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REVIEW

From Q Fever to *Coxiella burnetii* Infection: a Paradigm Change

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(continued)

SUMMARY *Coxiella burnetii* is the agent of Q fever, or "query fever," a zoonosis first described in Australia in 1937. Since this first description, knowledge about this pathogen and its associated infections has increased dramatically. We review here all the progress made over the last 20 years on this topic. *C. burnetii* is classically a strict intracellular, Gram-negative bacterium. However, a major step in the characterization of this pathogen was achieved by the establishment of its axenic culture. *C. burnetii* infects a wide range of animals, from arthropods to humans. The genetic determinants of virulence are now better known, thanks to the achievement of determining the genome sequences of several strains of this species and comparative genomic analyses. Q fever can be found worldwide, but the epidemiological features of this disease vary according to the geographic area considered, including situations where it is endemic or hyperendemic, and the occurrence of large epidemic outbreaks. In recent years, a major breakthrough in the understanding of the natural history of human infection with *C. burnetii* was the breaking of the old dichotomy between "acute" and "chronic" Q fever. The clinical presentation of *C. burnetii* infection depends on both the virulence of the infecting *C. burnetii* strain and specific risks factors in the infected patient. Moreover, no persistent infection can exist without a focus of infection. This paradigm change should allow better diagnosis and management of primary infection and long-term complications in patients with *C. burnetii* infection.

KEYWORDS *Coxiella burnetii*, diagnosis, Q fever, treatment, epidemiology, genomics

INTRODUCTION

Q fever has long been considered a rare and regionally restricted disease. In recent years, spectacular advances have been made in the knowledge of this disease and its causative agent, *Coxiella burnetii*. First, the worldwide role of *Coxiella burnetii* as a cause of endocarditis has been recognized in most countries performing systematic serology. Moreover, the classification of *C. burnetii* by the CDC as a potential bioterrorism agent resulted in the disease becoming reportable in many countries, such as in the United States, which revealed that the disease is more common than previously thought. Third, the recent war in the Middle East (1) and research in the tropics (2, 3) have shown that Q fever may be a very common cause of fever in the intertropical area. Finally, a very large outbreak in the Netherlands has shown that this disease could become a major public health problem (4).

Furthermore, knowledge about *Coxiella burnetii* has evolved, with the sequencing of multiple genomes of bacterial strains and their culture in axenic medium. This breakthrough enables genetic transformation and opens a new era. Moreover, redefining the clinical forms of Q fever is necessary, because of a lack of consensus on the distinction between acute Q fever and chronic Q fever (5). This redefinition, by more precisely qualifying the different clinical forms of the disease, will improve the exchange of medical and scientific knowledge about the disease throughout the world.

SEARCH STRATEGY

We searched in Medline and Google scholar for references with no language restriction and no restriction of publication status, using the key words "*Coxiella burnetii*" OR "Q fever" AND the other key words "diagnosis, treatment, epidemiology, human, animal, pathophysiology, chronic, acute." We applied no time restriction. This search yielded 12,887 references. We removed duplicates and assessed the remaining

references for eligibility. Finally, 687 references were included in the qualitative analysis. Data were collected and extracted from the selected studies and synthesized in the appropriate section.

MICROBIOLOGY

The Bacterium

C. burnetii has a cell wall similar to that of Gram-negative bacteria. However, this small coccobacillus (0.2 to 0.4 μm wide and 0.4 to 1 μm long) is not stainable with the Gram technique. The Gimenez method is used to stain *C. burnetii* isolated in culture or directly in clinical samples (6). The estimated doubling time of the bacterium is between 20 and 45 h in *in vitro* cell culture (7). It is an intracellular pathogen, replicating in eukaryotic cells. Its vacuole of replication progressively acquires phagolysosome-like characteristics, such as an acidic pH, acid hydrolysates, and cationic peptides (8). The bacterium actively participates in the genesis of this intracellular vacuole and has several strategies for adaptation to this exceptionally stressful environment. First, *C. burnetii* encodes an important number of basic proteins that are probably involved in the buffering of the acidic environment of the phagolysosome-like vacuole (9). Also, four sodium-proton exchangers and transporters for osmoprotectants are found in the *C. burnetii* genome, allowing this bacterium to confront osmotic and oxidative stresses.

Two forms of this microorganism can be observed, corresponding to a biphasic developmental cycle. The large-cell variant (LCV) of the bacterium is an exponentially replicating form, whereas the small-cell variant (SCV) is a stationary nonreplicating form (10). SCVs are small rods (0.2 to 0.5 μm long) characterized by condensed chromatin, a thick envelope, and an unusual internal membrane system. LCVs have a larger size ($>$0.5 μm), a dispersed chromatin, and an envelope similar to that of classical Gram-negative bacteria. SCVs are typical of the stationary phase. They are observed after prolonged culture (21 days) in Vero cells and in axenic acidified cysteine citrate medium 2 (ACCM2) (11). SCVs are stable in the environment and are highly resistant to osmotic, mechanical, chemical, heat, and desiccation stresses. These properties led to the adoption of a high temperature (71.7°C) for pasteurization in the 1950s (12). The transcriptome analysis of the SCV has revealed upregulated genes involved in the oxidative stress response, cell wall remodeling, and arginine acquisition (10). Also, SCVs show an unusually high number of cross-links in their peptidoglycan, which probably are involved in their exceptional environmental resistance (10). They can survive for 7 to 10 months on wool at ambient temperature, for more than 1 month on fresh meat, and for more than 40 months in milk (7). Although the SCVs are destroyed by 2% formaldehyde, they have been isolated from tissues stored in formaldehyde for 4 to 5 months (7). The high virulence of *C. burnetii*, the possibility of its aerosolization, and its environmental stability and have led the U.S. Centers for Disease Control and Prevention to classify this bacterium as a category B biological threat agent. A bioterrorism attack with this pathogen, although not associated with the high death rates observed for class A agents, could cause significant disability and possibly long-term consequences due to persistent infection in the population.

Antibiotic Susceptibility and the Role of pH

Methods for AST. For years, the antibiotic susceptibility testing (AST) of *C. burnetii* was difficult, owing to the obligate intracellular lifestyle of this bacterium. Antibiotic activity was evaluated first in animal models, then in embryonated-egg models and finally in cell culture systems. Inoculation in guinea pigs was used for susceptibility testing of streptomycin in early studies (13). The embryonated-egg method involved the ability of the tested antibiotic to prolong the survival time of the embryo in eggs infected with *C. burnetii*. This method was used in old studies to test the activities of streptomycin, chloramphenicol, oxytetracycline, and aureomycin against *C. burnetii* (14).

Cell culture systems were then implemented and remain the reference method for *C. burnetii* AST. In 1987, Yeaman et al. used *C. burnetii*-infected L929 fibroblast cells and

compared the percentage of infected cells (after Gimenez staining of bacteria) in antibiotic-treated cultures to that in drug-free controls (15). The more convenient shell vial assay was then developed, using specific antibodies and the immunofluorescence assay for detection of intracellular *C. burnetii* (16–18). In 2003, Brennan and Samuel developed a variant of the shell vial assay by using quantitative PCR (qPCR) for determination of *C. burnetii* intracellular counts (19). This method was found to be more repeatable and likely more sensitive than the indirect immunofluorescence assay (IFA) (19). It was then applied in another study from our team, which confirmed its excellent reproducibility for MIC determination (20). In recent years, both the IFA and qPCR tests targeting *com1* or *apoB* have been used for *C. burnetii* AST in various eukaryotic cell lines (21–24). We recently developed a new method using flow cytometry and specific immunofluorescent probes. This technique allows a very sensitive counting of *C. burnetii* cells because of specific morphological characteristics (25).

Main susceptibility features. Doxycycline is the most effective drug against *C. burnetii*, with MICs of $<$2 mg/liter in most reports (21, 23, 25). However, strains with acquired resistance to doxycycline have been described and represent a worrisome situation. The first resistant strain was isolated from a patient who died from *C. burnetii* endocarditis. The doxycycline MIC was 8 mg/liter, as determined using the shell vial assay and qPCR (26). In the same study, Rolain et al. found a correlation between the ratio of serum concentration to MIC for doxycycline and the rate of decline of anti-*C. burnetii* antibody titers in patients with *C. burnetii* endocarditis. For 16 *C. burnetii* strains isolated from cardiac valves removed from endocarditis patients, a ratio of serum concentration to MIC of $>$1 correlated with a rapid decline in specific antibody titers. A ratio between 0.5 and 1 was associated with a slower reduction in antibody titers. The only patient who died from endocarditis had a ratio of $<$0.5 (26). The whole genome of the *C. burnetii* strain infecting that patient (Cb109) was determined, but no specific sequence could be correlated with doxycycline resistance (27). Since then, two other isolates have been found to be resistant to doxycycline, including one goat isolate and another human isolate from a patient with acute Q fever (28).

In early studies, the fluoroquinolones were found to be one of the most effective agents in eliminating *C. burnetii* from L929 cells (15, 29). For that reason, in 1989 it was proposed to combine doxycycline with a fluoroquinolone to treat persistent forms of *C. burnetii* infection (30). Fluoroquinolones are also recommended for treatment of acute meningitis caused by *C. burnetii* because of the good cerebrospinal fluid penetration of these drugs (31). Pefloxacin- or ciprofloxacin-resistant strains of *C. burnetii* have been selected *in vitro* by Spyridaki et al. and Musso et al., with MICs up to 64 mg/liter (32, 33). These authors identified point mutations in the *gyrA* gene that could allow PCR-restriction fragment length polymorphism (PCR-RFLP) detection of these resistant strains (32). However, to date, clinical isolates of *C. burnetii* remain susceptible to levofloxacin, moxifloxacin, and to a lesser extent ciprofloxacin (12, 13, 16, 20).

