burnetii* organisms survived inside human monocytes, whereas avirulent bacteria were eliminated (Capo et al., 1999). In addition, they were phagocytozed by host cells at markedly lower efficiency than avirulent variants. *C. burnetii* enters monocytes/macrophages, the only known target cells, by phagocytosis that differs in phase I and phase II cells. Attachment of phase I bacteria is mediated by $\alpha v\beta 3$ integrin only, whereas phase II attachment is mediated by both $\alpha v\beta 3$ and complement receptor CR3 (Capo et al., 1999). As the efficiency of CR3-mediated phagocytosis depends on CR3 activation via $\alpha v\beta 3$ integrin, the low phagocytic efficiency observed with virulent *C. burnetii* results from the interference with integrin cross-talk and a pre-treatment of monocytes with virulent bacteria prevents CR3-mediated phagocytosis and CR3 activation. Virulent bacteria stimulate the formation of pseudopodal extensions and transient reorganization of filamentous actin, whereas avirulent agents have no effect (Meconi et al., 1998). Finally, specific inhibitors of src-related kinases prevent *C. burnetii* stimulated reorganization of the cytoskeleton (Meconi et al., 2001).

The adaptation of *C. burnetii* to intracellular life is linked with acidic pH of its phagosome and both virulent and avirulent bacteria are found in phagosomes. Acidic pH allows the entry of nutrients necessary for *C. burnetii* metabolism and also protects bacteria from antibiotics by altering their activity (Hackstadt and Williams, 1981). The survival of *C. burnetii* in human macrophages is based on the control of phagocytosis and the prevention of ultimate phagosome lysosome fusion. This is based on the fact that virulent organisms are presented in phagosomes that express endosomal markers such as the mannose 6-phosphate receptor, LAMP1 and proton ATPase, but they do not acquire a marker such as cathepsin D. On the other hand, avirulent agents are presented in phagosomes that colocalize with cathepsin D. Finally, defective phagosome maturation is induced by exogenous IL-10 in monocytes from patients with microbicidal competence and corrected IL-10 neutralization in patients with chronic Q fever which means that phagosome maturation and *C. burnetii* killing are linked in Q fever and are controlled by cytokines (Ghigo et al., 2004).

Toll-like receptor 4 (TLR4) has also a role in the uptake of virulent *C. burnetii*, since it is involved in the recognition of lipopolysaccharide, and in membrane ruffling induced by phase I lipopolysaccharide (Honstettre et al., 2004). TLR2 is also involved in *C. burnetii* infection and Zamboni et al. showed that TLR2 is involved in TNF and interferon-γ (IFN-γ) production (Zamboni et al., 2004). Myeloid dendritic cells (DCs) can be infected by *C. burnetii* and DCs constitute a protective niche for the bacteria as organisms replicate within DCs (Shannon et al., 2005). In contrast, avirulent bacteria which are eliminated by the host immune response stimulate DC maturation and IL-12 production. Phase I bacteria escape intracellular killing by inhibiting the final phagosome maturation step-cathepsin fusion (Ghigo et al., 2002). IFN-γ restores this fusion step and allows intracellular killing of *C. burnetii* by recruting the GTPase Rab7, which is involved in traffic regulation (Raoult et al., 2005). Moreover IFN-γ induces the killing of *C. burnetii* through the apoptosis of *C. burnetii*-infected macrophages by inducing the expression of membrane tumour necrosis factor (Raoult et al., 2005).

The control of the primary Q fever infection involves systemic cell-mediated immune response and granuloma formation. The granulomatous lesions have a central open

space and a fibrin ring, and are referred to as doughnut granulomas. Immune control of *C. burnetii* is T-cell dependent but does not lead to *C. burnetii* eradication (Honstettre et al., 2004). *C. burnetii* DNA can also be found in circulating monocytes or bone marrow of people infected months or years earlier (Capo et al., 2003). Specific immunoglobulins are secreted following infection. IgG is mainly directed against phase II antigen, whereas IgM is directed against both phase I and II cells (Maurin and Raoult, 1999).

C. burnetii infection may become chronic. Once established, chronic Q fever is characterised by defective cell-mediated immunity, thus emphasizing the major role of cell-mediated immunity in the protection against *C. burnetii*. Lymphocytes from patients with Q fever endocarditis do not proliferate in response to *C. burnetii* antigen, in contrast to lymphocytes from patients with acute Q fever (Koster et al., 1985). The mechanisms of this specific unresponsiveness may include alterations in T-cell subsets, but CD4 T-cell lymphopenia was observed in patients with Q fever endocarditis (Sabatier et al., 1997). Finally, a severe inflammation is found in almost every patient with Q fever endocarditis as they exhibit up-regulated levels of TNF and IL-6, two inflammatory cytokines, type II TNF receptors and IL-1 receptor antagonist (Mege, 2007).

7. Clinical manifestations

The main characteristic of Q fever is its clinical polymorphism, so that diagnosis can only be made by systematic tests. It is likely that factors such as the route of infection and the inoculum size, affect the expression of *C. burnetii* infection. Indeed the respiratory route is associated with pneumonia and the intraperitoneal route with hepatitis (Marrie et al., 1996). High inocula are associated with myocarditis (Maurin and Raoult, 1999). Gender and age also affect the expression of *C. burnetii* infection. Men are symptomatic more often than women despite comparable exposure and seroprevalence (Tissot-Dupont et al., 1992; Maltezou and Raoult, 2002). Moreover, the prevalence of clinical cases in children significantly increases with age and symptomatic Q fever occurs more frequently in people over 15 years old (Maltezou and Raoult, 2002).

8. Acute Q fever

In an epidemiological survey that took place in Marseille between 1985 and 1998, Q fever diagnosis was 1070 patient with a male/female sex ratio of 2.45 (Raoult et al., 2000). The mean age of acute Q fever patients was 45.32 ± 16.56 years (range, 6–87 years). There was no statistically significant age difference according to sex. Occupation was studied for 477 patients and 8% of them were farmers or veterinarians, a rural existence was noted for 162 (37.9%), ingestion of farm goat cheese was noted for 85/366 (23.2%), and contact with newborn or pregnant animals for 142/401 (35.4%). Immunosuppression was noted for 20 patients (4.7%) (Raoult et al., 2000).

The incubation period has been estimated to be approximately 20 days (range, 14–39 days). There is no typical form of acute Q fever and the clinical signs vary greatly from patient to patient. The most frequent clinical manifestation of acute Q fever is probably a self-limited febrile illness (91%) which is associated with severe headaches (51%), myalgias (37%), arthralgias (27%) and cough (34%) (Tissot-Dupont and Raoult, 2007). The main symptoms fever, pulmonary signs, and elevated liver enzyme levels can coexist. Of 323 hospitalized patients with acute Q fever in France, 25% presented with the three symptoms, 40% presented with fever and elevated liver enzyme levels, 17% presented with fever and pulmonary signs, and 4% presented with only fever, pulmonary signs, or elevated liver enzyme levels (Tissot-Dupont et al., 1992). Atypical pneumonia is also a major clinical presentation and abnormal chest X rays can be found in 27% of the patients (Tissot-Dupont and Raoult, 2007). After primary infection, 60% of the patients will exhibit a symptomatic seroconversion, and only 4% of the symptomatic patients will be admitted to hospitals. A chronic disease will develop in at risk patients.

8.1. Prolonged fever

Prolonged fever is usually accompanied by severe headaches. The fever may reach from 39 to 40 °C, usually remaining elevated all day. Fever typically increases to a plateau within 2–4 days, and then after 5–14 days the temperature returns rapidly to normal. However, in untreated patients, fever may last from 5 to 57 days (Derrick, 1973). The duration of fever is longer in elderly patients (Derrick, 1973).

8.2. Pneumonia

Atypical pneumonia is one of the most commonly recognized forms of acute Q fever. 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 (Raoult et al., 1990b). 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 (Tissot-Dupont et al., 1992). Pneumonia is the major manifestation of acute Q fever in Nova Scotia, Canada, in the Basque country in Spain, and in Switzerland, while in France, Ontario, California, and Australia, hepatitis is the predominant form of acute Q fever (Fournier et al., 1998).

