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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.

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 Gam-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 phylogenic 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 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).

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

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 to 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).

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 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 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 αvβ3 integrin only, whereas phase II attachment is mediated by both αvβ3 and complement receptor CR3 (Capo *et al.*, 1999). As the efficiency of CR3-mediated phagocytosis depends on CR3 activation via αvβ3 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. burnetti*, 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. burnetti* 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

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

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 to 4 days, and then after 5 to 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).

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).

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).

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 reponse 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.

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

443 **Immunodetection**. The detection of *C. burnetii* in tissues is especially informative in
444 patients who are undergoing treatment for chronic Q fever. Samples can be tested
445 fresh or after formalin fixation and paraffin embedding. Immunodetection is carried
446 out using immunoperoxidase techniques or immunofluorescence with polyclonal or
447 monoclonal antibodies (Maurin and Raoult, 1999). Only this last technique can be
448 used on paraffin-embedded samples (Raoult *et al.*, 1994). Recently, Lepidi *et al.*
449 proposed a new method named autoimmunohistochemistry for the detection of the *C.*
450 *burnetii* endocarditis (Lepidi *et al.*, 2006).

451 **Molecular biology**. During the last years, several PCR based diagnostic assays were
452 developed to detect *C. burnetii* DNA in cell cultures and in clinical samples. These
453 assays used conventional PCR, nested PCR or real-time PCR conditions with
454 LightCycler, SYBR Green or TaqMan chemistry (Klee *et al.*, 2006). The Light-
455 Cycler Nested PCR (LCN-PCR), a rapid nested-PCR assay that uses serum as a
456 specimen and the LightCycler as a thermal cycler, targeting a multicopy 20-copy
457 htpAB-associated element sequence has been adapted for the diagnosis of both acute
458 and chronic Q fever (Fenollar and Raoult, 2007). The LCN-PCR assay may be helpful
459 in establishing an early diagnosis of chronic Q fever (Fenollar *et al.*, 2004). Due to its
460 high sensitivity and specificity, the repetitive element, IS 11-11, is the best target gene
461 for the detection of *C. burnetii* in patients with active Q fever (Fenollar and Raoult,
462 2004). Recently, the complete sequences of the genome of *C. burnetii* became
463 available, allowing a large choice of DNA targets.

464 **Serology**. Since the clinical diagnosis is difficult, in most instances, the diagnosis of
465 Q fever relies upon serology. A variety of serological techniques are available, but the
466 indirect microimmunofluorescent antibody test has become the reference technique.
467 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

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;Baca *et al.*, 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 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

543 The guideline recommendations for the treatment of Q fever are summarised in Table
544 2.

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

554 **Acute Q fever in children**. In children younger than 8 years, co-trimoxazole has been
555 recommended, because of the adverse effects of tetracyclines and quinolones in this
556 age group. However, it has now been admitted that age is not a contraindication to
557 doxycycline, when the antibiotic is specific of the disease (Tissot-Dupont and Raoult,
558 2007). Moreover, in patients with prolonged fever, the addition of corticosteroids to
559 treatment might prove beneficial and interferon γ was successfully administered to a
560 3-year-old child with a prolonged fever unresponsive to appropriate treatment against
561 *C. burnetti* (Maltezou and Raoult, 2002).

562 **Acute Q fever during pregnancy.** Specific treatment using cotrimoxazole (800/160)
563 BID, until delivery, associated to folinic acid (25 mg OD) is recommended (Raoult *et*
564 *al.*, 2002). Recently, Carcopino *et al*. compared the incidence of obstetric and
565 maternal Q fever complications for women who received long-term cotrimoxazole
566 treatment with that for women who did not receive long-term cotrimoxazole treatment
567 (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:8µg/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 (Landais *et al.*, 2007). Those with phase I IgG antibody titers ≥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

618 average seroprevalence (41.6%) than sheep (16.5%) or cattle (3.4%) (McQuiston and
619 Childs, 2002). Q fever remains primarily an occupational hazard in persons in contact
620 with domestic animals such as cattle, sheep and, less frequently, goats. Persons at risk
621 from Q fever include farmers, veterinarians, abattoir workers, those in contact with
622 dairy products, and laboratory personnel performing *C. burnetii* culture and more
623 importantly working with *C. burnetii*-infected animals.

624 It is important to mention that during the last years the prevalence of chronic
625 Q fever in the USA has increased because of the Iraq war. Q fever is apparently
626 hyper-endemic in Iraq and many US soldiers serving in this area have been exposed to
627 *C. burnetii* and diagnosed as suffering by Q fever. As Q fever may reveal more then
628 10 years after primo infection (symptomatic or not and diagnosed or not), it is
629 possible that the Iraq war veterans will be an important reservoir of potential chronic
630 Q fever cases that will increase the real prevalence of the disease.