Erythromycin was proposed as an empirical treatment for *C. burnetii* pneumonia. However, in 1991, Raoult et al. found that 6 of 13 clinical isolates of *C. burnetii* were resistant to this antibiotic (16), and such resistance was more recently observed in 6 isolates from Cayenne, French Guiana (11, 20). Conversely, clarithromycin was found to be active, with MICs between 2 and 4 mg/liter (12, 13). For azithromycin, higher MICs, up to 8 mg/liter, have been observed (16, 20). Telithromycin was considered active against *C. burnetii*, with MICs between 0.5 and 2 mg/liter for 13 clinical isolates (28). However, we recently isolated a strain from French Guiana which was resistant to this antibiotic (25).

No resistance to sulfamethoxazole-trimethoprim has been reported to date, suggesting that this agent is useful during pregnancy. Anecdotal reports observed susceptibility to tigecycline and linezolid and proposed them as alternative agents (13, 18). Unsworth et al. recently reported susceptibility of *C. burnetii* to antimicrobial peptides (24). Other nonantibiotic agents have been reported to display *in vitro* activity against *C. burnetii*. Lovastatin and pentamidine can inhibit *C. burnetii* growth *in vitro* (683, 684).

Also, omeprazole is effective in reducing the size of *C. burnetii* intracellular vacuoles (34).

Role of pH in persistent infection. The antibiotic treatment of persistent *C. burnetii* infection has long been challenging, because no antibiotic has displayed a bactericidal effect. In 1990, it was demonstrated that the acidic environment of the phagolysosome-like vacuole where *C. burnetii* multiplies inhibited antibiotic activity. The acidification of the *C. burnetii* replication vacuole was stable over time for the three strains tested in an *in vitro* persistent cell infection model (35). For that reason, Raoult et al. combined alkalinizing agents with doxycycline and observed a restoration of the bactericidal effect of doxycycline (36). Among alkalinizing agents, chloroquine and amantadine were used, and doxycycline was the antibiotic for which restoration of the bactericidal activity was the highest. These results were subsequently confirmed clinically by comparing the outcomes of patients with *C. burnetii* endocarditis treated with the combination of either doxycycline plus a fluoroquinolone or doxycycline plus hydroxychloroquine. Patients who benefited from the latter combination had a shorter duration of treatment and less frequent relapses (37).

Recent Advances in Culture Techniques

For years, the strict intracellular nature of *C. burnetii* had been an experimental obstacle, with time-consuming culture methods. Our laboratory developed an empirical medium based on Vero cell extract (VCEM) that allows host cell-free cultivation of *C. burnetii* (38). However, adding fresh medium every 48 h was necessary to sustain *C. burnetii* growth, and to date no growth has been obtained on the solid agarose-VCEM (38).

Another axenic medium, called acidified cysteine citrate medium 2 (ACCM2), was developed. First, Omsland et al. in 2008 elaborated a complex *Coxiella* medium (CCM) composed of an acidic citrate buffer and a mixture of three complex nutrient sources (neopeptone, fetal bovine serum, and RPMI medium) (39). This composition was based on an *in silico* genomic analysis of metabolic deficiencies. The authors observed sustained metabolic activity of *C. burnetii* in this medium, which was measured with [^{35}S]Cys-Met incorporation (39). Other metabolic requirements were then analyzed using transcriptomic microarray, genomic reconstruction, and metabolite typing. This work led to the formulation of ACCM2, a medium with an acidic pH incubated in a 2.5% oxygen atmosphere. This medium allowed a substantial growth of about 3 logs of *C. burnetii* after 7 days of incubation. Microcolonies of *C. burnetii* were observed on solid agar plates (40). This medium was subsequently improved by adding methyl-β-cyclodextrin. An increased growth of 4 to 5 logs was obtained at day 7, and an easier isolation from animal tissue and genetic transformation was achieved with solid ACCM2 (41).

EPIDEMIOLOGICAL CHARACTERISTICS OF *C. BURNETII* INFECTION

Overview

Q fever cases have been reported almost everywhere they have been sought, except in New Zealand. The main reservoirs of *C. burnetii* are cattle, sheep, and goats. However, in recent years, an increasing number of animals have been reported to shed the bacterium, including domestic mammals, marine mammals, reptiles, ticks, and birds (42). Birth products contain the highest concentration of bacteria, but *C. burnetii* is also found in urine, feces, and milk of infected animals (7, 43, 44). Transmission to humans is most frequently due to inhalation of aerosolized bacteria that are spread in the environment by infected animals after delivery or abortion.

Because Q fever is a zoonosis, with only anecdotal reports of human-to-human transmission, the epidemiology of human infections always reflects the circulation of the bacterium in animal reservoirs. The prevalence of Q fever is highly variable from one country to another, due to epidemiological disparities and whether or not the disease is reportable. For example, Q fever became a reportable disease only in 1999 in the United States, which led to an increase of 250% in the number of human cases between

2000 and 2004 due to better recognition of cases (45). Depending on the geographic area, endemic or outbreak situations are observed. In areas of endemicity, Q fever occurs as sporadic cases, usually after identifiable at-risk activities (farming, slaughterhouse work, or rural tourism). This is the predominant presentation in France, Spain, and the United States. In these countries some hyperendemic foci also can be identified, as in Martigues, a city in southeastern France, where the Q fever incidence rate reaches 34.5/100,000 inhabitants due to dissemination of spores by the local mistral wind from sheep herds breeding in the local plains (46). Small outbreaks (especially familial outbreaks) may occur after exposure to a common source, such as *C. burnetii*-infected parturient pets such as dogs or cats (47, 48). Also, an anecdotal outbreak was recently reported in the United States in five patients, due to intramuscular injection of fetal sheep cells by a German doctor practicing "live cell therapy," a xenotransplantation practice with no published evidence of efficacy (49).

Finally, large-scale outbreaks can occur at a country level, which happened in the Netherlands between 2007 and 2010, with more than 4000 reported cases. In the following section, we detail three of the most striking epidemiological profiles observed in recent years; the hyperendemicity situation in Africa, the major outbreak in the Netherlands, and the epidemic in Cayenne, French Guiana, associated with unique virulence and reservoir features.

The Different Epidemiological Profiles

Q fever in Africa. In 1955, Kaplan and Bertagna reported the first cases of Q fever in nine African countries, from Morocco to South Africa, suggesting that the infection was widespread in that continent (50). Seroprevalence studies then revealed the highest seropositivity rates in Mali, Burkina Faso, Nigeria, and Central African Republic, which are countries with the highest density of domestic ruminants (>100 per 100 inhabitants) (51). Q fever seroprevalence rates in humans varied from 1% in Chad (52) to 16% in Egypt (53). More recently, a seroprevalence study among blood donors in Namibia identified a 26% seropositivity rate (54). In an agropastoral region of Algeria, seroprevalence rates of 15%, with peaks up to 30% in villages where the disease is hyperendemic, have been observed (55). In Senegal, 24.5% of the population tested seropositive for *C. burnetii* in a rural village, and the bacterium was detected by PCR in 6 of 511 febrile patients (2, 56).

Because there are no easy diagnostic tools available in most African countries, the global impact of Q fever on public health has largely been underestimated. In a recent study, *C. burnetii* was found as the etiological agent in 5% of 109 severe pneumonia cases in Tanzania (57). In the same country, an investigation in a cohort of severely ill febrile patients found 26.2% zoonoses, among which 30% were Q fever (58). In Tunisia and Algeria, *C. burnetii* accounts for 1 to 3% of infective endocarditis (59). In Burkina Faso, Q fever is responsible for 5% of acute febrile illnesses (59). In Cameroon, 9% of community-acquired pneumonia in those aged >15 tested positive for *C. burnetii* (59). In that country, *C. burnetii* was the third most frequent agent of pneumonia, after *Streptococcus pneumoniae* and *Mycoplasma pneumoniae* (60). These studies may be the tip of the iceberg, indicating widespread circulation of the bacterium in rural Africa.

Moreover, in most African countries, seroprevalence rates are elevated in domestic ruminants. Surveys in cattle showed rates ranging from 4% in Senegal to 55% in Nigeria (61, 62). In Egypt, a high rate of 33% seropositivity was observed in sheep herds (63). Goat seropositivity for *C. burnetii* ranged from 13% in Chad to 23% in Egypt. Also, Schelling et al. found 80% of camels with *C. burnetii* seropositivity (52), and a similar rate of 70% was found in Egypt (64), suggesting that these animals are significant reservoirs of the disease. In rural regions of most of these countries, human households are in close vicinity to domestic ruminants, making transmission easier than it is elsewhere. As a consequence, *C. burnetii* DNA was detected in 2% to 22% of household samples in rural Senegal (65), which correlated with the prevalence of Q fever cases in the population. Consumption of unpasteurized raw milk may be a source of human contamination, as *C. burnetii* has been detected in up to 63% of cattle milk samples in

Nigeria (59). A few *C. burnetii* genotypes (including genotypes 2, 6, 16, 19, 30, 35, 36, and 52) have been characterized in Africa, mainly in ticks (56, 66). Only genotypes 19 and 35 have been detected so far in humans (56).