8.3. 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. Hepatitis is the most common presentation worldwide, particularly in France and Australia. Alkaline phosphatase, AST, and ALT levels are usually mildly elevated to two to three times the normal level (Marrie, 1988). Q fever hepatitis is usually accompanied clinically by fever and less frequently by abdominal pain (especially in the right hypochondrium),

anorexia, nausea, vomiting, and diarrhea. Progressive jaundice and palpation of a mass in the right hypochondrium have also been reported. Frequently, patients with hepatitis exhibit autoantibodies, including antibodies directed to smooth muscle, anticardiolipin antibodies, antiphospholipid antibodies, circulating anticoagulant, and antinuclear antibodies (Tissot-Dupont and Raoult, 2007).

8.4. Cardiac involvement

Cardiac involvement is found in 2% of the acute Q fever cases and myocarditis is the leading cause of death (Fournier et al., 2001). The pathophysiology of the heart damage is still not clear, although a relationship has been demonstrated between the onset of a myocarditis and the inoculum size in an experimental model (La Scola et al., 1997). Myocarditis may be associated with pericarditis, and a pericardial effusion may be observed on chest radiographs. Clinical manifestations of Q fever pericarditis are not specific and most often correspond to a fever with thoracic pain. However, *C. burnetii* is the main cause of pericarditis in southern France and a frequent one in Spain and in the United Kingdom (Tissot-Dupont and Raoult, 2007).

8.5. Skin rash

Skin lesions have been found in 5–21% of Q fever patients in different series. The Q fever rash is nonspecific and may correspond to pink macular lesions or purpuric red papules of the trunk (Maurin and Raoult, 1999).

8.6. Neurologic signs

There are 3 major neurological entities associated with Q fever: (1) meningoencephalitis or encephalitis; (2) lymphocytic meningitis and (3) peripheral neuropathy (Bernit et al., 2002). Patients with central nervous system involvement do not demonstrate differences in predisposing conditions, but more frequently have occupational exposure to goats than patients with acute Q fever but no neurological involvement (Bernit et al., 2002).

9. Chronic Q fever

Chronic Q fever may develop many months to years after initial infection, manifesting as bacterial culture-negative endocarditis in up to 75% of cases (Gami et al., 2004). Chronic Q fever occurs almost exclusively in patients with predisposing conditions, including those with heart valve lesions, vascular abnormalities, and immunosuppression (Fenollar et al., 2001). Fig. 1 shows the natural history of Q fever in the absence of treatment.

9.1. Endocarditis

The most frequent and studied preservation of chronic Q fever is endocarditis (Fig. 1). More than 800 cases were reported in various studies between 1949 and 2005 (Tissot-Dupont and Raoult, 2007). The main series were studied in the United Kingdom and in Ireland (227 cases), in France (264 cases), in Spain (62 cases), in Israel (35 cases), in Switzerland (21 cases), in Australia (18 cases) and in Canada (10 cases). Q fever endocarditis is often a severe disease associated with a long diagnostic delay. Q fever represents 5% of endocarditis cases in France (Brouqui and Raoult, 2006). It occurs almost exclusively in patients with a previous cardiac defect or in immunocompromised patients. The aortic and mitral valves are mostly involved. Q fever prosthetic valve endocarditis has been increasingly reported over recent years (Maurin and Raoult, 1999). The male/female ratio is 75%, and most patients are older than 40 years. The clinical presentation has changed over the

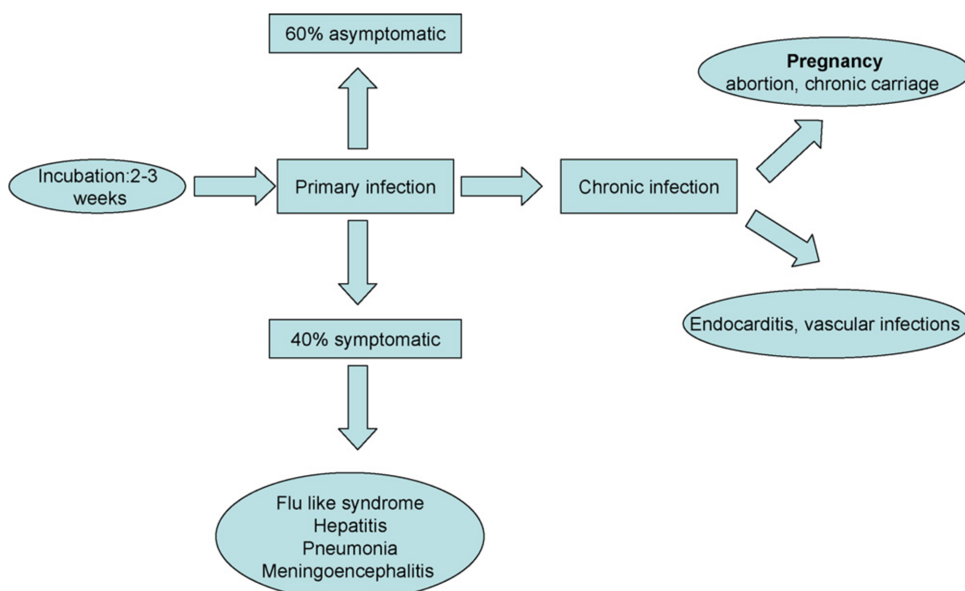

Fig. 1. Q fever natural history in the absence of treatment.

last 30 years. With faster diagnoses, the prevalence of heart failure, hepatomegaly, inflammatory syndrome, anaemia and leucopenia and abnormal liver function tests have decreased significantly (Houpikian et al., 2002).

The prognosis of chronic Q fever was dramatically improved over the course of just a few years. The mortality rate was 37% in a series of 79 patients reported in 1987 (Raoult et al., 1987), whereas it was only 15% in more recent series of 116 patients, between 1997 and 2000 (Tissot-Dupont and Raoult, 2007). Among the most recently diagnosed patients the death rate was under 5% (Raoult et al., 1999) an improvement that is probably related to the earlier diagnosis, the efficient treatment and the better follow up (Siegman-Igra et al., 1997).

10. Other clinical manifestations of chronic Q fever

Vascular infection is the second most frequent presentation of Q fever. An aortic aneurism can be infected by *C. burnetii*, leading to an intestinal fistula or a spondylitis, as well as a vascular graft. The prognosis is poor in the absence of treatment (Botelho-Nevers et al., 2007).

Other manifestations of chronic Q fever are osteoarticular infections, including osteomyelitis, osteoarthritis, and aortic graft infection with contiguous spinal osteomyelitis (Maurin and Raoult, 1999), chronic hepatitis in alcohol addicts (Raoult et al., 2000), pseudotumors of the spleen, of the lung (Lipton et al., 1987), infection of a ventriculo-peritoneal drain (Lohuis et al., 1994). Chronic fatigue syndrome has also been reported infrequently as a possible clinical manifestation following acute Q fever. The latter may be associated with cytokine dysregulation and presents as fatigue, myalgia, arthralgia, night sweats, mood changes and sleep disturbance.

