



Q fever

Emmanouil Angelakis, Didier Raoult

► To cite this version:

Emmanouil Angelakis, Didier Raoult. Q fever. Veterinary Microbiology, 2010, 140 (3-4), pp.297. 10.1016/j.vetmic.2009.07.016 . hal-00556051

HAL Id: hal-00556051

<https://hal.science/hal-00556051>

Submitted on 15 Jan 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Q fever

Authors: Emmanouil Angelakis, Didier Raoult

PII: S0378-1135(09)00338-1
DOI: doi:10.1016/j.vetmic.2009.07.016
Reference: VETMIC 4508

To appear in: *VETMIC*

Received date: 28-11-2008
Revised date: 3-7-2009
Accepted date: 30-7-2009



Please cite this article as: Angelakis, E., Raoult, D., Q fever, *Veterinary Microbiology* (2008), doi:10.1016/j.vetmic.2009.07.016

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **Q fever**

2

3 Emmanouil Angelakis and Didier Raoult*

4

5 ¹Unité des Rickettsies, CNRS UMR 6020, IFR 48, Faculté de Médecine, Université
6 de la Méditerranée, 27 Bd Jean Moulin, 13385 Marseille Cedex 05, France

7

8 Corresponding author.

9 Mailing address: Unité des Rickettsies, CNRS UMR 6020, IFR 48, Faculté de
10 Médecine, Université de la Méditerranée, 27 Bd Jean Moulin, 13385 Marseille Cedex
11 05, France

12

13 Phone: (33) 491 38 55 17

14 Fax: (33) 491 83 03 90

15 Email: didier.raoult@gmail.com

16 Abstract =222

17 Text word count = 7312

18 Tables: 1

19 Figures: 2

20

21

22

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.

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 (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 (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

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 to 45 hours (Mertens K. 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 phylogenetic neighbours include *Legionellae* spp, *Francisella tularensis*, and *Rickettsiella* spp. (Raoult *et al.*, 2005).

C. burnetii possesses a small circular chromosome of approximately 5Mbp. Most isolates harbor additionally one of four previously described plasmids of 32 to 51kb in size, which carry about 2% of the genome information. Strains without a resident

plasmid carry instead a 16kb 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 transposons and 20 IS elements are also identified, with 21 copies of a unique IS110-related isotype IS1111, five IS30 and three ISAs1 family elements (Seshadri *et al.*, 2003).

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).

Routes of transmission to humans

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, III and Kadull, 1966).

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).

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

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.

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-Wierzbanska, 1993) and viable *C. burnetii* has been found in bull semen (Kruszewska and Tylewska-Wierzbanska, 1997).

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)

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.

171 Animals which live in close contact can become infected with *C. burnetii*.
 172 Sanford *et al.* described abortions that occurred in goat herds that were exposed to
 173 three goats from another herd that kidded prematurely during a fair (Sanford *et al.*,
 174 1994). Twenty one days after exposure abortions began and affected 20 to 46% of the
 175 pregnant animals in each herd. Moreover, when cows were imported into an area of
 176 endemic infection, 40% of uninfected cows became *C. burnetii* infected within 6
 177 months (Huebner and Bell, 1951).

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

184 **Pathogenicity**

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

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).

Acute Q fever

In an epidemiological survey that took place in Marseille between 1985 and 1998, Q fever diagnosis was 1,070 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 to 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

295 seroconversion, and only 4% of the symptomatic patients will be admitted to
 296 hospitals. A chronic disease will develop in at-risk patients.

297 **Prolonged fever.** Prolonged fever is usually accompanied by severe headaches. The
 298 fever may reach from 39 to 40°C, usually remaining elevated all day. Fever typically
 299 increases to a plateau within 2 to 4 days, and then after 5 to 14 days the temperature
 300 returns rapidly to normal. However, in untreated patients, fever may last from 5 to 57
 301 days (Derrick, 1973). The duration of fever is longer in elderly patients (Derrick,
 302 1973).

303 **Pneumonia.** Atypical pneumonia is one of the most commonly recognized forms of
 304 acute Q fever. Most cases are clinically asymptomatic or mild, characterized by a
 305 nonproductive cough, fever, and minimal auscultatory abnormalities, but some
 306 patients present with acute respiratory distress (Raoult *et al.*, 1990b). Pleural effusion
 307 can also be present. Findings on the chest radiograph are nonspecific. The duration of
 308 symptoms varies from 10 to 90 days. The mortality rate ranges from 0.5 to 1.5%,
 309 depending upon the series (Tissot-Dupont *et al.*, 1992). Pneumonia is the major
 310 manifestation of acute Q fever in Nova Scotia, Canada, in the Basque country in
 311 Spain, and in Switzerland, while in France, Ontario, California, and Australia,
 312 hepatitis is the predominant form of acute Q fever (Fournier *et al.*, 1998).

313 **Hepatitis.** Three major forms of hepatitis may be encountered: an infectious hepatitis-
 314 like form of hepatitis with hepatomegaly but seldom with jaundice, clinically
 315 asymptomatic hepatitis, and prolonged fever of unknown origin with characteristic
 316 granulomas on liver biopsy. Hepatitis is the most common presentation worldwide,
 317 particularly in France and Australia. Alkaline phosphatase, AST, and ALT levels are
 318 usually mildly elevated to two to three times the normal level (Marrie, 1988). Q fever
 319 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).

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).

Skin rash. Skin lesions have been found in 5 to 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).

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).

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). Figure 1 shows the natural history of Q fever in the absence of treatment.