Q fever is not commonly considered a tropical disease. Clinicians should think of this diagnosis in febrile patients coming back from African countries. The fact that it has been reported in travelers returning from a safari tour illustrates this aspect (67).

Q fever outbreak in the Netherlands. Between 2007 and 2010, the Netherlands faced the largest Q fever outbreak ever reported, with more than 4,000 reported cases and an estimation of probably more than 40,000 total cases (4, 68, 69). The outbreak occurred in a population with low previous Q fever seroprevalence (2.4%) (70). The regions with the highest rates of infection were the Noord-Brabant province in the southern part of the country and the provinces of Gelderland and Limburg (71). Noord-Brabant province has 2.4 million inhabitants and 6.4 million farm animals, and goat farming is concentrated in this region. The outbreak correlated with the development of intense dairy goat farming in proximity to urban areas (72). The importation of a huge number of animals, with a 75-fold increase in the goat population between 1985 and 2009, could have contributed to the introduction of *C. burnetii*-infected animals in this country (4). Thanks to a retrospective study, it is now known that the epizooty had begun in 2005, with abortion rates above 60% on some farms (73). Thus, the combination of a large number of infected animals, the location of farms near populated areas, a lack of surveillance, and the low level of immune protection in this population probably explain the magnitude of the Q fever epidemic.

A public health strategy for controlling the outbreak had to be developed by Dutch authorities and was implemented in the spring of 2008. The notification of abortion cases in herds and vaccination became mandatory in June 2008 for goat or sheep farms with more than 50 animals in affected areas (74). Due to the inefficiency of these measures, and while fatal cases in humans were being reported, it was decided in December 2009 to systematically cull gestating goats and ewes, leading to the culling of more than 50,000 goats (75). In 2010, the number of reported cases in humans began to decrease, probably due to these veterinary measures and to increasing the immunization of the population.

As a consequence of the epidemic, many patients with underlying cardiac valve (or vascular) defects or prostheses could have been exposed to *C. burnetii* and might develop life-threatening endocarditis or vascular infections (76–78). Also, a high number of pregnant women could have been exposed to *C. burnetii* and might develop obstetrical complications and spontaneous abortions (79). Therefore, the long-term public health consequences of the Q fever epidemic, especially the potential development of a high number of persistent infections, are still a major concern.

This exceptional outbreak highlights that despite the microbiological knowledge about *C. burnetii* infection, its sudden emergence and spread cannot be predicted.

Hyperendemic Q fever in Cayenne, French Guiana. In Cayenne, the capital city of French Guiana, *C. burnetii* causes 24% of community-acquired pneumonia (CAP), which is the highest prevalence ever reported in the world (80). The first Q fever case was reported in 1955 in Cayenne, in a slaughterhouse worker (81). Sporadic cases were then reported in the following 4 decades. In the 1990s, a dramatic increase in the incidence of Q fever was observed, with seroprevalence rates rising from 2% in 1992 to 24% in 1996 in a cohort of febrile patients (3). The same year, a patient died of respiratory distress syndrome due to acute Q fever (82). The incidence continued to rise, with up to 150 cases per 100,000 inhabitants in 2005 (3). The majority of cases (81%) occurred in the Cayenne area and its suburbs, in contrast with the usual rural distribution of the disease. However, a more sensitive analysis of the incidence found a heterogenous distribution, with seven areas of high incidence. In these areas, rainforest hills are near houses (83). Living near the forest and seeing wild animals (bats or marsupials) around the house were risk factors for Q fever (82). No classical at-risk exposure was found in acute Q fever cases in Cayenne. Low Q fever seroprevalence was found in cattle, sheep, and goats, as well as in tested pets (82). In an initial study performed by Gardon et al.,

no significant seroprevalence was observed among wild mammals and arthropods, so that no reservoir was identified (82). In a recent study, *C. burnetii* was detected by qPCR in the ticks, spleen, and stools of a dead three-toed sloth in Cayenne (84). In addition, the incidence of acute Q fever in Cayenne is correlated with the rainy season, and we observed a 1- to 2-month lag in the correlation between the incidence of acute Q fever and the number of births of three-toed sloths in Cayenne (85). Also, a retrospective study of an outbreak in a military camp in Cayenne in 2013 found that having carried a three-toed sloth in the arms in the month preceding symptoms was an independent risk factor for acute Q fever (unpublished data).

The same study found that 100% of cases (defined by a positive serology and/or qPCR in blood) in this military camp were symptomatic, which is an exceptional feature compared to the usual rate of 60% symptomatic cases found in most areas of endemicity. Clinically, the pneumonias caused by *C. burnetii* in Cayenne show a more severe initial presentation, with more frequent headaches, chills, and night sweats than pneumonia caused by other microorganisms (80). Moreover, patients tend to exhibit higher levels of phase I IgG, despite the acute presentation. These atypical features seem to be correlated with the presence of a single clone of *C. burnetii*, MST 17, which to date has only been isolated from this area. This clone also appears to be more virulent in animal models (see Pathophysiology section below).

The Reservoirs

Coxiella burnetii can infect a broad range of vertebrate and invertebrate hosts (86–88). Also, the bacterium can persist for prolonged periods in the environment, owing to a pseudosporulation process. Among mammals, cattle, sheep, and goats are the most frequent reservoir leading to human transmission. However, wildlife can also constitute a reservoir, as illustrated by an acute Q fever case reported after contact with kangaroos and wallabies in Australia (89) or by the involvement of the three-toed sloth in Cayenne (84). *C. burnetii* has also been isolated in many tick species, suggesting that these arthropods play a role in the transmission of the bacterium. Finally, it has been shown that *C. burnetii* is able to grow within amoebae, suggesting a participation of these hosts in the environmental persistence of the bacterium.

Domestic ruminants. Since domestic ruminants are the main reservoir responsible for human outbreaks, the detection and control of infected herds are important issues. However, the identification of infected animals is challenging, because dairy animals can shed the bacterium without being symptomatic. The main clinical manifestations in ruminants are reproductive disorders such as infertility, stillbirth, abortion, endometritis, and mastitis (87). Increased abortion rates in infected caprine herds have been described, with up to 90% abortions in pregnant animals (90). Infected females shed a huge amount of bacteria in birth products and in urine, feces, and milk. This shedding can persist for several months in vaginal mucus, feces, and milk (91). In nonpregnant animals, *C. burnetii* infection is most frequently asymptomatic.

Rodolakis et al. have compared the shedding of *C. burnetii* in bovine, caprine, and ovine herds from France (44). Cows were mostly asymptomatic and shed *C. burnetii* mainly in milk, with a few of them (5%) shedding in vaginal mucus. Ovine flocks were the most heavily infected, with shedding of the bacterium in feces, vaginal mucus, and milk, and ewes shed the bacterium for up to 2 months of follow-up. Goats shed the bacterium mainly in milk, and a minority shed *C. burnetii* in vaginal mucus or feces (44). These differences in shedding routes could explain why human outbreaks are most frequently related to small ruminants rather than to bovine herds, which is what was observed in the Netherlands. Interestingly, in the same study, seropositivity for *C. burnetii* was not strongly correlated with shedding of the bacterium. Fewer than 10% of ovine flocks shedding *C. burnetii* tested positive for *C. burnetii* antibodies by enzyme-linked immunosorbent assay (ELISA), and the proportions were 35% and 50% for bovine and caprine herds, respectively (44). As a consequence, serology does not seem to be an efficient tool in estimating the real rate of contamination of herds. For

monitoring purposes, PCR testing of bulk tank milk is a useful method to detect shedding of *C. burnetii* at the farm level (92).

Preventive veterinary measures are a key point in the control of Q fever. Antibiotic treatment with oxytetracycline (20 mg/kg) during the last months of pregnancy has been proposed to reduce abortion rates and *C. burnetii* shedding in pregnant animals (93). However, this strategy is not always effective, since Astobiza et al. found no difference in rates of bacterial shedding between treated and untreated sheep (94).

Vaccination is another available option to decrease abortion rates and spread of the infection. The phase I vaccine Coxevac has been effective in decreasing abortion rates and bacterial load in vaginal mucus, feces, and milk in goats (95). The vaccination should be administered in noninfected ruminants before their first pregnancy (74). When ruminants are already infected, vaccination cannot prevent abortion (90, 96). Also, standard hygiene measures should be taken to prevent dissemination of *C. burnetii* in the environment and between animals. Infected parturient females should be isolated from the herd, and placentas and fetuses should be collected and destroyed by burning or burying (90). Moreover, the spreading of manure in infected farms should be avoided during windy conditions. Animals exhibited at fairs should also be carefully selected. In Germany, an infected ewe lambing in a farmer's market led to an outbreak of 299 Q fever cases in the people present (97). In Briançon, a town in France located in the Alps, the investigation of a slaughterhouse-related outbreak found that contamination resulted from airborne transmission of contaminated sheep waste, facilitated by the wind caused by a nearby heliport (98, 99). Finally, when no preventive measures can be applied and if too many contaminated animals are involved, culling of herds is the ultimate solution, and this was performed in the Netherlands to control the epidemic (90).

The main routes of introduction of *C. burnetii* on a farm are the aerosolized spore-like forms transported by the wind (100) and the introduction of an infected animal. Then, depending on the size and immune status of the herd, the spread of the bacterium can lead to "abortion storms," such as what occurred in the Netherlands in 2005 and 2009 (90).