11. Pregnant women

When a woman is infected by *C. burnetii* during pregnancy, the bacteria settle in the uterus and in the mammary glands. The consequences are of great importance: (a) there is an immediate risk for the mother; (b) there is an immediate risk for the fetus as 100% of the fetuses abort when the infection occurs during the first trimester and there is a risk of preterm delivery, or low birth weight if infection occurs during the second or third trimester; (c) there is a long-term risk of chronic Q fever in the mother. Few data are available on the consequences of Q fever during pregnancy. To date, only 38 cases have been published, demonstrating that Q fever in pregnant women is associated with high morbidity and mortality (Carcopino et al., 2007). Thus, Q fever during pregnancy can result in spontaneous abortion (26%), intrauterine fetal death (5.3%), premature delivery (44.7%), or intrauterine growth retardation (5.3%) (Carcopino et al., 2007). Normal obstetric outcome is possible (15.8%). Transplacental infection of the fetus in utero is possible, but its consequences are still unknown, and its association with obstetric complications remains hypothetical (Carcopino et al., 2007). In a work of our laboratory it was shown that Q fever, when contracted during pregnancy, can result in abortions or neonatal deaths (9 cases, 38%), premature births, low birth weight (8 cases, 33%), or no abnormalities (7 cases, 29%) (Raoult et al., 2002). Q fever during pregnancy also has important consequences for the mother, with higher risk of chronic form and spontaneous abortions of future pregnancies. Although most infected pregnant women present with fever, flu-like illness, severe thrombocytopenia, and atypical pneumonia have also been reported (Maurin and Raoult, 1999). However, Q fever in pregnant women may also be asymptomatic (Marrie, 1993). Serological profiles at the time of diagnosis were suggestive of acute Q fever in 14 (58.3%) of 24 pregnant women for whom serology was performed and of chronic Q fever in 10 (41.7%) (Maurin and Raoult, 1999).

12. Clinical manifestations in animals

In contrast to acute human Q fever animal infection with *C. burnetii* is, in most cases, so strikingly asymptomatic that the term coxiellosis is considered a more appropriate designation than animal Q fever (Lang, 1988). In animals, during the acute phase, *C. burnetii* can be found in the blood, lungs, spleen, and liver whereas during the chronic phase it is presented as a persistent shedding of *C. burnetii* in feces and urine. Most animals remain totally asymptomatic, including a lack of fever. However, low birth weight animals can occur (Marrie et al., 1996). Aborted fetuses usually appear normal and the abortion rates can range from 3 to 80% (Marrie, 2007). Infected placentas exhibit exudates and intracotyledonary fibrous thickening. A severe inflammatory response is noted in the myometrium of goats and metris is frequently a unique manifestation of the disease in cattle (Arricau-Bouvery and Rodolakis, 2005). *C. burnetii* can also be recovered from milk for up to 32 months. Goat shed *C. burnetii* in feces before and after kidding and the mean duration of excretion is 20 days.

13. Diagnosis

13.1. Collection and storage of specimens

C. burnetii virulence is particularly high and 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. All specimens, excluding whole blood which should be kept at 4 °C, should be stored at −80 °C and should be forwarded on dry ice to the diagnostic laboratory (Fournier et al., 1998).

13.2. Culture

C. burnetii isolation from biological samples is carried out on HEL cells using the Shell Vial centrifugation technique (Marrero and Raoult, 1989). Cell monolayers in shell vials are inoculated with 1 ml of clinical specimen and centrifuged ($700 \times g$ at 20 °C) for 1 h to enhance attachment and penetration of *C. burnetii* into cells. Inoculated monolayers are incubated at 37 °C in 5% CO_2

for 5–7 days. *C. burnetii* is usually observed by microscopic examination of cell monolayers after Gimenez or immunofluorescence staining.

13.3. Immunodetection

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. Immunodetection is carried out using immunoperoxidase techniques or immunofluorescence with polyclonal or monoclonal antibodies (Maurin and Raoult, 1999). Only this last technique can be used on paraffin-embedded samples (Raoult et al., 1994). Recently, Lepidi et al. proposed a new method named autoimmunohistochemistry for the detection of the *C. burnetii* endocarditis (Lepidi et al., 2006) (Fig. 2).

13.4. Molecular biology

During the last years, several PCR-based diagnostic assays were developed to detect *C. burnetii* DNA in cell cultures and in clinical samples. These assays used conventional PCR, nested PCR or real-time PCR conditions with Light-Cycler, SYBR Green or TaqMan chemistry (Klee et al., 2006). The Light-Cycler Nested PCR (LCN-PCR), a rapid nested PCR assay that uses serum as a specimen and the Light-Cycler as a thermal cycler, targeting a multicopy 20-copy htpAB-associated element sequence has been adapted for the diagnosis of both acute and chronic Q fever (Fenollar and Raoult, 2007). The LCN-PCR assay may be helpful in establishing an early diagnosis of chronic Q fever (Fenollar et al., 2004). Due to its high sensitivity and specificity, the repetitive element, IS 11-11, is the best target gene for the detection of *C. burnetii* in patients with active Q fever (Fenollar and Raoult, 2004). Recently, the complete sequences of the genome of *C. burnetii* became available, allowing a large choice of DNA targets.

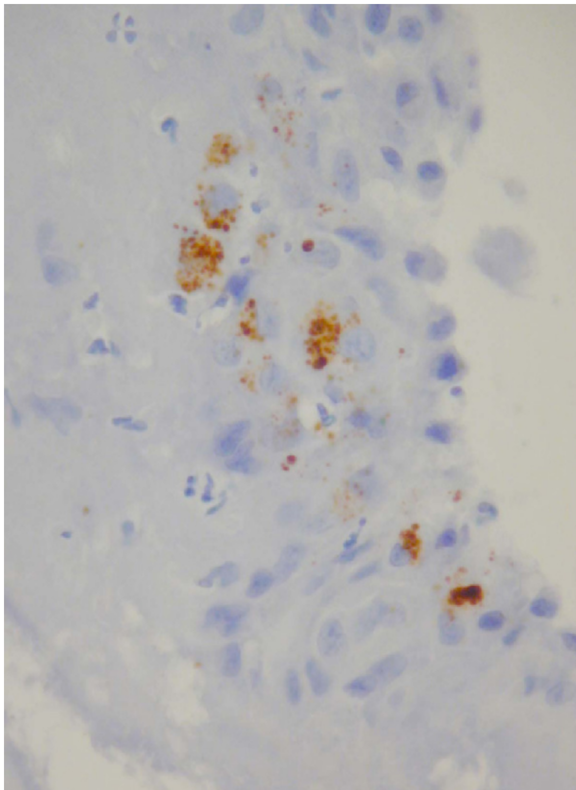

Fig. 2. Immunohistochemical detection of *C. burnetii* in a resected cardiac valve from a patient with a Q fever endocarditis, using a monoclonal antibody and hematoxylin counterstain. Note the intracellular location of the bacteria in the macrophage cytoplasm (original magnification ×400).

13.5. Serology

Since the clinical diagnosis is difficult, in most instances, the diagnosis of Q fever relies upon serology. A variety of serological techniques are available, but the indirect microimmunofluorescent antibody test has become the reference technique. Immunoglobulin M antibodies reactive with phase II *C. burnetii* appear rapidly, reach high titers within 14 days and persist for 10–12 weeks (Maurin and Raoult, 1999). Immunoglobulin M antibodies reactive with phase I antigens are usually at a much lower titer during acute infection. Immunoglobulin G antibodies reactive with phase II antigens reach peak titers about 8 weeks after the onset of symptoms, while those reactive with phase I antigens develop only very slowly and remain at lower titers than antibodies to phase II antigens, even after a year. In chronic Q fever, where there is persistence of organisms, the IgG titers to phase I and phase II antigens may both be high, and the presence of IgA antibody to phase I antigen is usually, although not exclusively, associated with chronic infection. Seroconversion or a fourfold increase in titer indicates acute infection. Elevated levels of IgG ($>$1/200) and IgM ($>$1/25) to phase II antigens also indicate a recent infection. High titers of IgG (1/800) and/or IgA ($>$1/50) to phase I antigen are found in chronic infections. Serology should be used to follow up patients with acute Q fever to determine if treatment was successful and to enable the early diagnosis of chronic infections (Landais et al., 2007).

13.6. Diagnosis in animals

Isolation of *C. burnetii* is not performed for routine diagnosis in veterinary medicine. Routine diagnosis of Q fever in animals is usually established by examination of fixed impressions or smears prepared from the placenta stained by the Stamp, Gimenez or Machiavello methods, associated with serological tests. The CF test, which is the OIE prescribed serological test, is weakly sensitive and the antigen used in this test frequently fails to detect antibodies in sheep or goats (Kovacova et al., 1998). The ELISA test is more sensitive than the CF test but it does not allow individual identification of animals that shed *C. burnetii* in faeces or milk. PCR kits are becoming available and provide a specific, sensitive and rapid tool for the detection of *C. burnetii* in various clinical samples (Berri et al., 2003).