Endocarditis. The most frequent and studied preservation of chronic Q fever is endocarditis (Figure 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 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).

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.

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).

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 metritis 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.

Diagnosis

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).

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 x 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% CO₂ for 5 to 7 days. *C. burnetii* is usually observed by microscopic examination of cell monolayers after Gimenez or immunofluorescence staining

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).

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 LightCycler, SYBR Green or TaqMan chemistry (Klee *et al.*, 2006). The LightCycler Nested PCR (LCN-PCR), a rapid nested-PCR assay that uses serum as a specimen and the LightCycler 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.

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 to 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).

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).

Antimicrobial Susceptibility Testing

493 Antibiotic susceptibility testing of *C. burnetii* is difficult because this
 494 organism is an obligate intracellular bacterium. Three models of infection have been
 495 developed: animals, chick embryos and cell culture. The current method used to test
 496 the antibiotic susceptibility of *C. burnetii* is based on cell culture models (Rolain,
 497 2007).

498 A number of cell lines have been used to test antibiotic activity against
 499 intracellular *C. burnetii*, including murine macrophage-like cell lines (P388D1 and
 500 J774) and a murine fibroblast cell line (L929) (Akporiaye *et al.*, 1983; Baca *et al.*,
 501 1981; Baca *et al.*, 1985; Burton *et al.*, 1978; Roman *et al.*, 1986). Yeaman *et al.*
 502 described an acute *C. burnetii* infection model in acutely infected L929 cells (Yeaman
 503 *et al.*, 1989). Bacteriostatic activity was demonstrated against *C. burnetii* Nine Mile
 504 and Priscilla isolates with doxycycline (10 mg/ml), rifampin (1 mg/ml), and ofloxacin
 505 (5 mg/ml). Moreover, they used chronically infected L929 cells to test the bactericidal
 506 activity of antibiotics against *C. burnetii* (Yeaman *et al.*, 1987). They found that the
 507 percentage of infected cells was not reduced by tetracycline, erythromycin, or
 508 sulfamethoxazole at concentrations up to 10 mg/ml and was only slightly reduced by
 509 chloramphenicol, doxycycline, and trimethoprim, suggesting that these drugs were not
 510 bactericidal. In contrast, the quinolone compounds and rifampin reduced the
 511 percentage of infected cells from 100% to 2, 2, 7, and 4%, respectively, after 10 days
 512 of continuous culture treatment. Torres and Raoult have developed a Shell-Vial assay
 513 with human embryonic lung cells (HEL) for assessment of the bacteriostatic effect of
 514 antibiotics (Torres and Raoult, 1993). By this technique, amikacin and amoxicillin
 515 were not effective against *C. burnetii*, ceftriaxone and fucidic acid were inconsistently
 516 active (Torres and Raoult, 1993), whereas cotrimoxazole, rifampin, doxycycline,
 517 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 to 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).

Treatment

The guideline recommendations for the treatment of Q fever are summarised in Table 2.

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).

Acute Q fever in children. In children younger than 8 years, co-trimoxazole 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. burnetii* (Maltezou and Raoult, 2002).

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)

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 one 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: $8 \mu\text{g/mL}$) from patients with Q fever endocarditis (Rolain *et al.*, 2005a; Rolain *et al.*, 2005b)

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 (Landaïs *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

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.

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 1,346 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 hyper-endemic 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 than 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 immunosuppressed) 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.

Reference List

- Akporiaye ET, Rowatt JD, Aragon AA, Baca OG: Lysosomal response of a murine macrophage-like cell line persistently infected with *Coxiella burnetii*. Infect immun 40:1155-1162 (1983).
- Amano K, Williams JC, Missler SR, Reinhold VN: Structure and biological relationships of *Coxiella burnetii* lipopolysaccharides. J Biol Chem 262:4740-4747 (1987).
- Arricau-Bouvery N, Rodolakis A: Is Q fever an emerging or re-emerging zoonosis? Vet Res 36:327-349 (2005).
- Babudieri B: Q fever: A zoonosis. Adv Vet Sci 5:82-182 (1959).
- Baca OG, Akporiaye ET, Aragon AS, Martinez IL, Robles MV, Warner NL: Fate of phase I and phase II *Coxiella burnetii* in several macrophage-like tumor cell lines. Infect immun 33:258-266 (1981).
- Baca OG, Scott TO, Akporiaye ET, DeBlassie R, Crissman HA: Cell cycle distribution patterns and generation times of L929 fibroblast cells persistently infected with *Coxiella burnetii*. Infect immun 47:366-369 (1985).

- 688 Banerjee-Bhatnagar N, Bolt CR, Williams JC: Pore-forming activity of *Coxiella*
 689 *burnetii* outer membrane protein oligomer comprised of 29.5- and 31-kDa
 690 polypeptides. Inhibition of porin activity by monoclonal antibodies 4E8 and
 691 4D6. Ann N Y Acad Sci 791:378-401 (1996).
- 692 Benson WW, Brock DW, Mather J: Serologic analysis of a penitentiary group using
 693 raw milk from a Q fever infected herd. Public Health Rep 78:707-710 (1963).
- 694 Bernit E, Pouget J, Janbon F, Dutronc H, Martinez P, Brouqui P, Raoult D:
 695 Neurological involvement in acute Q fever: a report of 29 cases and review of
 696 the literature. Arch Intern Med 162:693-700 (2002).
- 697 Berri M, Rousset E, Champion JL, Russo P, Rodolakis A: Goats may experience
 698 reproductive failures and shed *Coxiella burnetii* at two successive parturitions
 699 after a Q fever infection. Res Vet Sci 83:47-52 (2007).
- 700 Berri M, rricau-Bouvery N, Rodolakis A: PCR-based detection of *Coxiella burnetii*
 701 from clinical samples. Methods Mol Biol 216:153-161 (2003).
- 702 Botelho-Nevers E, Fournier PE, Richet H, Fenollar F, Lepidi H, Foucault C,
 703 Branchereau A, Piquet P, Maurin M, Raoult D: *Coxiella burnetii* infection of
 704 aortic aneurysms or vascular grafts: report of 30 new cases and evaluation of
 705 outcome. Eur J Clin Microbiol Infect Dis 26:635-640 (2007).