Role of ticks. *C. burnetii* strains were first isolated from hard ticks: *Dermacentor andersoni* (Nine Mile isolate) collected in Montana (101) and *Haemaphysalis humerosa* from Australia (102). Hematophagy is the essential factor for the acquisition of *C. burnetii* by arthropods. Following the "classical" epidemiological pattern of zoonotic infections, *C. burnetii* likely circulates among animals with the help of hematophagous arthropod vectors. However, in contrast to the case for most vector-borne diseases, the presence of an arthropod vector is not necessary for the transmission of the infectious agent from the reservoir to host mammals.

Another important aspect of the epizootic cycle of *C. burnetii* is the absence of any vector specificity. This bacterium was isolated from more than 40 hard tick species, at least 14 soft tick species, and many other arthropods, including bed bugs, flies, and mites (Table 1). Even more arthropod species (including human lice and fleas) were shown to be susceptible to *C. burnetii* infection under experimental conditions (103), although they were not able to transmit the agent to experimental animals or to their progeny.

(i) Susceptibility of arthropods. Hematophagous arthropods at all stages of their development can be easily infected with *C. burnetii* when taking a blood meal from an infected mammal (104). However, in experimental situations, not all ticks feeding on a *C. burnetii*-infected animal become infected; e.g., in the case of a *Haemaphysalis humerosa* infection model, only half of the ticks became infected (102, 105). A similar situation was described for the experimental infection of *Ixodes ricinus* and *Ornithodoros papillipes* ticks fed on infected guinea pigs (104).

(ii) Localization inside the tick. The first experiments on the localization and dissemination of *C. burnetii* in ticks were performed by D. J. W. Smith on *Haemaphysalis humerosa* and *Haemaphysalis bispinosa* (105, 106). Bacteria were abundant in the epithelial cells and lumen of the gut. However, the transovarial transmission

TABLE 1 Arthropods from which *C. burnetii* has been isolated

Arthropod species from which strain was isolated	Host(s)	Reference(s)
Hard ticks (Ixodidae)		
Amblyomma americanum	Deer, cattle	103
Amblyomma cajennense	Dogs	670
Amblyomma flavomaculatum	Monitors	103, 671
Amblyomma nuttalli	Reptiles	103, 671
Amblyomma paulopunctatum	Suidae	103, 671
Amblyomma splendidum	Ungulates	103, 671
Amblyomma triguttatum	Kangaroos	672
Amblyomma variegatum	Cattle	103, 109, 671
Dermacentor andersoni	Deer, cattle	673
Dermacentor marginatus	Deer, cattle	(186)
Dermacentor nuttalli	Ruminants	(186)
Dermacentor occidentalis	Deer, cattle	103
Dermacentor silvarum	Deer, cattle	(186)
Haemaphysalis bispinosa	Kangaroos	674
Haemaphysalis humerosa	Marsupial bandicoot	102
Haemaphysalis leachi	Dogs	103, 671
Haemaphysalis leporis-palustris	Hares	103
Haemaphysalis punctata	Rodents	103, 186
Hyalomma asiaticum	Ruminants	104, 675
Hyalomma anatolicum	Cattle	104
Hyalomma marginatum	Cattle	103, 671
Hyalomma detritum	Ruminants	103, 104
Hyalomma dromedarii	Camels	103, 104
Hyalomma excavatum	Cattle	103
Hyalomma lusitanicum	Horses	103
Hyalomma plumbeum	Cattle	103, 108
Hyalomma scupense	Cattle	104
Ixodes crenulatus	Weasels	104
Ixodes dentatus	Rodents, rabbits	103
Ixodes frontalis	Birds	104
Ixodes holocyclus	Dogs, rodents	674
Ixodes lividus	Birds	104
Ixodes persulcatus	Ruminants	104, 676
Ixodes redikorzevi	Argali	677
Ixodes ricinus	Ruminants	23, 103
Ixodes trianguliceps	Rodents	104
Rhipicephalus annulatus	Cattle	104
Rhipicephalus bursa	Cattle	103, 108
Rhipicephalus cuspidatus	Aardvarks	103, 671
Rhipicephalus decoloratus	Cattle	103, 671
Rhipicephalus sanguineus	Dogs	671, 678
Rhipicephalus simus	Carnivores	103, 671
Rhipicephalus turanicus	Ruminants	104
Soft ticks (Argasidae)		
Argas persicus	Birds	104, 671
Argas reflexus	Birds	104
Argas vespertilionis	Bats	679
Ornithodoros alactagalis	Rodents	104
Ornithodoros erraticus	Rodents	103
Ornithodoros gurneyi	Kangaroos	105
Ornithodoros hermsi		680
Ornithodoros lahorensis		103
Ornithodoros moubata		103, 113, 680
Ornithodoros papillipes		679
Ornithodoros sonrai	Rodents, insectivores	56
Ornithodoros tartakovskyi	Rodents	679
Ornithodoros turicata		110
Otobius megnini	Horses, cattle	103
Other arthropods		
Musca domestica		103, 186
Cimex lectularius	Humans	186, 681
Hematophagous *Mesostigmata* mites: at least 14 species, including *Liponyssoides sanguineus*, *Ornithonyssus bacoti*, *Haemolaelaps glasgowi*, *Dermanyssus hirundinis*, and *Haemogamasus nidi*	Rodents	104, 186, 682

of *C. burnetii* was reported for *H. humerosa*, suggesting that these ticks could serve as a long-time reservoir for this pathogen. Using an *Ornithodoros moubata* model, von Weyer later showed the generalized dissemination of *C. burnetii* in this tick's body, affecting the gut, hemocytes, salivary glands, and ovaries (107). Tarasevich, working on *Haemaphysalis plumbeum* ticks infected intracoelomically with *C. burnetii*, demonstrated that this bacterium was visible in hemocytes for several hours postinfection and was detectable in salivary glands and ovaries from the third day postinfection (108). Similar data were observed in different experiments on *Dermacentor nuttalli*, *Haemaphysalis asiaticum*, and *Ornithodoros papillipes* (103, 104).

(iii) Survival of *C. burnetii* in ticks. It is clear that *C. burnetii*, once infecting tick cells, is capable of remaining viable inside the tick's body for a very long time, between 200 (109) and 1,000 days, depending on the tick species (110). However, in several cases, much longer survival times have been reported: 1,301 days in *O. moubata* (107) and even 6 to 10 years in *O. papillipes* (104). Low temperatures, starvation, and feeding on an immunized host do not influence the viability of *C. burnetii* (104).

(iv) Transstadial and transovarial transmission. Most soft and hard ticks transmit *C. burnetii* transstadially in 100% of cases (104). Similarly, it is thought that most hard and soft ticks are able to transmit *C. burnetii* transovarially. Several exceptions include *Ixodes holocyclus*, *Ornithodoros hermsi*, and *Ornithodoros turicata* (104). However, transovarial transmission is not very effective and may vary from 30 to 60% (102, 104, 107).

(v) Excretion of *C. burnetii*. Massive excretion of highly infective phase I *C. burnetii* (11) in tick feces occurs on the skin of the animal host at feeding time. This is the direct consequence of the multiplication of *C. burnetii* primarily inside gut epithelial cells. Feces may contain a huge number of bacteria, from 10^3 to 10^8 in different experiments (102, 104, 106, 111). Excreted bacteria stay viable in tick feces for many days (up to 635), although infectivity decreases with time (104). As for other bacteria excreted in arthropod feces, abraded skin may serve as a portal of entry for infection (112).

Soft ticks may also excrete *C. burnetii* in coxal fluid (6, 20). This may even be the most important method of *C. burnetii* excretion for soft ticks with no direct connection between the rectum and the midgut, such as in *O. moubata* (113).

(vi) *C. burnetii* influence on tick fitness. No deleterious effects of *C. burnetii* in ticks has been reported (104). Slight metabolic changes between infected and noninfected ticks, such as oxygen consumption and CO_2 excretion, have been noted. *C. burnetii* infection is considered to be harmless to ticks (104).

In livestock, ticks are probably nonessential for transmission of *C. burnetii* (103). However, they seem to play a role in the transmission of *C. burnetii* among other vertebrates (rodents, wild birds, and lagomorphs) (103, 114).

Detection and strain isolation of *C. burnetii* from bed bugs, lice, and flies (Table 1) (103) have been reported, but the role of these arthropods in the natural cycle of *C. burnetii* is unknown.

Role of free-living amoebae. *C. burnetii* is an intracellular bacterium that multiplies in monocytes and macrophages of infected hosts (115). Prokaryotes colonized Earth before eukaryotes, and unicellular eukaryotes emerged before multicellular ones. It has been emphasized that most intracellular bacteria infecting humans and animals are able to multiply within unicellular protozoa such as amoebae (116). Because these protozoa are widespread in nature, many bacteria have developed mechanisms to resist the phagosomal pathway of amoebae. These microorganisms have then used similar mechanisms to resist attack by the phagocytic cells of multicellular eukaryotes. *Legionella pneumophila*, the agent of legionellosis, is a paradigm for such an adaptive evolution (117). This bacterium can multiply in the vegetative form of amoebae. Under deleterious environmental conditions, *L. pneumophila* survives in encysted amoebae until they recover their vegetative form. Thus, amoebae allow long-term persistence of *L. pneumophila* in water and soil environments. *In vitro*, *C. burnetii* can multiply in amoebae, which may represent a long-term environmental reservoir for this bacterium (118). A report from Amitai et al. (119, 120) of a Q fever outbreak in an Israeli school occurring after exposure to an air conditioning system favored such a hypothesis.