14. Antimicrobial susceptibility testing

Antibiotic susceptibility testing of *C. burnetii* is difficult because this organism is an obligate intracellular bacterium. Three models of infection have been developed: animals, chick embryos and cell culture. The current method used to test the antibiotic susceptibility of *C. burnetii* is based on cell culture models (Rolain, 2007).

A number of cell lines have been used to test antibiotic activity against intracellular *C. burnetii*, including murine macrophage-like cell lines (P388D1 and J774) and a murine fibroblast cell line (L929) (Akporiaye et al., 1983; Baca et al., 1981, 1985; Burton et al., 1978; Roman et al., 1986). Yeaman et al. described an acute *C. burnetii* infection model in acutely infected L929 cells (Yeaman et al., 1989). Bacteriostatic activity was demonstrated against *C. burnetii* Nine Mile and Priscilla isolates with doxycycline (10 mg/ml), rifampin (1 mg/ml), and ofloxacin (5 mg/ml). Moreover, they used chronically infected L929 cells to test the bactericidal activity of antibiotics against *C. burnetii* (Yeaman et al., 1987). They found that the percentage of infected cells was not reduced by tetracycline, erythromycin, or sulfamethoxazole at concentrations up to 10 mg/ml and was only slightly reduced by chloramphenicol, doxycycline, and trimethoprim, suggesting that these drugs were not bactericidal. In contrast, the quinolone compounds and rifampin reduced the percentage of infected cells from 100% to 2, 2, 7, and 4%, respectively, after 10 days of continuous culture treatment. Torres and Raoult have developed a Shell Vial assay with human embryonic lung cells (HEL) for assessment of the bacteriostatic effect of antibiotics (Torres and Raoult, 1993). By this technique, amikacin and amoxicillin were not effective against *C. burnetii*, ceftriaxone and fucidic acid were inconsistently active (Torres and Raoult, 1993), whereas cotrimoxazole, rifampin, doxycycline, clarithromycin and quinolones were bacteriostatic (Rolain et al., 2005b; Maurin and Raoult, 1993). Raoult et al., using P288D1 and L929 cells, showed that pefloxacin, rifampin and doxycycline (Raoult et al., 1990a) as well as clarithromycin were bacteriostatic against *C. burnetii* (Maurin and Raoult, 1993). Moreover, Maurin et al. demonstrated that the addition of a lysosomotropic alkalinizing agent, chloroquine, to antibiotics improved the activities of doxycycline and pefloxacin which then became bactericidal (Maurin et al., 1992). That result has been corroborated by the demonstration of *in vivo* efficacy for the combination of doxycycline and hydroxychloroquine (Raoult et al., 1999).

High level resistance to fluoroquinolones due to an amino acid substitution of Gly instead of Glu at position 87 of the GyrA has been reported (Musso et al., 1996). Porins have been demonstrated in *C. burnetii* cells, but their potential role in antibiotic resistance associated with impermeability remains undefined (Banerjee-Bhatnagar et al., 1996). Moreover, *C. burnetii* strains have been found to present differences in susceptibility to erythromycin (Raoult et al., 1991) and in susceptibility to doxycycline, ciprofloxacin, and rifampin (Yeaman and Baca, 1990). In vitro selection of *C. burnetii* strains resistant to tetracyclines has been also performed (Brezina et al., 1975).

The real-time quantitative PCR (RT-PCR) assay has also been used for the determination of the antibiotic susceptibility of *C. burnetii* (Boulos et al., 2004; Brennan and Samuel, 2003). RT-PCR confirmed that MICs against doxycycline, fluoroquinolone compounds and rifampicin were in the range 1–4 mg/L and that telithromycin was the most effective macrolide compound (Boulos et al., 2004). By the use of this assay, for the first time a human isolate of *C. burnetii* resistant to doxycycline was found in a patient with Q fever endocarditis (Rolain et al., 2005a).

15. Treatment

The guideline recommendations for the treatment of Q fever are summarised in Table 1.

15.1. Treatment of Acute Q fever

The recommended regimen for acute Q fever associates doxycycline (200 mg daily for 14 days) to hydroxychloroquine, which alkalinizes the phagolysosomes (Maurin and Raoult, 1999). Fluoroquinolones are considered to be a reliable alternative and have been advocated for patients with Q fever meningoencephalitis, because they penetrate the cerebrospinal fluid (Maurin and Raoult, 1999). Cotrimoxazole and rifampin can be used in case of allergy to tetracyclines or contraindication (Tissot-Dupont and Raoult, 2007). Erythromycin and other new macrolides such as clarithromycin and roxithromycin, could be considered a reasonable treatment for acute *C. burnetii* infection (Gikas et al., 2001).

Table 1
Guidelines for the treatment of Q fever.

Clinical feature	Patient cohort	Treatment	Duration	Reference
Acute Q fever	Adults	Doxycycline (100 mg/day)	14 days	Maurin and Raoult (1999)
		Fluoroquinolones (200 mg three times a day or pefloxacin (400 mg)	14–21 days	Maurin and Raoult (1999)
		Rifampin (1200 mg/day)	21 days	Raoult (1993)
	Pregnant	Trimethoprim (320 mg) and sulfamethoxazole (1600 mg)	>5 weeks	Carcopino et al. (2007)
	Children	Doxycycline (100 mg/day)	10–14 days	Maurin and Raoult (1999)
Chronic Q fever	Adults	Doxycycline (100 mg/day) and hydroxychloroquine (600 mg)	>18 months	Carcopino et al. (2007)
	Children	Trimethoprim and sulfamethoxazole	>18 months	Nourse et al. (2004)

15.2. Acute Q fever in children

In children younger than 8 years, cotrimoxazole has been recommended, because of the adverse effects of tetracyclines and quinolones in this age group. However, it has now been admitted that age is not a contraindication to doxycycline, when the antibiotic is specific of the disease (Tissot-Dupont and Raoult, 2007). Moreover, in patients with prolonged fever, the addition of corticosteroids to treatment might prove beneficial and interferon γ was successfully administered to a 3-year-old child with a prolonged fever unresponsive to appropriate treatment against *C. burnetti* (Maltezou and Raoult, 2002).

15.3. Acute Q fever during pregnancy

Specific treatment using cotrimoxazole (800/160) BID, until delivery, associated to folinic acid (25 mg OD) is recommended (Raoult et al., 2002). Recently, Carcopino et al. compared the incidence of obstetric and maternal Q fever complications for women who received long-term cotrimoxazole treatment with that for women who did not receive long-term cotrimoxazole treatment (Carcopino et al., 2007). They found that long-term cotrimoxazole treatment protected against maternal chronic Q fever, placental infection, obstetric complications and especially of intrauterine fetal death. However, obstetric complications were observed in 81.1% of pregnant women who did not receive long-term cotrimoxazole therapy. After delivery, if the woman shows a chronic serology profile, she should be treated as a chronic case, in order to prevent endocarditis and relapsing abortions. Breast feeding is contraindicated (Raoult et al., 2002).

15.4. Chronic Q fever

Although the optimal duration of therapy is unknown, the current recommendations for the treatment of chronic Q fever are 100 mg of doxycycline orally twice daily with 600 mg of hydroxychloroquine by mouth once daily for at least 18 months. Serologic testing is recommended on a regular basis during therapy, and the main predictive criterion of clinical cure is a decrease of phase I IgG antibody titers to <200 (Karakousis et al., 2006). In general, the antibody titers decrease slowly with treatment (Raoult et al., 1999). However, the kinetics of antibody titer decrease in patients treated with doxycycline may vary, suggesting that some patients should be treated for >18 months to be cured (Rolain et al., 2003). Successful evolution is evaluated by the decrease of antibody titers (IgG and IgA) to phase I that should reach two dilutions in 1 year at the minimum. When available, the *C. burnetii* strain should be cultured from blood or valves in order to evaluate the doxycycline MIC: the doxycycline plasmatic level should be adjusted between 1.5 and 2 MICs (Rolain et al., 2005a). Hydroxychloroquine dosing should be adapted according to plasmatic levels (1 ± 0.2 mg/L). Recently, Rolain et al. found isolates of *C. burnetii* resistant to doxycycline (MIC:8 μg/mL) from patients with Q fever endocarditis (Rolain et al., 2005a,b).