- 706 Boulos A, Rolain JM, Maurin M, Raoult D: Evaluation of Antibiotic Susceptibilities
707 against *Coxiella burnetii* by real time PCR. Int J Antimicrob Agents 23:169-174
708 (2004).
- 709 Brennan RE, Samuel JE: Evaluation of *Coxiella burnetii* Antibiotic Susceptibilities by
710 Real- Time PCR Assay. J Clin Microbiol 41:1869-1874 (2003).
- 711 Brezina R, Schramek S, Kazar J: Selection of chlortetracycline-resistant strain of
712 *Coxiella burnetii*. Acta Virol 19:496 (1975).
- 713 Brouqui P, Raoult D: New insight into the diagnosis of fastidious bacterial
714 endocarditis. FEMS Immunol Med Microbiol 47:1-13 (2006).
- 715 Burnet FM, Freeman M: Experimental studies on the virus of "Q" fever. Med J
716 Australia 2:299-305 (1937).
- 717 Burton PR, Stueckemann J, Welsh RM, Paretsky D: Some ultrastructural effects of
718 persistent infections by the rickettsia *Coxiella burnetii* in mouse L cells and
719 green monkey kidney (Vero) cells. Infect immun 21:556-566 (1978).
- 720 Capo C, Lindberg FP, Meconi S, Zaffran Y, Tardei G, Brown EJ, Raoult D, Mege JL:
721 Subversion of monocyte functions by *Coxiella burnetii*: impairment of the cross-
722 talk between $\alpha_v\beta_3$ integrin and CR3. J Immunol 163:6078-6085 (1999).

- 723 Capo C, Moynault A, Collette Y, Olive D, Brown EJ, Raoult D, Mege JL: *Coxiella*
 724 *burnetii* avoids macrophage phagocytosis by interfering with spatial distribution
 725 of complement receptor 3. J Immunol 170:4217-4225 (2003).
- 726 Carcopino X, Raoult D, Bretelle F, Boubli L, Stein A: Managing Q fever during
 727 pregnancy: the benefits of long-term cotrimoxazole therapy. Clin Infect Dis
 728 45:548-555 (2007).
- 729 Chiu CK, Durrheim DN: A review of the efficacy of human Q fever vaccine
 730 registered in Australia. N S W Public Health Bull 18:133-136 (2007).
- 731 Derrick EH: "Q" fever, a new fever entity: clinical features, diagnosis and laboratory
 732 investigation. Med J Australia 2:281-299 (1937).
- 733 Derrick EH: The course of infection with *Coxiella burnetii*. Med J Australia 1:1051-
 734 1057 (1973).
- 735 Deutch DL, Peterson ET: Q fever : transmission from one human being to others. J
 736 Amer Med Assoc 143:348-354 (1950).
- 737 Dupuis G, Vouilloz M, Peter O, Mottiez MC: [Incidence of Q fever in Valais]. Rev
 738 Med Suisse Romande 105:949-954 (1985).
- 739 Editorial: Experimental Q fever in man. Br Med J 1:1000 (1950).

- 740 Fenollar F, Fournier PE, Carrieri MP, Habib G, Messana T, Raoult D: Risks factors
741 and prevention of Q fever endocarditis. Clin Infect Dis 33:312-316 (2001).
- 742 Fenollar F, Fournier PE, Raoult D: Molecular detection of *Coxiella burnetii* in the
743 sera of patients with Q fever endocarditis or vascular infection. J Clin Microbiol
744 42:4919-4924 (2004).
- 745 Fenollar F, Raoult D: Molecular genetic methods for the diagnosis of fastidious
746 microorganisms. APMIS 112:785-807 (2004).
- 747 Fenollar F, Raoult D: Molecular diagnosis of bloodstream infections caused by non-
748 cultivable bacteria. Int J Antimicrob Agents 30 Suppl 1:S7-15 (2007).
- 749 Fishbein DB, Raoult D: A cluster of *Coxiella burnetii* infections associated with
750 exposure to vaccinated goats and their unpasteurized dairy products. Am J Trop
751 Med Hyg 47:35-40 (1992).
- 752 Fournier PE, Etienne J, Harle JR, Habib G, Raoult D: Myocarditis, a rare but severe
753 manifestation of Q fever: report of 8 cases and review of the literature. Clin
754 Infect Dis 32:1440-1447 (2001).
- 755 Fournier PE, Marrie TJ, Raoult D: Diagnosis of Q fever. J Clin Microbiol 36:1823-
756 1834 (1998).
- 757 Gami AS, Antonios VS, Thompson RL, Chaliki HP, Ammash NM: Q fever
758 endocarditis in the United States. Mayo Clin Proc 79:253-257 (2004).