However, further studies are needed to isolate *C. burnetii*-infected amoebae from the natural environment.

Routes of Human Infection

A single study of experimental infection with *C. burnetii* in human volunteers was performed in 1950 in Portugal (121). This very old work is interesting because it summarizes the main possible routes of human infection. Fifty-one human volunteers took part in the experiment. Ten volunteers had intranasal inoculation, 11 ate infected food, and 29 were infected intradermally. The most efficient route of infection was intradermal inoculation, which resulted in 100% seroconversion. Three patients (27%) seroconverted after consumption of infected food and two patients (20%) after intranasal inoculation.

Aerosols. Most human infections occur after inhalation of infected aerosols of *C. burnetii* (86–88). Infection may occur after direct exposure to infected animals and their products (placenta, abortion products, hides, wool, manure, etc.), especially at the time of parturition or slaughtering (49–51). Because *C. burnetii* may persist for prolonged periods in the soil, these aerosols may also be produced long after the release of bacteria by infected animals. Moreover, bacterial aerosols can be delivered for at least 30 km by the wind (122), resulting in Q fever cases far away from the primary contaminated areas. Thus, Q fever cases are often diagnosed in persons with no recent contact with animals.

Digestive route. The hypothesis that consumption of dairy products from *C. burnetii*-infected animals can lead to foodborne Q fever in humans is controversial (91). In an early study, Huebner and Jellison found that pasteurization of dairy products could eliminate *C. burnetii* from infected milk (123). Other studies have shown higher seroprevalence rates and clinical disease in patients consuming raw milk (124). However, a significant bias of these studies is that people consuming raw milk may live more frequently in rural areas and be in contact with ruminants, so that contamination could also be the result of aerosol inhalation. However, during an epidemic in Newfoundland, Canada, eating pasteurized goat cheese was an independent risk factor for acute Q fever (125). Also, in an old study performed in a prison, *C. burnetii* serology was found to be more frequently positive in persons consuming raw milk than in persons who did not (126). Conversely, no seroconversion or clinical illness was observed in another study involving 34 volunteers consuming raw milk (127). *C. burnetii* DNA has been detected in up to 64% of dairy products in France (124) and more frequently in cow dairy products. However, no viable bacteria could be isolated from cheese and yogurt in that study. Consequently, even if the digestive route of contamination does not constitute a major public health threat, it may play a significant role in *C. burnetii* transmission.

Ticks. Although this mode of contamination has not been proven in humans, ticks may play a role in the transmission of *C. burnetii* infection. This is illustrated by the detection of *C. burnetii* coinfection with other arthropod-borne pathogens in ticks. In Italy, 85 *Rhipicephalus turanicus* and 33 *Ixodes ricinus* ticks were collected from a public park in Rome (128). Coinfection with *Rickettsia* of the spotted fever group and *C. burnetii* was observed in 5 (5%) *R. turanicus* ticks. Double infection with *C. burnetii* and *Borrelia burgdorferi* was detected in 7 (21%) of *I. ricinus* ticks, and a positive statistically significant correlation between these two pathogens was observed. These findings of different microorganisms in ticks from urban areas suggest a common mode of transmission for both human pathogens via arthropods (128).

Human-to-human transmission. Q fever pneumonia is considered a noncommunicable disease, although a recent case of respiratory nosocomial spread was reported (129). Anecdotal cases of human-to-human transmission through infected aerosols have been reported after autopsies (88). Birth products from infected parturient women are also a source of infection in obstetrical wards. A case of *C. burnetii* pneumonia was diagnosed in an obstetrician 7 days after he delivered the infant of an infected woman (130). Also, nosocomial transmission between two pregnant women sharing the same

room has been reported (131). In this case, the most probable route of infection was vaginally excreted aerosolized infectious particles.

C. burnetii infection through transfusion of blood collected from Q fever patients with bacteremia is plausible, since the bacterium can survive in stored human blood samples (132). In the Netherlands, screening of blood donors after the outbreak found 4.4% of donors with positive phase II antibodies (133). Another study found that during the outbreak, the probability of a donor being infected was estimated at 260/100,000 donors (134). As a consequence, systematic donor screening was the most useful strategy for reducing the risk of transmission through blood transfusion. A single case of Q fever was also reported after bone marrow transplantation (135). Another anecdotal case of possible sexual transmission of *C. burnetii* from a farmer to his wife has been reported (136).

Seasonality Patterns and the Role of Wind

In areas of endemicity, the Q fever incidence is variable through the year. Seasonal patterns have been observed. In the southeast of France, Tissot-Dupont et al. found a correlation between the incidence of the disease, sheep densities, and the local mistral wind in the city of Martigues (46). During the winter in 1998 to 1999, the high incidence of acute Q fever in this region was associated with an increased frequency of mistral wind shortly after the lambing season, 1 month before disease onset (122). In the UK, unusual wind speed from the south was observed shortly before a large epidemic in Birmingham (137). In Germany, a long-term survey on Q fever has revealed a changing pattern of seasonality, from winter-spring to spring-summer (138). This evolution is probably due to changes in sheep husbandry. Winter-spring lambing is associated with a type of nomadic husbandry that has considerably decreased throughout the years in this country. In Croatia, acute Q fever cases have been observed mainly in the spring.

In tropical areas, two examples suggest that acute Q fever incidence may be related to the rainy season. In Queensland, Australia, a clear seasonal peak of acute Q fever cases was observed in May, 3 months after a peak February rainfall (139). In Australia, the rainy season corresponds to an increase in the populations of macropods (wallabies and kangaroos) and other wildlife that potentially plays a role in the spread of the bacterium. Rates of up to 20.8% seroprevalence for *C. burnetii* have been observed in macropods (140). In Cayenne, acute Q fever incidence is the highest in July, with a 2-month correlation following a peak in rainfall in May, which is the breeding season of the three-toed sloth, which is a probable wild reservoir of the infection (85).

GENOMIC ASPECTS

The first genome of *C. burnetii* was sequenced in 2003 (9). This event led to significant progress in many fields of study of this bacterium. In particular, the intracellular nature of *C. burnetii* made the search for virulence determinants very difficult. Genomics, and more particularly comparative genomics studies, have demonstrated that the word "Q fever" covers a large range of epidemiological and pathogenicity characteristics, depending mainly upon the genetic characteristics of the *C. burnetii* strain involved.

Comparative Genomics and Pangenomic Analysis

The RSA493 strain, isolated from a tick in 1935, was the first strain to be sequenced (9). It revealed a QpH1 plasmid (37,393 bp) and a 1,995,275-bp chromosome. When comparing this genome with genomes from close relatives, including *Legionella*, *Chlamydia*, and *Rickettsia* species, several particularities were found. There were 719 hypothetical coding sequences, with no equivalent in gammaproteobacteria. There were more coding sequences than in the genomes of *Rickettsia prowazekii* and *Mycobacterium leprae*. The *C. burnetii* genome exhibited 83 pseudogenes, showing that genome reduction was in progress. It was also characterized by the presence of 29 insertion sequence (IS) elements, in favor of high genomic plasticity.

Regarding metabolic pathways, the *C. burnetii* genome contained a high number of transporters, reflecting the intracellular lifestyle of the bacterium, which finds its organic nutrients within the eukaryotic host cell. In comparison with other intracellular bacteria, the central carbon metabolism and bioenergetics pathways were mainly intact. Also, many ionic exchangers were present, playing a role in the survival of the bacterium by detoxifying the phagolysosome-like vacuole where *C. burnetii* multiplies. Many genes were very similar to eukaryotic genes, with no equivalent in prokaryotes, similar to what has been found in *Legionella*. Also, a new family of ankyrin repeat-containing proteins (Anks) was reported. Anks are mediators in protein-protein interactions and play a role in intracellular processes (141).

Since 2003, six new whole genomes of *C. burnetii* have become available in GenBank (142–144). Sixteen incomplete genomes can also be found (27, 47, 145–150). In 2009, Beare et al. performed a comparative genomic analysis of four strains and found a total of 125 pseudogenes, of which 65 were highly conserved, and 8 IS families (142). These features were consistent with genome reduction due to recent adaptation of *C. burnetii* to an intracellular lifestyle. More recently, D'Amato et al. performed a pangenomic analysis of the seven sequenced strains of *C. burnetii* (144). The Dugway strain exhibited the largest genome (2,158,758-bp chromosome and 54,179-bp QpDG plasmid). Cb175 from Cayenne, French Guiana, had the smallest genome, due to a unique 6,105-bp deletion in the coding region for the type 1 secretion system (T1SS) (1,989,565 bp chromosome, 37,398 bp QpH1 plasmid). Some strains had one of the plasmids QpRS, QpH1, and QpDV. The others had plasmid sequences integrated into the chromosome. A BLAST score ratio (BSR) analysis allowed the description of core accessory and unique genes. The core genome/pangenome ratio was 96%. A total of 13,542 core genes (shared by all strains), 498 accessory genes (found in some strains), and 88 unique genes (found in a single strain) were found (144). Seventy-four unique genes were found in the Dugway strain, 13 in the Q212 strain, and a single gene in the RSA331 strain.

The conclusion from this work is that *C. burnetii* strains share strong genomic similarity, with a closed pangenome. However, some particularities were observed regarding two epidemic strains, reflecting the links between genomic characteristics and virulence.