15.5. Q fever in patients at risk of chronic evolution

Acute Q fever in any patient presents a risk factor for chronic evolution (vascular damage, vascular or valvular graft, aneurism) and should be treated according to the same protocol as chronic cases (Fenollar et al., 2001). Patients with acute Q fever should be systematically tested – including those patients who do not have known underlying factors – 3 and 6 months after the onset of disease (Landais et al., 2007). Those with phase I IgG antibody titers $\geq$1:800 should be investigated for possible infective endocarditis using transesophageal echocardiography and PCR to allow for early detection of the disease.

16. Treatment in ruminants

In ruminants, antibiotic treatment generally consist in administering two injections of oxytetracycline (20 mg per kg bodyweight) during the last month of gestation, although this treatment does not totally suppress the abortions and the shedding of *C. burnetii* at lambing (Berri et al., 2007). In known infected herds, segregating pregnant animals indoors, burning or burying reproductive offal, or administering tetracycline (8 mg/(kg day)) prophylactically in the water supply prior to parturition may reduce spread of the organism.

17. Prevention

Epidemiological studies indicate Q fever as a public health problem in many countries, including France, the United Kingdom, Italy, Spain, Germany, Israel, Greece, and Canada (Nova Scotia). In Germany, 7.8% of 21,191 tested cattle, 1.3% of 1346 tested sheep, and 2.5% of 278 tested goats had evidence of *C. burnetii* infection (Hellenbrand et al., 2001). In Cyprus, the prevalence of IgG antibodies against *C. burnetii* phase II antigen was estimated at 48.2% for goats, 18.9% for sheep, and 24% for bovines (Psaroulaki et al., 2006). In Iran goats had a significantly higher average seroprevalence (65.78%) than cattle (10.75%) (Khalili and Sakhaee, 2009). In Zimbabwe, serological evidence of Q fever infection was found in 39% of cattle, and in 10% of goats (Kelly et al., 1993). In the USA goats had a significantly higher average seroprevalence (41.6%) than sheep (16.5%) or cattle (3.4%) (McQuiston and Childs, 2002). Q fever remains primarily an occupational hazard in persons in contact with domestic animals such as cattle, sheep and, less frequently, goats. Persons at risk from Q fever include farmers, veterinarians, abattoir workers, those in contact with dairy products, and laboratory personnel performing *C. burnetii* culture and more importantly working with *C. burnetii*-infected animals.

It is important to mention that during the last years the prevalence of chronic Q fever in the USA has increased because of the Iraq war. Q fever is apparently hyperendemic in Iraq and many US soldiers serving in this area have been exposed to *C. burnetii* and diagnosed as suffering by Q fever. As Q fever may reveal more then 10 years after primo infection (symptomatic or not and diagnosed or not), it is possible that the Iraq war veterans will be an

important reservoir of potential chronic Q fever cases that will increase the real prevalence of the disease.

In common with all zoonotic diseases, control of the disease in animals will influence the level of disease seen in man. Appropriate tick control strategies and good hygiene practice can decrease environmental contamination. Infected fetal fluids and membranes, aborted fetuses and contaminated bedding should be incinerated or buried. In addition, manure must be treated with lime or calcium cyanide 0.4% before spreading on fields; this must be done in the absence of wind to avoid spreading of the microorganism faraway. Antibiotic treatment may be performed to reduce the number of abortions and the quantity of *C. burnetii* shed at parturition. Although it is very expensive, infected animals should be removed from herds or provided with separate containment facilities in which to give birth. Workers in the animal industry should be fully informed about the risk factors of acquiring Q fever and laboratories should be provided with appropriate safety facilities and equipment.

Three types of vaccine have been proposed for providing human protection against Q fever: the attenuated live vaccine (produced and tested in Russia but subsequently abandoned because of concern about its safety); chloroform–methanol residue extracted vaccine or other extracted vaccines (tested in animals but not humans); and the whole-cell formalin-inactivated vaccine (Q-Vax), which is considered acceptably safe for humans (Chiu and Durrheim, 2007). The only economic study undertaken in Australia on Q fever vaccine was performed before the completion of the national vaccination program and assumed a Q-Vax efficacy of 98% (Chiu and Durrheim, 2007). Since Q fever in humans is often an occupational hazard, vaccination should be considered primarily in exposed populations (Maurin and Raoult, 1999). Moreover, vaccination should probably also be considered in persons not professionally exposed but at risk for chronic Q fever, including patients with cardiac valve defects, vascular aneurysms, or prostheses and immunocompromised patients.

Vaccines can prevent abortion in animals, and it is evident that a phase I vaccine must be used to control the disease and to reduce environmental contamination and thus, the risk of transmission to humans. The widespread application of such a vaccine in cattle in Slovakia in the 1970s and 1980s significantly reduced the occurrence of Q fever in that country (Kovacova and Kazar, 2002). Reducing exposure to raw milk for at risk people (pregnant women, patients with cardiac pathology or immunosupressed) and promoting the use of pasteurized milk and its products will also contribute to lowering the prevalence of Q fever.

Conflict of interest statement

None.

References

Akporiaye, E.T., Rowatt, J.D., Aragon, A.A., Baca, O.G., 1983. Lysosomal response of a murine macrophage-like cell line persistently infected with *Coxiella burnetii*. Infect. Immun. 40, 1155–1162.

Amano, K., Williams, J.C., Missler, S.R., Reinhold, V.N., 1987. Structure and biological relationships of *Coxiella burnetii* lipopolysaccharides. J. Biol. Chem. 262, 4740–4747.

Arricau-Bouvery, N., Rodolakis, A., 2005. Is Q fever an emerging or re-emerging zoonosis? Vet. Res. 36, 327–349.

Babudieri, B., 1959. Q fever. A zoonosis. Adv. Vet. Sci. 5, 82–182.

Baca, O.G., Akporiaye, E.T., Aragon, A.S., Martinez, I.L., Robles, M.V., Warner, N.L., 1981. Fate of phase I and phase II *Coxiella burnetii* in several macrophage-like tumor cell lines. Infect. Immun. 33, 258–266.

Baca, O.G., Scott, T.O., Akporiaye, E.T., DeBlassie, R., Crissman, H.A., 1985. Cell cycle distribution patterns and generation times of L929 fibroblast cells persistently infected with *Coxiella burnetii*. Infect. Immun. 47, 366–369.

Banerjee-Bhatnagar, N., Bolt, C.R., Williams, J.C., 1996. Pore-forming activity of *Coxiella burnetii* outer membrane protein oligomer comprised of 29.5- and 31-kDa polypeptides. Inhibition of porin activity by monoclonal antibodies 4E8 and 4D6. Ann. N.Y. Acad. Sci. 791, 378–401.

Benson, W.W., Brock, D.W., Mather, J., 1963. Serologic analysis of a penitentiary group using raw milk from a Q fever infected herd. Public Health Rep. 78, 707–710.

Bernit, E., Pouget, J., Janbon, F., Dutronc, H., Martinez, P., Brouqui, P., Raoult, D., 2002. Neurological involvement in acute Q fever. A report of 29 cases and review of the literature. Arch. Intern. Med. 162, 693–700.

Berri, M., Rousset, E., Champion, J.L., Russo, P., Rodolakis, A., 2007. Goats may experience reproductive failures and shed *Coxiella burnetii* at two successive parturitions after a Q fever infection. Res. Vet. Sci. 83, 47–52.