- 759 Gerth HJ, Leidig U, Riemenschneider T: Q-fieber Epidemie in einem Institut für
760 Humanpathologie. Deut Med Wochenschr 107:1391-1395 (1982).
- 761 Ghigo E, Capo C, Tung CH, Raoult D, Gorvel JP, Mege JL: *Coxiella burnetii* survival
762 in THP-1 monocytes involves the impairment of phagosome maturation: IFN-
763 gamma mediates its restoration and bacterial killing. J Immunol 169:4488-4495
764 (2002).
- 765 Ghigo E, Honstetter A, Capo C, Gorvel JP, Raoult D, Mege JL: Link between
766 impaired maturation of phagosomes and defective *Coxiella burnetii* killing in
767 patients with chronic Q fever. J Infect Dis 190:1767-1772 (2004).
- 768 Gikas A, Kofteridis DP, Manios A, Pediaditis J, Tselentis Y: Newer macrolides as
769 empiric treatment for acute Q fever infection. Antimicrob Agents Chemother
770 45:3644-3646 (2001).
- 771 Gonder JC, Kishimoto RA, Kastello MD, Pedersen CE, Jr., Larson EW: Cynomolgus
772 monkey model for experimental Q fever infection. J Infect Dis 139:191-196
773 (1979).
- 774 Hackstadt T, Williams JC: Biochemical stratagem for obligate parasitism of
775 eukaryotic cells by *Coxiella burnetii*. Proc Natl Acad Sci USA 78:3240-3244
776 (1981).
- 777 Harman JB: Q fever in Great Britain; clinical account of eight cases. Lancet 2:1028-
778 1030 (1949).

- 779 Hatchette T, Hudson R, Schlech W, Campbell N, Hatchette J, Ratnam S, Donovan C,
 780 Marrie T: Caprine-associated Q fever in Newfoundland. Can Comm Dis Rep
 781 26:17-19 (2000).
- 782 Hellenbrand W, Breuer T, Petersen L: Changing epidemiology of Q fever in
 783 Germany, 1947-1999. Emerg Infect Dis 7:789-796 (2001).
- 784 Honstettre A, Ghigo E, Moynault A, Capo C, Toman R, Akira S, Takeuchi O, Lepidi
 785 H, Raoult D, Mege JL: Lipopolysaccharide from *Coxiella burnetii* is involved in
 786 bacterial phagocytosis, filamentous actin reorganization, and inflammatory
 787 responses through Toll-like receptor 4. J Immunol 172:3695-3703 (2004).
- 788 Hoover TA, Vodkin MH, Williams JC: A *Coxiella burnetii* repeated DNA element
 789 resembling a bacterial insertion sequence. J Bacteriol 174:5540-5548 (1992).
- 790 Hotta A, Kawamura M, To H, Andoh M, Yamaguchi T, Fukushi H, Hirai K: Phase
 791 variation analysis of *Coxiella burnetii* during serial passage in cell culture by use
 792 of monoclonal antibodies. Infect immun 70:4747-4749 (2002).
- 793 Houplikian P, Habib G, Mesana T, Raoult D: Changing clinical presentation of Q
 794 fever endocarditis. Clin Infect Dis 34:E28-E31 (2002).
- 795 Huebner RJ, Bell JA: Q fever studies in Southern California. Summary of current
 796 results and a discussion of possible control measures. J Amer Med Assoc
 797 145:301-305 (1951).

- 798 Johnson JE, III, Kadull PJ: Laboratory acquired Q fever. A report of fifty cases. Amer
799 J Med 41:391-403 (1966).
- 800 Karakousis PC, Trucksis M, Dumler JS: Chronic Q fever in the United States. J Clin
801 Microbiol 44:2283-2287 (2006).
- 802 Kazar J: Q fever, Kazar J, Toman R (eds): Rickettsiae and Rickettsial Diseases, pp
803 353-362 (Slovak Academy of Sciences, Bratislava 1996).
- 804 Kelly PJ, Matthewman LA, Mason PR, Raoult D: Q fever in Zimbabwe. S Afr Med J
805 83:21-25 (1993).
- 806 Khalili M, Sakhaee E: An update on a serologic survey of Q Fever in domestic
807 animals in iran. Am J Trop Med Hyg 80:1031-1032 (2009).
- 808 Klee SR, Tyczka J, Ellerbrok H, Franz T, Linke S, Baljer G, Appel B: Highly
809 sensitive real-time PCR for specific detection and quantification of *Coxiella*
810 *burnetii*. BMC Microbiol 6:2 (2006).
- 811 Koster FT, Williams JC, Goodwin JS: Cellular immunity in Q fever: specific
812 lymphocyte unresponsiveness in Q fever endocarditis. J Infect Dis 152:1283-
813 1289 (1985).
- 814 Kovacova E, Kazar J: Q fever--still a query and underestimated infectious disease.
815 Acta Virol 46:193-210 (2002).