Genome and virulence. (i) Cb 175, a strain from Cayenne, French Guiana. The genome sequencing of strain Cb 175, isolated from a patient with endocarditis living in Cayenne, French Guiana, revealed an unexpected feature: a deletion of 6,105 bp resulting in a large genome reduction compared to the Nine Mile strain (139). A specific qPCR system was established with primers and probes targeting DNA sequences adjacent to the deleted region. This qPCR test was positive for all other clinical strains isolated from French Guiana. Conversely, this deletion was not found in any of the 298 *C. burnetii* isolates from other parts of the world (8/8 versus 0/298; $P < 0.001$). Alignment of the missing region with the genome of *Legionella pneumophila* revealed the presence of a conserved region of a type 1 secretion system (T1SS). Interestingly, the genes *lssB* and *lssD*, which were absent in Cb175, were also missing in *Legionella longbeachae* (144).

Several conclusions can be drawn from this study. First, genome reduction has been observed in many highly epidemic strains (151). For species like *Rickettsia* or *Mycobacterium* spp., hyperpathogenicity is driven by deletion of nonvirulence genes rather than by acquisition of virulence factors (152, 153). Cb175 and other strains of the genotype MST 17 specific for French Guiana are the most virulent strains ever described. They cause the highest prevalence of community-acquired pneumonia in the world. Consequently, the observed genome reduction is probably a mechanism leading to increased virulence in this *C. burnetii* clone. Also, it has been shown that highly pathogenic species have fewer secretion system proteins than their nonepidemic relatives (154). The fact that the deletion in Cb175 is located in the region of genes involved in the T1SS is consistent with this phenomenon. The role of T1SS in *C. burnetii* is not known, but it plays a role in the internalization of *Legionella pneumophila* in its host cell (155).

The exact pathophysiological consequences of the genetic deletion observed in both *L. longbeachae* and *C. burnetii* are currently unknown, and further experimental studies are warranted.

(ii) Strain Z3055, a strain close to the strain of the Netherlands outbreak. To date, the complete genome sequence of NL-Limburg, the strain causing the Netherlands outbreak is not available (150). Therefore, we sequenced the Z3055 strain isolated from a German ewe, which belonged to the same genotype (MST 33) as NL-Limburg (150). Compared with other *C. burnetii* genomes available in GenBank, we found a high proportion of mutations in genes coding for ankyrin repeat domain proteins, membrane proteins, and proteins involved in translation and transcription (143). Thus, we hypothesized that these mutations may have changed surface antigens, as in the case of influenza virus. These modifications in surface antigens could have led to an absence of immune recognition in a naive population, allowing the rapid spread of this specific clone in the Netherlands.

(iii) Strain DOG UTAD, from Canada. The majority of strains isolated so far in Canada belong to the MST 21 genotype. An interesting feature of Q fever outbreaks in Canada is the frequent exposure of patients to parturient pets (cats or dogs) (48, 156, 157). In 1994, three members of the same family had acute Q fever after assisting with the parturition of their dog in Nova Scotia (48). The whole genome of the strain isolated from this outbreak was sequenced. It was very close to the Q212 strain, which had been isolated from a patient with *C. burnetii* endocarditis in Canada. DOG UTAD was plasmidless and showed 70 mutations, of which 47 were nonsynonymous compared to Q212. Both strains belong to the same genotype, MST 21, which has been isolated only in Canada to date (47).

(iv) Plasmids and virulence. A correlation between plasmid types and virulence was proposed previously. In this hypothesis, strains with a QpH1 plasmid were associated with "acute Q fever" and strains harboring QpRS were associated with "chronic Q fever" (158). The substratum for such a hypothesis was that specific sequences in plasmids correlated with pathogenicity. For example, the gene *cbbE′* was found to be specific for QpRS, and *cbhE′* was found to be specific for QpH1 (159, 160). However, this hypothesis was ruled out by several studies. First, Stein and Raoult showed that *cbhE′* was not systematically detected in strains from "acute Q fever" and conversely could be detected in isolates from chronic Q fever (161). The description of a new plasmid type, named QpDV, which can be associated with both forms of the disease, has been another argument against the existence of plasmid pathotypes (162). Finally, Thiele and Willems also found endocarditis isolates harboring QpH1 sequences (163). Nevertheless, in a recent study, Angelakis et al. found that the QpDV plasmid was associated with strains causing abortion (164). However, this correlation could be related to the confounding factor represented by genotype, so further studies are needed to confirm this point.

***C. burnetii* genotyping.** Genotyping of bacteria is a key tool in the understanding of the epidemiology of infectious diseases. With regard to a zoonosis like Q fever, it is of tremendous importance, helping to find the animal source of human outbreaks. In initial studies, 16S rRNA sequencing, 16S-23S rRNA sequencing, RNA polymerase β-subunit (*rpoB*) sequencing, and internal transcribed spacer (ITS) sequencing were used as epidemiological markers but showed insufficient discriminant power for *C. burnetii* genotyping (165). Then, restriction fragment length polymorphism (RFLP) analysis and PCR-RFLP targeting several genes were developed, but these methods could not be used on a routine basis (165). Comparative genomic hybridization (CGH), thanks to whole-genome microarray techniques, was performed when genome sequences became available. Different "genomotypes" were defined based on polymorphisms in plasmid open reading frames (ORFs) and chromosomal sequences (166), but this method is time-consuming. Loftis et al. have tried to describe the diversity of strains by detection of different plasmid sequences, but this has a poor discriminant power (167). Currently, the three main discriminant genotyping methods used are multiple-

locus variable-number tandem repeat (VNTR) analysis (MLVA), multispacer sequence typing (MST), and single nucleotide polymorphism (SNP) genotyping.

(i) VNTR-MLVA genotyping. VNTR-MLVA genotyping was established by Svraka et al., who amplified VNTR sequences from 21 *C. burnetii* isolates (168). They identified five main clusters and nine MLVA types. Arricau-Bouvery et al. then analyzed 42 isolates and found 36 MLVA types. They proposed using two panels of markers to have a better discriminatory power (169). In the literature, there are 17 references for studies that have used this genotyping method, almost all from Europe and mainly among ruminants (66, 73, 170–184). The clone causing the Netherlands outbreak was identified as CbNL01 using this method, and a very similar clone was detected in goats in Belgium (178). However, MLVA is based on the analysis of relatively unstable repetitive DNA elements and can produce results that are too discriminatory (185). Moreover, it significantly lacks interlaboratory reproducibility (185).

(ii) MST genotyping and "geotyping." MST genotyping was introduced by Glazunova et al., who identified 10 highly variable spacers located between ORFs (186). This typing method identified 30 different genotypes and three monophyletic groups among 173 *C. burnetii* isolates. These groups were partially correlated with plasmid types. The first group contained strains with the QpDV or QpRS plasmid, the second contained only strains with the QpH1 plasmid, and the third group contained plasmidless strains or strains with QpH1. This method is very discriminant and has been used most frequently in different studies around the world. Seventeen studies have used MST genotyping from human, animal, or environmental *C. burnetii* strains (2, 56, 66, 84, 164, 172, 173, 176, 179, 183, 184, 186–191), providing a worldwide database allowing interlaboratory comparison (Fig. 1). MST genotyping helps to trace the spread of *C. burnetii* from one region to another and from animal reservoirs to humans. Some MSTs are present across the five continents, whereas others are very specific to epidemic situations. For example, MST 20 has been described in ruminants in Europe and in humans and ruminants in the United States, suggesting a spread of the disease by infected animals historically brought to the New World. In contrast, MST 17 has been isolated only from French Guiana to date, causing severe forms of the disease (192). In the Netherlands, MST genotyping identified MST 33 in goats and humans, allowing confirmation that the source of the epidemic was goat herds rather than cow herds, which predominantly harbored MST20 (176). The phylogenetic analysis showed that MST 33 may have spread from Germany to the Netherlands via France.

For these reasons, MST genotyping has been qualified as a "geotyping method" (Fig. 1). This "geotyping" scheme is still incomplete and has to be implemented in further studies to provide a comprehensive cartography of the genetic diversity of *C. burnetii*.

(iii) SNP genotyping. SNP genotyping was developed by a Dutch team during the outbreak in the Netherlands to provide a method directly applicable to animal and human samples without the need for enrichment by a culture step (193). Ten discriminatory SNPs were selected using five *C. burnetii* whole-genome sequences available in GenBank. (RSA493, RSA331, CbuG_Q212, Cbuk_Q154, and Dugway). Detection of SNPs by reverse transcription-PCR (RT-PCR) was performed in 14 human and 26 animal samples, allowing identification of five different genotypes. This method has also been used in Belgium and in the United States for livestock and tick strains (178, 194–196). Karlsson et al. developed an SNP genotyping method targeting 10 phylogenetically stable synonymous canonical SNPs (canSNPs) (197). Finally, Hornstra et al. developed an SNP method derived from MST genotyping (189). They extracted SNP signatures in MST loci and designed 14 SNP-base assays. These assays allowed genotyping of 43 previously untyped isolates when using classic MST, increasing the database available for worldwide comparison of "geotypes" (Fig. 1).