Berri, M., rricau-Bouvery, N., Rodolakis, A., 2003. PCR-based detection of *Coxiella burnetii* from clinical samples. Methods Mol. Biol. 216, 153–161.

Botelho-Nevers, E., Fournier, P.E., Richet, H., Fenollar, F., Lepidi, H., Foucault, C., Branchereau, A., Piquet, P., Maurin, M., Raoult, D., 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, 635–640.

Boulos, A., Rolain, J.M., Maurin, M., Raoult, D., 2004. Evaluation of antibiotic susceptibilities against *Coxiella burnetii* by real time PCR. Int. J. Antimicrob. Agents 23, 169–174.

Brennan, R.E., Samuel, J.E., 2003. Evaluation of *Coxiella burnetii* antibiotic susceptibilities by real-time PCR assay. J. Clin. Microbiol. 41, 1869–1874.

Brezina, R., Schramek, S., Kazar, J., 1975. Selection of chlortetracycline-resistant strain of *Coxiella burnetii*. Acta Virol. 19, 496.

Brouqui, P., Raoult, D., 2006. New insight into the diagnosis of fastidious bacterial endocarditis. FEMS Immunol. Med. Microbiol. 47, 1–13.

Burnet, F.M., Freeman, M., 1937. Experimental studies on the virus of "Q" fever. Med. J. Aust. 2, 299–305.

Burton, P.R., Stueckemann, J., Welsh, R.M., Paretsky, D., 1978. Some ultrastructural effects of persistent infections by the rickettsia *Coxiella burnetii* in mouse L cells and green monkey kidney (Vero) cells. Infect. Immun. 21, 556–566.

Capo, C., Lindberg, F.P., Meconi, S., Zaffran, Y., Tardei, G., Brown, E.J., Raoult, D., Mege, J.L., 1999. Subversion of monocyte functions by *Coxiella burnetii* impairment of the cross-talk between a_vb_3 integrin and CR3. J. Immunol. 163, 6078–6085.

Capo, C., Moynault, A., Collette, Y., Olive, D., Brown, E.J., Raoult, D., Mege, J.L., 2003. *Coxiella burnetii* avoids macrophage phagocytosis by interfering with spatial distribution of complement receptor 3. J. Immunol. 170, 4217–4225.

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, 548–555.

Chiu, C.K., Durrheim, D.N., 2007. A review of the efficacy of human Q fever vaccine registered in Australia. N.S.W. Public Health Bull. 18, 133–136.

Derrick, E.H., 1937. Q fever, a new fever entity clinical features, diagnosis and laboratory investigation. Med. J. Aust. 2, 281–299.

Derrick, E.H., 1973. The course of infection with *Coxiella burnetii*. Med. J. Aust. 1, 1051–1057.

Deutch, D.L., Peterson, E.T., 1950. Q fever transmission from one human being to others. J. Am. Med. Assoc. 143, 348–354.

Dupuis, G., Vouilloz, M., Peter, O., Mottiez, M.C., 1985. Incidence of Q fever in Valais. Rev. Med. Suisse Romande 105, 949–954.

Editorial, 1950. Editorial: experimental Q fever in man. Br. Med. J. 1, 1000.

Fenollar, F., Fournier, P.E., Carrieri, M.P., Habib, G., Messana, T., Raoult, D., 2001. Risks factors and prevention of Q fever endocarditis. Clin. Infect. Dis. 33, 312–316.

Fenollar, F., Fournier, P.E., Raoult, D., 2004. Molecular detection of *Coxiella burnetii* in the sera of patients with Q fever endocarditis or vascular infection. J. Clin. Microbiol. 42, 4919–4924.

Fenollar, F., Raoult, D., 2004. Molecular genetic methods for the diagnosis of fastidious microorganisms. APMIS 112, 785–807.

Fenollar, F., Raoult, D., 2007. Molecular diagnosis of bloodstream infections caused by non-cultivable bacteria. Int. J. Antimicrob. Agents 30 (Suppl 1), S7–S15.

Fishbein, D.B., Raoult, D., 1992. A cluster of *Coxiella burnetti* infections associated with exposure to vaccinated goats and their unpasteurized dairy products. Am. J. Trop. Med. Hyg. 47, 35–40.

Fournier, P.E., Etienne, J., Harle, J.R., 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, 1440–1447.

Fournier, P.E., Marrie, T.J., Raoult, D., 1998. Diagnosis of Q fever. J. Clin. Microbiol. 36, 1823–1834.

Gami, A.S., Antonios, V.S., Thompson, R.L., Chaliki, H.P., Ammash, N.M., 2004. Q fever endocarditis in the United States. Mayo Clin. Proc. 79, 253–257.

Gerth, H.J., Leidig, U., Riemenschneider, T., 1982. Q-fieber Epidemie in einem Institut fór Humanpathologie. Deut. Med. Wochenschr. 107, 1391–1395.

Ghigo, E., Capo, C., Tung, C.H., Raoult, D., Gorvel, J.P., Mege, J.L., 2002. *Coxiella burnetii* survival in THP-1 monocytes involves the impairment of phagosome maturation. IFN-gamma mediates its restoration and bacterial killing. J. Immunol. 169, 4488–4495.

Ghigo, E., Honstettre, A., Capo, C., Gorvel, J.P., Raoult, D., Mege, J.L., 2004. Link between impaired maturation of phagosomes and defective *Coxiella burnetii* killing in patients with chronic Q fever. J. Infect. Dis. 190, 1767–1772.

Gikas, A., Kofteridis, D.P., Manios, A., Pediaditis, J., Tselentis, Y., 2001. Newer macrolides as empiric treatment for acute Q fever infection. Antimicrob. Agents Chemother. 45, 3644–3646.

Gonder, J.C., Kishimoto, R.A., Kastello, M.D., Pedersen Jr., C.E., Larson, E.W., 1979. Cynomolgus monkey model for experimental Q fever infection. J. Infect. Dis. 139, 191–196.

Hackstadt, T., Williams, J.C., 1981. Biochemical stratagem for obligate parasitism of eukaryotic cells by *Coxiella burnetii*. Proc. Natl. Acad. Sci. U.S.A. 78, 3240–3244.

Harman, J.B., 1949. Q fever in Great Britain; clinical account of eight cases. Lancet 2, 1028–1030.

Hatchette, T., Hudson, R., Schlech, W., Campbell, N., Hatchette, J., Ratnam, S., Donovan, C., Marrie, T., 2000. Caprine-associated Q fever in Newfoundland. Can. Commun. Dis. Rep. 26, 17–19.

Hellenbrand, W., Breuer, T., Petersen, L., 2001. Changing epidemiology of Q fever in Germany, 1947–1999. Emerg. Infect. Dis. 7, 789–796.

Honstettre, A., Ghigo, E., Moynault, A., Capo, C., Toman, R., Akira, S., Takeuchi, O., Lepidi, H., Raoult, D., Mege, J.L., 2004. Lipopolysaccharide from *Coxiella burnetii* is involved in bacterial phagocytosis, filamentous actin reorganization, and inflammatory responses through Toll-like receptor 4. J. Immunol. 172, 3695–3703.

Hoover, T.A., Vodkin, M.H., Williams, J.C., 1992. A *Coxiella burnetii* repeated DNA element resembling a bacterial insertion sequence. J. Bacteriol. 174, 5540–5548.

Hotta, A., Kawamura, M., To, H., Andoh, M., Yamaguchi, T., Fukushi, H., Hirai, K., 2002. Phase variation analysis of *Coxiella burnetii* during serial passage in cell culture by use of monoclonal antibodies. Infect. Immun. 70, 4747–4749.

Houpikian, P., Habib, G., Mesana, T., Raoult, D., 2002. Changing clinical presentation of Q fever endocarditis. Clin. Infect. Dis. 34, E28–E31.

Huebner, R.J., Bell, J.A., 1951. Q fever studies in Southern California. Summary of current results and a discussion of possible control measures. J. Am. Med. Assoc. 145, 301–305.

Johnson III, J.E., Kadull, P.J., 1966. Laboratory acquired Q fever. A report of fifty cases. Am. J. Med. 41, 391–403.