- 816 Kovacova E, Kazar J, Spanelova D: Suitability of various *Coxiella burnetii* antigen
 817 preparations for detection of serum antibodies by various tests. Acta Virol
 818 42:365-368 (1998).
- 819 Krumbiegel ER, Wisniewski HJ: Q fever in Milwaukee. II. Consumption of infected
 820 raw milk by human volunteers. Arch Environ Health 21:63-65 (1970).
- 821 Kruszevska D, Tylewska-Wierzbanowska S: Isolation of *Coxiella burnetii* from bull
 822 semen. Res Vet Sci 62:299-300 (1997).
- 823 Kruszevska D, Tylewska-Wierzbanowska ST: *Coxiella burnetii* penetration into the
 824 reproductive system of male mice, promoting sexual transmission of infection.
 825 Infect immun 61:4188-4195 (1993).
- 826 La Scola B, Lepidi H, Raoult D: Pathologic changes during acute Q fever: influence
 827 of the route of infection and inoculum size in infected guinea pigs. Infect
 828 immun 2443-2447 (1997).
- 829 Landais C, Fenollar F, Thuny F, Raoult D: From acute Q fever to endocarditis:
 830 serological follow-up strategy. Clin Infect Dis 44:1337-1340 (2007).
- 831 Lang GH: Serosurvey of *Coxiella burnetii* infection in dairy goat herds in Ontario.
 832 Can J Vet Res 52:37-41 (1988).
- 833 Lang GH: Coxiellosis (Q fever) in animals, Marrie TJ (ed): Q fever, the disease, pp
 834 23-48 (CRC press, Boca Raton 1990).

- 835 Leone M, Honstettre A, Lepidi H, Capo C, Bayard F, Raoult D, Mege JL: Effect of
 836 sex on *Coxiella burnetii* infection: protective role of 17beta-estradiol. J Infect
 837 Dis 189:339-345 (2004).
- 838 Lepidi H, Coulibaly B, Casalta JP, Raoult D: Autoimmunohistochemistry: a new
 839 method for the histologic diagnosis of infective endocarditis. J Infect Dis
 840 193:1711-1717 (2006).
- 841 Lipton JH, Fong TC, Gill MJ, Burgess K, Elliott PD: Q fever inflammatory
 842 pseudotumor of the lung. Chest 92:756-757 (1987).
- 843 Lohuis PJFM, Ligtenberg PC, Dieperslost RJA, de Graaf M: Q fever in a patient with
 844 a ventriculo-peritoneal drain.case report and short review of the literature.
 845 Netherlands J Med 44:60-64 (1994).
- 846 Mallavia LP: Genetics of Rickettsiae. Eur J Epidemiol 7:213-221 (1991).
- 847 Maltezou HC, Raoult D: Q fever in children. Lancet Infect Dis 2:686-691 (2002).
- 848 Mantovani A, Benazzi P: The isolation of *Coxiella burnetii* from *Rhipicephalus*
 849 *sanguineus* on naturally infected dogs. J Am Vet Med Assoc 122:117-118
 850 (1953).
- 851 Marmion BP, Stoker MGP, Walker CBV, Carpenter RG: Q fever in Great Britain -
 852 epidemiological information from a serological survey of healthy adults in Kent
 853 and East Anglia. J Hyg 54:118-140 (1956).

- 854 Marrero M, Raoult D: Centrifugation-shell vial technique for rapid detection of
855 Mediterranean spotted fever rickettsia in blood culture. Am J Trop Med Hyg
856 40:197-199 (1989).
- 857 Marrie TJ: Q fever in pregnancy: report of two cases. Infect Dis Clinical Practice
858 2:207-209 (1993).
- 859 Marrie TJ: Epidemiology of Q fever: Rickettsial Diseases, pp 281-289 (2007).
- 860 Marrie TJ, Langille D, Papukna V, Yates L: Truckin' pneumonia - an outbreak of Q
861 fever in a truck repair plant probably due to aerosols from clothing contaminated
862 by contact with newborn kittens
863 Epidemiol Infect 102:119-127 (1989).
- 864 Marrie TJ, Raoult D: Update on Q fever, including Q fever endocarditis. Curr Clin
865 Top Infect Dis 22:97-124.:97-124 (2002).
- 866 Marrie TJ, Stein A, Janigan D, Raoult D: Route of infection determines the clinical
867 manifestations of acute Q fever. J Infect Dis 173:484-487 (1996).
- 868 Marrie T: Liver involvement in acute Q fever. Chest 94:896-898 (1988).
- 869 Maurin M, Benoliel AM, Bongrand P, Raoult D: Phagolysosomal alkalinization an
870 the bactericidal effect of antibiotics : the *Coxiella burnetii* paradigm. J Infect Dis
871 166:1097-1102 (1992).

- 872 Maurin M, Raoult D: In vitro susceptibilities of spotted fever group rickettsiae and
873 *Coxiella burnetii* to clarithromycin. Antimicrob Agents Chemother 37:2633-
874 2637 (1993).
- 875 Maurin M, Raoult D: Q fever. Clin Microbiol Rev 12:518-553 (1999).
- 876 McDade JE: Historical aspects of Q fever, Marrie TJ (ed): Q fever. Volume I: The
877 Disease, pp 5-21 (CRC Press, Boston 1990).
- 878 McQuiston JH, Childs JE: Q fever in humans and animals in the United States. Vector
879 Borne Zoonotic Dis 2:179-191 (2002).
- 880 Meconi S, Capo C, Remacle-Bonnet M, Pommier G, Raoult D, Mege JL: Activation
881 of protein tyrosine kinases by *Coxiella burnetii*: role in actin cytoskeleton
882 reorganization and bacterial phagocytosis. Infect Immun 69:2520-2526 (2001).
- 883 Meconi S, Jacomo V, Boquet P, Raoult D, Mege JL, Capo C: *Coxiella burnetii*
884 induces reorganization of the actin cytoskeleton in human monocytes. Infect
885 immun 66:5527-5533 (1998).
- 886 Mege JL: Immune Response to Q fever: Rickettsial Diseases, pp 271-281 2007).
- 887 Mertens K., Samuel JE: Bacteriology of *Coxiella*: Rickettsial Diseases, pp 257-270
888 2007).