(iv) Other methods. Some other genotyping methods have been reported on the basis of small samples, giving very local information. In Spain, Jado et al. developed a genotyping method based on the detection of seven ORFs and on the presence or not of the acute disease antigen A (adaA) (198). They identified seven genomic groups and 10 different genotypes among 90 samples from ruminants and humans. In that study,

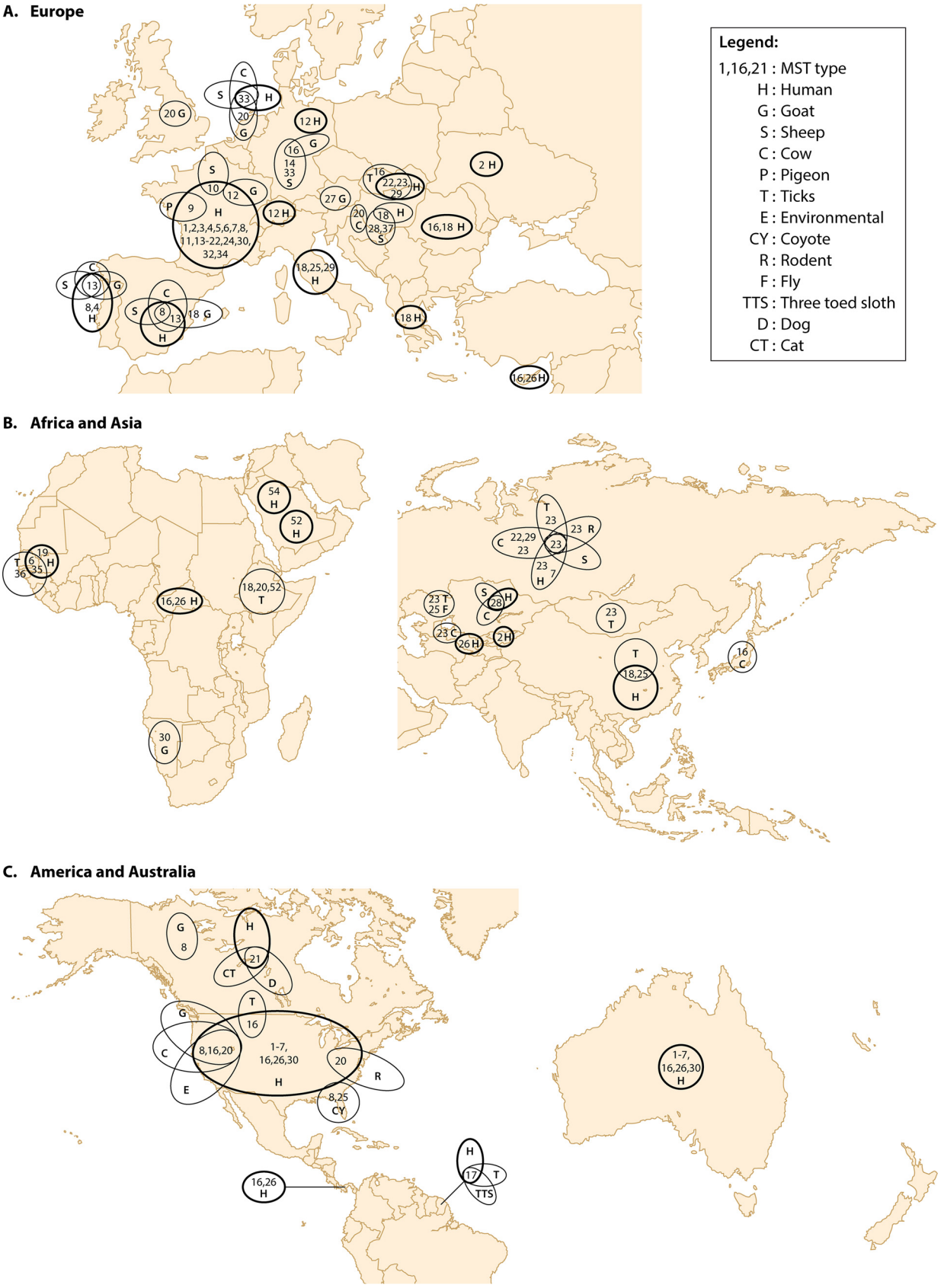

FIG 1 *C. burnetii* geotyping.

sheep, wild boars, goats, ticks, and rats shared common genotypes with humans, while cattle harbored a different one (198). In France, Sidi-Boumedine et al. used the randomly amplified polymorphic DNA (RAPD) method for genotyping of 10 isolates from goats, sheep, and cows (199). This method was more discriminant than the MLVA method among these strains.

Phylogenetic Aspects

Historically, *C. burnetii* was considered a member of the *Rickettsiaceae* family in the class of *Alphaproteobacteria*. Actually, the sequencing of the 16S rRNA gene has shown that it is a member of the *Gammaproteobacteria*, close to *Rickettsiella grylli* and *Legionella pneumophila* (200) (Fig. 2). For years, *C. burnetii* was the only species of the *Coxiella* genus, so that phylogenetic studies were not accurate. However, comparison with some close genera revealed interesting features. Like *Legionella*, *C. burnetii* can survive in amoebae (118). *C. burnetii* has a sterol-delta-reductase gene very similar to that of *Legionella drancourtii*. This gene is eukaryotic and is not present in other close relatives (118, 201). This suggests horizontal gene transfer between these two bacteria within the amoeba (201). Similarly, nontuberculous mycobacteria that can live in amoebae have a pyridine nucleotide disulfide oxidoreductase (pyr-redox) very close to that of *C. burnetii* (202).

Coxiella cheraxi, another member of the genus, was described in 2000, showing 95.5% similarity with *C. burnetii* in 16S rRNA (203). To date, its genome sequence is still not available. The most recent advances in the description of *C. burnetii* phylogeny have resulted from the study of *Coxiella*-like endosymbionts from ticks (204–207). These microorganisms have been identified mainly thanks to 16S rRNA sequencing. A *Coxiella*-like endosymbiont from the tick *Amblyomma americanum* (CLEAA) has been studied by Smith et al. (204). CLEAA is phylogenetically closely related to *C. burnetii* but has a reduced genome and does not seem to derive from it. These two species are both derived from a probable common ancestor (204). Recently, the phylogenetic analysis of a third species, the *Coxiella*-like symbiont of *Rhipicephalus turanicus* ticks (CRt), suggested that it also shares the same common ancestor (207). However, compared to these two other species, the genome of *C. burnetii* shows a high number of unique genes, suggesting gene gain events in its lineage (207). A deeper analysis among *Coxiella*-like tick endosymbionts has been performed by Duron et al. (205). They combined a new multilocus typing method and whole-genome sequencing for the phylogenetic analysis of *Coxiella*-like DNA from 637 specimens of ticks. They found a high diversity of *Coxiella*-like organisms, which could be divided in four clades (A to D) (Fig. 3). These *Coxiella*-like microorganisms were widely distributed among the different tick species, suggesting a long coevolution of *Coxiella* and ticks. This work also revealed that *C. burnetii* belonged to a unique subclade within clade A of *Coxiella*-like endosymbionts associated with soft ticks. Thus, *C. burnetii* has probably recently emerged from a tick-borne ancestor.

The genomes of *Coxiella*-like organisms are small (0.7 and 1.7 Mbp) (207). All known genomes of C. *burnetii* are larger (2.0 to 2.1 Mbp) than genomes of *Coxiella*-like organisms. Thus, it has been proposed that *C. burnetii* recently evolved to vertebrate pathogenicity from an inherited endosymbiont of ticks, due to spontaneous mutations or horizontal gene transfers from pathogens that coinfected the same tick or vertebrate host (205, 206). We recently detected a new *Coxiella*-like species, "*Candidatus* Coxiella massiliensis," from ticks and skin biopsy specimens of patients presenting with eschars and SENLAT (scalp eschar and neck lymphadenopathy after tick bite). This microorganism may be one of the etiological agents of this syndrome. Further studies are needed to know if "*Candidatus* Coxiella massiliensis" is a representative agent of the evolution from tick endosymbiont to human pathogen (208).

Genetic Transformation

The first genetic transformation experiment with *C. burnetii* was achieved in 1996 by Suhan et al. (209). Before that, the same authors performed cloning in *Escherichia coli*