Karakousis, P.C., Trucksis, M., Dumler, J.S., 2006. Chronic Q fever in the United States. J. Clin. Microbiol. 44, 2283–2287.

Kazar, J., 1996. Q fever. In: Kazar, J., Toman, R. (Eds.), Rickettsiae and Rickettsial Diseases. Slovak Academy of Sciences, Bratislava, pp. 353–362.

Kelly, P.J., Matthewman, L.A., Mason, P.R., Raoult, D., 1993. Q fever in Zimbabwe. S. Afr. Med. J. 83, 21–25.

Khalili, M., Sakhaee, E., 2009. An update on a serologic survey of Q Fever in domestic animals in Iran. Am. J. Trop. Med. Hyg. 80, 1031–1032.

Klee, S.R., Tyczka, J., Ellerbrok, H., Franz, T., Linke, S., Baljer, G., Appel, B., 2006. Highly sensitive real-time PCR for specific detection and quantification of *Coxiella burnetii*. BMC Microbiol. 6, 2.

Koster, F.T., Williams, J.C., Goodwin, J.S., 1985. Cellular immunity in Q fever. Specific lymphocyte unresponsiveness in Q fever endocarditis. J. Infect. Dis. 152, 1283–1289.

Kovacova, E., Kazar, J., 2002. Q fever—still a query and underestimated infectious disease. Acta Virol. 46, 193–210.

Kovacova, E., Kazar, J., Spanelova, D., 1998. Suitability of various *Coxiella burnetii* antigen preparations for detection of serum antibodies by various tests. Acta Virol. 42, 365–368.

Krumbiegel, E.R., Wisniewski, H.J., 1970. Q fever in Milwaukee. II. Consumption of infected raw milk by human volunteers. Arch. Environ. Health 21, 63–65.

Kruszewska, D., Tylewska-Wierzbanowska, S., 1997. Isolation of *Coxiella burnetii* from bull semen. Res. Vet. Sci. 62, 299–300.

Kruszewska, D., Tylewska-Wierzbanowska, S.T., 1993. *Coxiella burnetii* penetration into the reproductive system of male mice, promoting sexual transmission of infection. Infect. Immun. 61, 4188–4195.

La Scola, B., Lepidi, H., Raoult, D., 1997. Pathologic changes during acute Q fever. Influence of the route of infection and inoculum size in infected guinea pigs. Infect. Immun. 1, 2443–2447.

Landais, C., Fenollar, F., Thuny, F., Raoult, D., 2007. From acute Q fever to endocarditis. Serological follow-up strategy. Clin. Infect. Dis. 44, 1337–1340.

Lang, G.H., 1988. Serosurvey of *Coxiella burnetii* infection in dairy goat herds in Ontario. Can. J. Vet. Res. 52, 37–41.

Lang, G.H., 1990. Coxiellosis (Q fever) in animals. In: Marrie, T.J. (Ed.), Q Fever, the Disease. CRC Press, Boca Raton, pp. 23–48.

Leone, M., Honstettre, A., Lepidi, H., Capo, C., Bayard, F., Raoult, D., Mege, J.L., 2004. Effect of sex on *Coxiella burnetii* infection. Protective role of 17beta-estradiol. J. Infect. Dis. 189, 339–345.

Lepidi, H., Coulibaly, B., Casalta, J.P., Raoult, D., 2006. Autoimmunohistochemistry. A new method for the histologic diagnosis of infective endocarditis. J. Infect. Dis. 193, 1711–1717.

Lipton, J.H., Fong, T.C., Gill, M.J., Burgess, K., Elliott, P.D., 1987. Q fever inflammatory pseudotumor of the lung. Chest 92, 756–757.

Lohuis, P.J.F.M., Ligtenberg, P.C., Dieperslost, R.J.A., de Graaf, M., 1994. Q fever in a patient with a ventriculo-peritoneal drain. Case report and short review of the literature. Netherlands J. Med. 44, 60–64.

Mallavia, L.P., 1991. Genetics of Rickettsiae. Eur. J. Epidemiol. 7, 213–221.

Maltezou, H.C., Raoult, D., 2002. Q fever in children. Lancet Infect. Dis. 2, 686–691.

Mantovani, A., Benazzi, P., 1953. The isolation of *Coxiella burnetii* from *Rhipicephalus sanguineus* on naturally infected dogs. J. Am. Vet. Med. Assoc. 122, 117–118.

Marmion, B.P., Stoker, M.G.P., Walker, C.B.V., Carpenter, R.G., 1956. Q fever in Great Britain—epidemiological information from a serological survey of healthy adults in Kent ans East Anglia. J. Hyg. 54, 118–140.

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, 197–199.

Marrie, T.J., 1993. Q fever in pregnancy. Report of two cases. Infect. Dis. Clin. Pract. 2, 207–209.

Marrie, T.J., 2007. Epidemiology of Q fever. Rickettsial diseases. 281–289.

Marrie, T.J., Langille, D., Papukna, V., Yates, L., 1989. Truckin pneumonia—an outbreak of Q fever in a truck repair plant probably due to aerosols from clothing contaminated by contact with newborn kittens. Epidemiol. Infect. 102, 119–127.

Marrie, T.J., Raoult, D., 2002. Update on Q fever, including Q fever endocarditis. Curr. Clin. Top. Infect. Dis. 22, 97–124.

Marrie, T.J., Stein, A., Janigan, D., Raoult, D., 1996. Route of infection determines the clinical manifestations of acute Q fever. J. Infect. Dis. 173, 484–487.

Marrie, T., 1988. Liver involvement in acute Q fever. Chest 94, 896–898.

Maurin, M., Benoliel, A.M., Bongrand, P., Raoult, D., 1992. Phagolysosomal alkalinization an the bactericidal effect of antibiotics. The *Coxiella burnetii* paradigm. J. Infect. Dis. 166, 1097–1102.

Maurin, M., Raoult, D., 1993. In vitro susceptibilities of spotted fever group rickettsiae and *Coxiella burnetii* to clarithromycin. Antimicrob. Agents Chemother. 37, 2633–2637.

Maurin, M., Raoult, D., 1999. Q fever. Clin. Microbiol. Rev. 12, 518–553.

McDade, J.E., 1990. Historical aspects of Q fever. In: Marrie, T.J. (Ed.), Q fever. Volume I. The Disease. CRC Press, Boston, pp. 5–21.

McQuiston, J.H., Childs, J.E., 2002. Q fever in humans and animals in the United States. Vector Borne Zoonotic Dis. 2, 179–191.

Meconi, S., Capo, C., Remacle-Bonnet, M., Pommier, G., Raoult, D., Mege, J.L., 2001. Activation of protein tyrosine kinases by *Coxiella burnetii*. Role in actin cytoskeleton reorganization and bacterial phagocytosis. Infect. Immun. 69, 2520–2526.

Meconi, S., Jacomo, V., Boquet, P., Raoult, D., Mege, J.L., Capo, C., 1998. *Coxiella burnetii* induces reorganization of the actin cytoskeleton in human monocytes. Infect. Immun. 66, 5527–5533.

Mege, J.L., 2007. Immune Response to Q fever. Rickettsial Diseases. 271–281.