- 889 Milazzo A, Hall R, Storm PA, Harris RJ, Winslow W, Marmion BP: Sexually
890 transmitted Q fever. Clin Infect Dis 33:399-402 (2001).
- 891 Musso D, Drancourt M, Osscini S, Raoult D: Sequence of the quinolone resistance-
892 determining region of the GYRA gene for clinical isolates and for a stepwise-
893 selected quinolone resistant of *Coxiella burnetii*. Antimicrob Agents Chemother
894 40:870-873 (1996).
- 895 Nourse C, Allworth A, Jones A, Horvath R, McCormack J, Bartlett J, Hayes D,
896 Robson JM: Three cases of Q fever osteomyelitis in children and a review of the
897 literature. Clin Infect Dis 39:e61-e66 (2004).
- 898 Philip CB: Comments on the name of the Q fever organism. Public Health Rep 63:58-
899 59 (1948).
- 900 Psaroulaki A, Hadjichristodoulou C, Loukaides F, Soteriades E, Konstantinidis A,
901 Papastergiou P, Ioannidou MC, Tselentis Y: Epidemiological study of Q fever in
902 humans, ruminant animals, and ticks in Cyprus using a geographical information
903 system. Eur J Clin Microbiol Infect Dis 25:576-586 (2006).
- 904 Raoult D: Treatment of Q fever. Antimicrob Agents Chemother 37:1733-1736 (1993).
- 905 Raoult D, Drancourt M, Vestris G: Bactericidal effect of Doxycycline associated with
906 lysosomotropic agents on *Coxiella burnetii* in P388D1 cells. Antimicrob Agents
907 Chemother 34:1512-1514 (1990a).

- 908 Raoult D, Etienne J, Massip P, Iacono S, Prince MA, Beaurain P, Benichou S,
 909 Auvergnat JC, Mathieu P, Bachet P: Q fever endocarditis in the south of France.
 910 J Infect Dis 155:570-573 (1987).
- 911 Raoult D, Fenollar F, Stein A: Q fever during pregnancy: diagnosis, treatment, and
 912 follow-up. Arch Intern Med 162:701-704 (2002).
- 913 Raoult D, Houpihan P, Tissot Dupont H, RISS JM, Arditi-Djane J, Brouqui P:
 914 Treatment of Q fever endocarditis: comparison of two regimens containing
 915 doxycycline and ofloxacin or hydroxychloroquine. Arch Int Med 159:167-173
 916 (1999).
- 917 Raoult D, Laurent JC, Mutillod M: Monoclonal antibodies to *Coxiella burnetii* for
 918 antigenic detection in cell cultures and in paraffin embedded tissues. Am J Clin
 919 Pathol 101:318-320 (1994).
- 920 Raoult D, Levy PY, Harle JR, Etienne J, Massip P, Goldstein F, Micoud M, Beytout
 921 J, Gallais H, Remy G, Capron JP: Chronic Q fever: Diagnosis and follow up.
 922 Ann N Y Acad Sci 590:51-60 (1990b).
- 923 Raoult D, Marrie T, Mege J: Natural history and pathophysiology of Q fever. Lancet
 924 Infect Dis 5:219-226 (2005).
- 925 Raoult D, Tissot-Dupont H, Foucault C, Gouvernet J, Fournier PE, Bernit E, Stein A,
 926 Nesri M, Harle JR, Weiller PJ: Q fever 1985-1998 - Clinical and epidemiologic
 927 features of 1,383 infections. Medicine 79:109-123 (2000).

- 928 Raoult D, Torres H, Drancourt M: Shell-vial assay: Evaluation of a new technique for
 929 determining antibiotic susceptibility, tested in 13 isolates of *Coxiella burnetii*.
 930 Antimicrob Agents Chemother 35:2070-2077 (1991).
- 931 Rolain JM: Antimicrobial Susceptibility of Rickettsial Agents: Rickettsial Diseases,
 932 pp 361-369 2007).
- 933 Rolain JM, Boulos A, Mallet MN, Raoult D: Correlation between ratio of serum
 934 doxycycline concentration to MIC and rapid decline of antibody levels during
 935 treatment of Q fever endocarditis. Antimicrob Agents Chemother 49:2673-2676
 936 (2005a).
- 937 Rolain JM, Lambert F, Raoult D: Activity of telithromycin against thirteen new
 938 isolates of *C. burnetii* including three resistant to doxycycline. Ann N Y Acad
 939 Sci 1063:252-256 (2005b).
- 940 Rolain JM, Mallet MN, Raoult D: Correlation between serum levels of doxycycline
 941 and serology evolution in patients treated for *Coxiella burnetii* endocarditis. J
 942 Infect Dis 9:1322-1325 (2003).
- 943 Roman MJ, Coriz PD, Baca OG: A proposed model to explain persistent infection of
 944 host cells with *Coxiella burnetii*. J Gen Microbiol 132:1415-1422 (1986).
- 945 Sabatier F, Dignat-George F, Mege JL, Brunet C, Raoult D, Sampil J: CD4⁺ T-cell
 946 lymphopenia in Q fever endocarditis. Clin Diag Lab Immunol 4:89-92 (1997).