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

643 Three types of vaccine have been proposed for providing human protection
644 against Q fever: the attenuated live vaccine (produced and tested in Russia but
645 subsequently abandoned because of concern about its safety); chloroform– methanol
646 residue extracted vaccine or other extracted vaccines (tested in animals but not
647 humans); and the whole-cell formalin-inactivated vaccine (Q-Vax), which is
648 considered acceptably safe for humans (Chiu and Durrheim, 2007). The only
649 economic study undertaken in Australia on Q fever vaccine was performed before the
650 completion of the national vaccination program and assumed a Q-Vax efficacy of
651 98% (Chiu and Durrheim, 2007). Since Q fever in humans is often an occupational
652 hazard, vaccination should be considered primarily in exposed populations (Maurin
653 and Raoult, 1999). Moreover, vaccination should probably also be considered in
654 persons not professionally exposed but at risk for chronic Q fever, including patients
655 with cardiac valve defects, vascular aneurysms, or prostheses and
656 immunocompromised patients.

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

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667

668 **Conflict of Interest Statement**

669 None.

670
671 **Reference List**
672

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Table 1. Guidelines for the treatment of Q fever

Figure legends

Figure1. Q fever natural history in the absence of treatment

Figure 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 x 400).

Table1

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 *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 (600mg)	>18 months	(Carcopino *et al.*, 2007)

	children	trimethoprim and sulfamethoxazole	>18 months	(Nourse *et al.*, 2004)

1 Figure1.

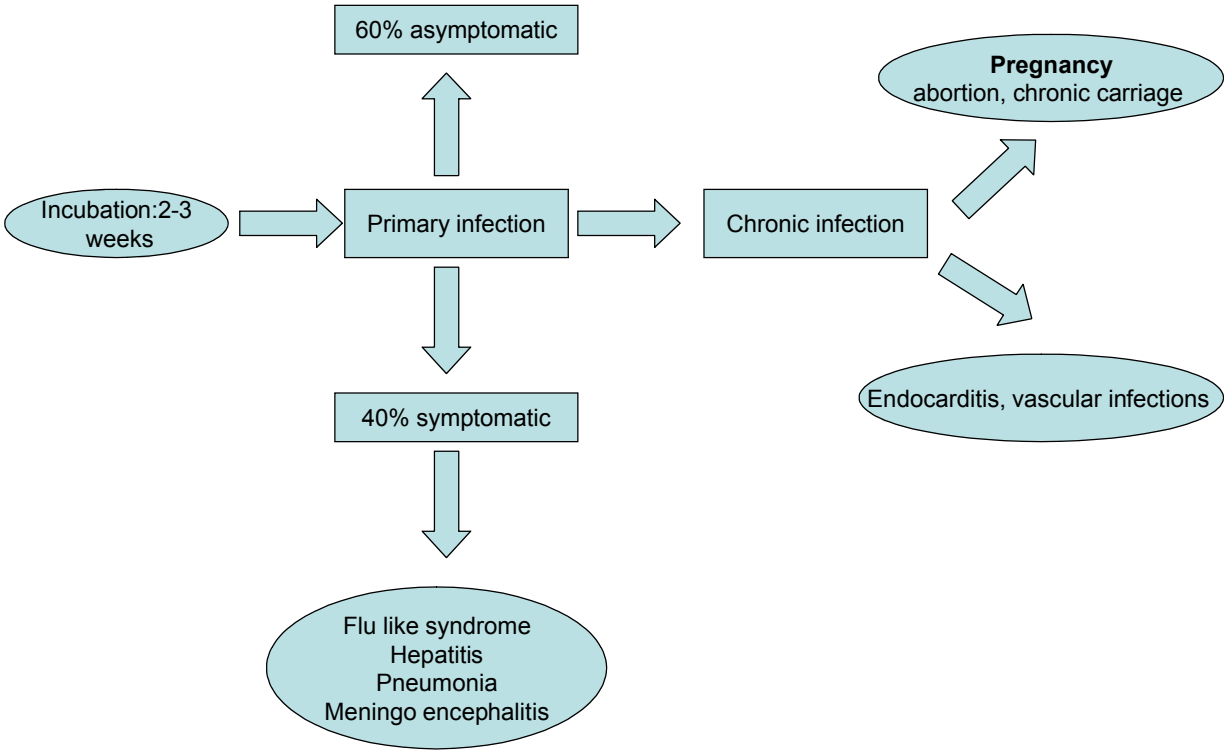

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Figure2.