Mertens, K., Samuel, J.E., 2007. Bacteriology of Coxiella: Rickettsial diseases. 257–270.
Milazzo, A., Hall, R., Storm, P.A., Harris, R.J., Winslow, W., Marmion, B.P., 2001. Sexually transmitted Q fever. Clin. Infect. Dis. 33, 399–402.
Musso, D., Drancourt, M., Osscini, S., Raoult, D., 1996. Sequence of the quinolone resistance-determining region of the GYRA gene for clinical isolates and for a stepwise-selected quinolone resistant of *Coxiella burnetii*. Antimicrob. Agents Chemother. 40, 870–873.
Nourse, C., Allworth, A., Jones, A., Horvath, R., McCormack, J., Bartlett, J., Hayes, D., Robson, J.M., 2004. Three cases of Q fever osteomyelitis in children and a review of the literature. Clin. Infect. Dis. 39, e61–e66.
Philip, C.B., 1948. Comments on the name of the Q fever organism. Public Health Rep. 63, 58–59.
Psaroulaki, A., Hadjichristodoulou, C., Loukaides, F., Soteriades, E., Konstantinidis, A., Papastergiou, P., Ioannidou, M.C., Tselentis, Y., 2006. Epidemiological study of Q fever in humans, ruminant animals, and ticks in Cyprus using a geographical information system. Eur. J. Clin. Microbiol. Infect. Dis. 25, 576–586.
Raoult, D., 1993. Treatment of Q fever. Antimicrob. Agents Chemother. 37, 1733–1736.
Raoult, D., Drancourt, M., Vestris, G., 1990a. Bactericidal effect of Doxycycline associated with lysosomotropic agents on *Coxiella burnetii* in P388D1 cells. Antimicrob. Agents Chemother. 34, 1512–1514.
Raoult, D., Etienne, J., Massip, P., Iaocono, S., Prince, M.A., Beaurain, P., Benichou, S., Auvergnat, J.C., Mathieu, P., Bachet, P., 1987. Q fever endocarditis in the south of France. J. Infect. Dis. 155, 570–573.
Raoult, D., Fenollar, F., Stein, A., 2002. Q fever during pregnancy. Diagnosis, treatment, and follow-up. Arch. Intern. Med. 162, 701–704.
Raoult, D., Houpikian, P., Tissot Dupont, H., Riss, J.M., 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, 167–173.
Raoult, D., Laurent, J.C., Mutillod, M., 1994. Monoclonal antibodies to *Coxiella burnetii* for antigenic detection in cell cultures and in paraffin embedded tissues. Am. J. Clin. Pathol. 101, 318–320.
Raoult, D., Levy, P.Y., Harle, J.R., Etienne, J., Massip, P., Goldstein, F., Micoud, M., Beytout, J., Gallais, H., Remy, G., Capron, J.P., 1990b. Chronic Q fever. Diagnosis and follow up. Ann. N.Y. Acad. Sci. 590, 51–60.
Raoult, D., Marrie, T., Mege, J., 2005. Natural history and pathophysiology of Q fever. Lancet Infect. Dis. 5, 219–226.
Raoult, D., Tissot-Dupont, H., Foucault, C., Gouvernet, J., Fournier, P.E., Bernit, E., Stein, A., Nesri, M., Harle, J.R., Weiller, P.J., 2000. Q fever 1985–1998—clinical and epidemiologic features of 1383 infections. Medicine 79, 109–123.
Raoult, D., Torres, H., Drancourt, M., 1991. Shell-vial assay. Evaluation of a new technique for determining antibiotic susceptibility, tested in 13 isolates of *Coxiella burnetii*. Antimicrob. Agents Chemother. 35, 2070–2077.
Rolain, J.M., 2007. Antimicrobial susceptibility of Rickettsial agents: Rickettsial diseases. 361–369.
Rolain, J.M., Boulos, A., Mallet, M.N., Raoult, D., 2005a. 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, 2673–2676.
Rolain, J.M., Lambert, F., Raoult, D., 2005b. Activity of telithromycin against thirteen new isolates of *C. burnetii* including three resistant to doxycycline. Ann. N.Y. Acad. Sci. 1063, 252–256.
Rolain, J.M., Mallet, M.N., Raoult, D., 2003. Correlation between serum levels of doxycycline and serology evolution in patients treated for *Coxiella burnetii* endocarditis. J. Infect. Dis. 9, 1322–1325.
Roman, M.J., Coriz, P.D., Baca, O.G., 1986. A proposed model to explain persistent infection of host cells with *Coxiella burnetii*. J. Gen. Microbiol. 132, 1415–1422.
Sabatier, F., Dignat-George, F., Mege, J.L., Brunet, C., Raoult, D., Sampol, J., 1997. $CD4^+$ T-cell lymphopenia in Q fever endocarditis. Clin. Diag. Lab. Immunol. 4, 89–92.
Sanford, E.S., Josephson, G.K.A., MacDonald, A., 1994. *Coxiella burnetii* (Q fever) abortion storms in goat herds after attendance at an annual fair. Can. Vet. J. 35, 376–378.
Seshadri, R., Paulsen, I.T., Eisen, J.A., Read, T.D., Nelson, K.E., Nelson, W.C., Ward, N.L., Tettelin, H., Davidsen, T.M., Beanan, M.J., Deboy, R.T., Daugherty, S.C., Brinkac, L.M., Madupu, R., Dodson, R.J., Khouri, H.M., Lee, K.H., Carty, H.A., Scanlan, D., Heinzen, R.A., Thompson, H.A., Samuel, J.E., Fraser, C.M., Heidelberg, J.F., 2003. Complete genome sequence of the Q-fever pathogen *Coxiella burnetii*. Proc. Natl. Acad. Sci. U.S.A. 100, 5455–5460.
Shannon, J.G., Howe, D., Heinzen, R.A., 2005. Virulent *Coxiella burnetii* does not activate human dendritic cells. Role of lipopolysaccharide as a shielding molecule. Proc. Natl. Acad. Sci. U.S.A. 102, 8722–8727.
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, 41–49.
Stein, A., Raoult, D., 1999. Pigeon pneumonia in Provence. A bird borne Q fever outbreak. Clin. Infect. Dis. 29, 617–620.
Stoker, M.G., Marmion, B.P., 1995. The spread of Q fever from animals to man. The natural history of a rickettsial disease. Bull WHO781-806.
Tiggert, W.D., Benenson, A.S., 1956. Studies on Q fever in man. Trans. Assoc. Am. Phys. 69, 98–104.
Tissot-Dupont, H., Raoult, D., 2007. Clinical aspects, diagnosis and treatment of Q fever: Rickettsial diseases. 291–301.
Tissot-Dupont, H., Raoult, D., Brouqui, P., Janbon, F., Peyramond, D., Weiller, P.J., Chicheportiche, 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, 427–434.
Torres, H., Raoult, D., 1993. In vitro activities of ceftriaxone and fusidic acid against 13 isolates of *Coxiella burnetii*. Determined using the shell Vial Assay. Antimicrob. Agents Chemother. 37, 491–494.
Vodkin, M.H., Williams, J.C., 1986. Overlapping deletion in two spontaneous phase variants of *Coxiella burnetii*. J. Gen. Microbiol. 132, 2587–2594.
Webster, J.P., LLoyd, G., Macdonald, D.W., 1995. Q fever (*Coxiella burnetii*) reservoir in wild brown rat (*Rattus norvegicus*) populations in the UK Wildlife conservation Research Unit, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS - centre for Applied Microbiology and research, Division of Pathology, Porton Down, Salisbury, Wiltshire SP4 OJG. Parasitology 110, 31–35.
Yeaman, M.R., Baca, O.G., 1990. Unexpected antibiotic susceptibility of a chronic isolate of *Coxiella burnetii*. Ann. N.Y. Acad. Sci. 590, 297–305.
Yeaman, M.R., Mitscher, L.A., Baca, O.G., 1987. In vitro susceptibility of *Coxiella burnetii* to antibiotics, including several quinolones. Antimicrob. Agents Chemother. 31, 1079–1084.
Yeaman, M.R., Roman, M.J., Baca, O.G., 1989. Antibiotic susceptibilities of two *Coxiella burnetii* isolates implicate in distinct clinical syndromes. Antimicrob. Agents Chemother. 33, 1052–1057.
Zamboni, D.S., Campos, M.A., Torrecilhas, A.C., Kiss, K., Samuel, J.E., Golenbock, D.T., Lauw, F.N., Roy, C.R., Almeida, I.C., Gazzinelli, R.T., 2004. Stimulation of toll-like receptor 2 by *Coxiella burnetii* is required for macrophage production of pro-inflammatory cytokines and resistance to infection. J. Biol. Chem. 279, 54405–54415.