- 947 Sanford ES, Josephson GKA, MacDonald A: *Coxiella burnetii* (Q fever) abortion
 948 storms in goat herds after attendance at an annual fair. Can Vet J 35:376-378
 949 (1994).
- 950 Seshadri R, Paulsen IT, Eisen JA, Read TD, Nelson KE, Nelson WC, Ward NL,
 951 Tettelin H, Davidsen TM, Beanan MJ, Deboy RT, Daugherty SC, Brinkac LM,
 952 Madupu R, Dodson RJ, Khouri HM, Lee KH, Carty HA, Scanlan D, Heinzen
 953 RA, Thompson HA, Samuel JE, Fraser CM, Heidelberg JF: Complete genome
 954 sequence of the Q-fever pathogen *Coxiella burnetii*. Proc Natl Acad Sci U S A
 955 100:5455-5460 (2003).
- 956 Shannon JG, Howe D, Heinzen RA: Virulent *Coxiella burnetii* does not activate
 957 human dendritic cells: role of lipopolysaccharide as a shielding molecule. Proc
 958 Natl Acad Sci U S A 102:8722-8727 (2005).
- 959 Siegman-Igra Y, Kaufman O, Keysary A, Rzotkiewicz S, Shalit I: Q fever
 960 endocarditis in Israel and a worldwide review. Scand J Infect Dis 29:41-49
 961 (1997).
- 962 Stein A, Raoult D: Pigeon pneumonia in Provence. A bird borne Q fever outbreak.
 963 Clin Infect Dis 29:617-620 (1999).
- 964 Stoker MG, Marmion BP: The spread of Q fever from animals to man. The natural
 965 history of a rickettsial disease. Bull WHO 78:1-806 (1995).

- 966 Tiggert WD, Benenson AS: Studies on Q fever in man. Trans Assoc Am Phys 69:98-
967 104 (1956).
- 968 Tissot-Dupont H, Raoult D: Clinical Aspects, Diagnosis and Treatment of Q fever:
969 Rickettsial Diseases , pp 291-301 2007).
- 970 Tissot-Dupont H, Raoult D, Brouqui P, Janbon F, Peyramond D, Weiller PJ,
971 Chicheportiche C, Nezri M, Poirier R: Epidemiologic features and clinical
972 presentation of acute Q fever in hospitalized patients: 323 French cases. Am J
973 Med 93:427-434 (1992).
- 974 Torres H, Raoult D: In vitro activities of ceftriaxone and fusidic acid against 13
975 isolates of *Coxiella burnetii*, Determined Using the shell Vial Assay. Antimicrob
976 Agents Chemother 37:491-494 (1993).
- 977 Vodkin MH, Williams JC: Overlapping deletion in two spontaneous phase variants of
978 *Coxiella burnetii*. J Gen Microbiol 132:2587-2594 (1986).
- 979 Webster JP, LLOYD G, Macdonald DW: Q fever (*coxiella burnetii*) reservoir in wild
980 brown rat (*rattus norvegicus*) populations in the UK
981 Wildlife conservation Research Unit, Department of Zoology, University of
982 Oxford, South Parks Road, Oxford OX1 3PS - centre for Applied Microbiology
983 and research, Division of Pathology, Porton Down, Salisbury, Wiltshire SP4
984 OJG. Parasitology 110:31-35 (1995).

- 985 Yeaman MR, Baca OG: Unexpected antibiotic susceptibility of a chronic isolate of
986 *Coxiella burnetii*. Ann N Y Acad Sci 590:297-305 (1990).
- 987 Yeaman MR, Mitscher LA, Baca OG: In vitro susceptibility of *Coxiella burnetii* to
988 antibiotics, including several quinolones. Antimicrob Agents Chemother
989 31:1079-1084 (1987).
- 990 Yeaman MR, Roman MJ, Baca OG: Antibiotic susceptibilities of two *Coxiella*
991 *burnetii* isolates implicate in distinct clinical syndromes. Antimicrob Agents
992 Chemother 33:1052-1057 (1989).
- 993 Zamboni DS, Campos MA, Torrecilhas AC, Kiss K, Samuel JE, Golenbock DT,
994 Lauw FN, Roy CR, Almeida IC, Gazzinelli RT: Stimulation of toll-like receptor
995 2 by *Coxiella burnetii* is required for macrophage production of pro-
996 inflammatory cytokines and resistance to infection. J Biol Chem 279:54405-
997 54415 (2004).
- 998
999
1000

1001 Table 1. Guidelines for the treatment of Q fever

1002

1003 **Figure legends**

1004 Figure1. Q fever natural history in the absence of treatment

1005 Figure 2. Immunohistochemical detection of *C. burnetii* in a resected cardiac valve

1006 from a patient with a Q fever endocarditis, using a monoclonal antibody and

1007 hematoxylin counterstain. Note the intracellular location of the bacteria in the

1008 macrophage cytoplasm (original magnification x 400).

1009

Table 1

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 to 21 days	(Maurin and Raoult, 1999)
		rifampin (1,200 mg/day)	21 days	(Raoult, 1993)
	Pregnant	trimethoprim (320 mg) and sulfamethoxazole (1600 mg)	>5 weeks	(Carcopino <i>et al.</i> , 2007)
Chronic Q fever	Children	doxycycline (100 mg/day)	10-14 days	(Maurin and Raoult, 1999)
	Adults	doxycycline (100 mg/day) and hydroxychloroquine (600mg)	>18 months	(Carcopino <i>et al.</i> , 2007)

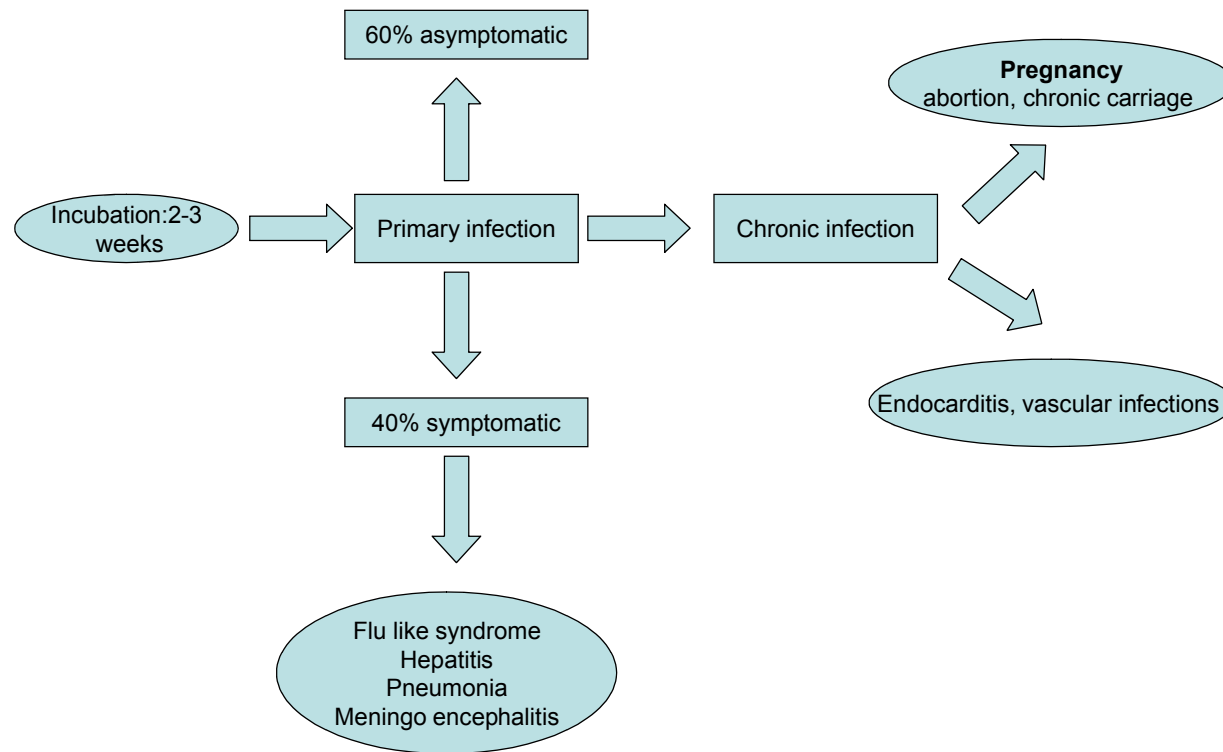
children

trimethoprim and sulfamethoxazole

>18 months

(Nourse *et al.*, 2004)

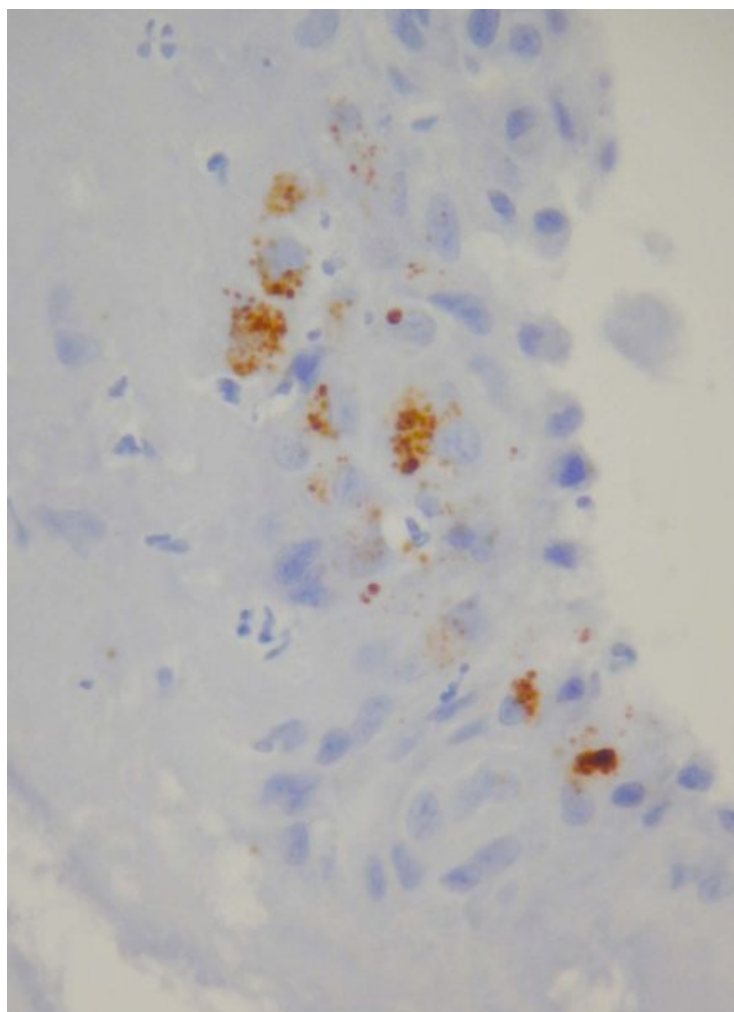
1 Figure1.



2

3 Figure2.

4



5

Accepted Manuscript