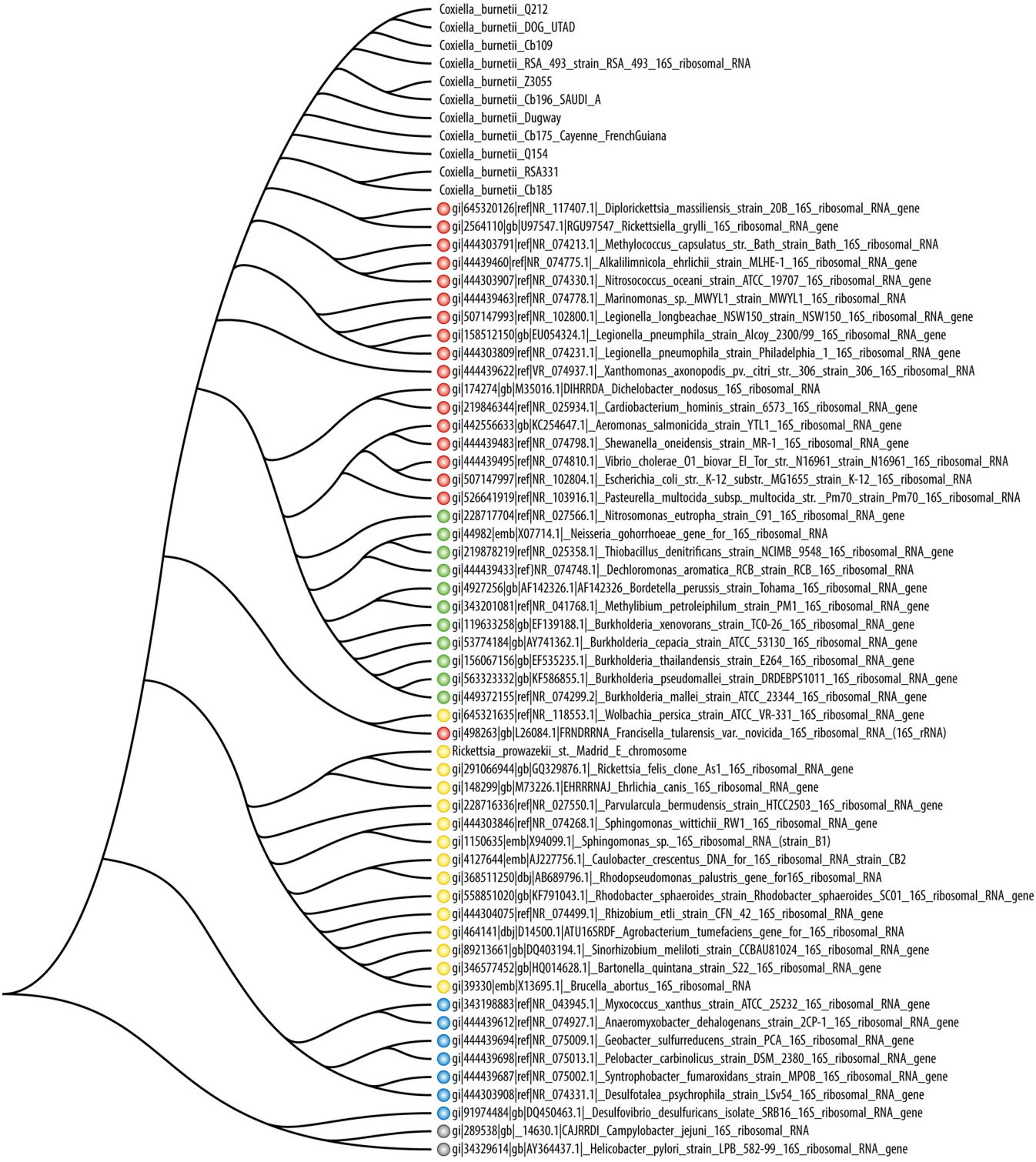

FIG 2 Phylogeny estimation of proteobacterial 16S rRNA genes. (Adapted from reference 165 with permission of Future Medicine Ltd.)

to identify an autonomous replicating sequence (ARS) of 403 bp (210). This sequence was used to transform *C. burnetii* to ampicillin resistance. The transformation was successfully achieved by using a plasmid containing the ARS and a ColE1-type replicon encoding beta-lactamase, which was introduced with electroporation (209). Some years later, Lukacova et al. proposed improving the technique by adding a green fluorescent protein as a marker of transformation (211). Then, in 2009, Beare et al. reported the first

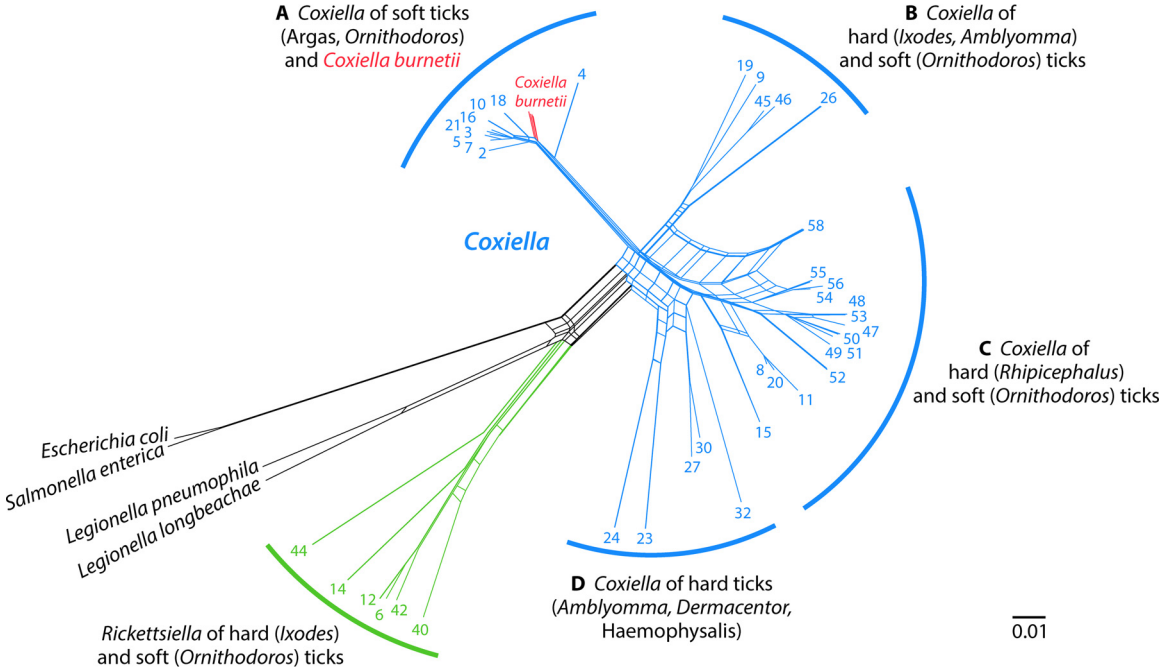

FIG 3 Phylogenetic network with concatenated 16S rRNA, 23S rRNA, *groEL*, *rpoB*, and *dnaK* sequences (3,009 unambiguously aligned base pairs), including 71 *Coxiella*-like strains of ticks, 15 *C. burnetii* reference strains, and bacterial outgroups. (Adapted from reference 205.)

defined gene mutation obtained with the *Himar* transposon system inserted in the gene for FtsZ, a protein critical for cell division (212). However, at that time the absence of an axenic medium made the procedure very cumbersome, requiring 8 to 12 weeks to obtain mutants. The elaboration of the axenic medium ACCM2, allowing host cell-free growth of *C. burnetii*, has been a real breakthrough in this domain. Thanks to axenic culture, Omsland et al. reduced the time to obtain transformants to 16 days: 4 days for transformant recovery in ACCM2 and 12 days for colony development and expansion (41). Recently, Beare et al. reported two methods of targeted gene deletion in *C. burnetii* grown axenically. They used a Cre-*lox*-mediated or a loop-in/loop-out system for recombination and deletion (213). These two systems were used for the deletion of *dotA* and *dotB*, which are genes involved in the T4SS. Both strategies used a *sacB*-mediated sucrose counterselection to select for mutants (*sacB* produces an enzyme that converts sucrose to levans, which are toxic for most Gram-negative bacteria). Both systems were successful in producing *dotA* and *dotB* mutants, which presented a significant lack of intracellular replication owing to failure in the production of the intracellular vacuole. These new possibilities of targeted gene deletion will significantly improve the understanding of *C. burnetii* virulence mechanisms. Putative virulence and nonvirulence genes will be easily knocked out because of genetic transformation.

PATHOPHYSIOLOGY

Role of the Strain in Virulence

Primary infection. (i) Comparison of strain virulence in animal models. Since the 1960s, several animal models, including mice, guinea pigs, and anecdotally rabbits and nonhuman primates, have been used to describe *C. burnetii* pathogenicity (214–220). However, none of these models is able to mimic the disease observed in humans. Murine rodents are poorly susceptible to *C. burnetii* infection, and consequently a high dose of this bacterium is necessary to induce organ lesions. The A/J mouse strain was described as the strain most sensitive to *C. burnetii* infection, with a higher mortality rate than BALB/c and C57L/6 mice (221, 222). It has been considered the best mouse model to evaluate the immune response to *C. burnetii* infection and vaccine efficacy (221). BALB/c and C57BL/6 mice showed minimal and transient signs of infection and

no deaths. They were considered resistant to *C. burnetii* infection. In contrast, severe combined immunodeficiency (SCID) and nude mice present durable and severe signs of the disease and even die. The 50% lethal dose of *C. burnetii* Nine Mile strain in SCID mice was 10^8 lower than that in BALB/c mice, making SCID mice the model of choice to study the virulence of this bacterium (223). Clinical signs of discomfort, bacterial burden, and histological findings were stronger and occurred earlier in SCID mice than in A/J, BALB/c, and CB57BL/6 mice (86, 224). Nude mice or athymic mice are useful models for evaluating the role of cell-mediated immunity in the host defense against Q fever (225). Interleukin-10 (IL-10) transgenic mice, which constitutively overexpress IL-10 in their macrophage cells, are used to study persistent *C. burnetii* infection (226, 227). Guinea pigs are globally most susceptible to Q fever, which represents a life-threatening disease in these animals. They are used to study *C. burnetii* virulence, dissemination, and persistent infection (223, 228, 229). Rabbits were anecdotally used to study the ability of the Priscilla and Nine Mile strains to induce endocarditis (217). Finally, the nonhuman primate model of cynomolgus monkeys showed a *C. burnetii* infection very close to that observed in humans. These animals were used to study *C. burnetii* vaccine efficacy (219, 220, 224).

The pathogenicity and virulence of *C. burnetii* depend on the infected animal species, the route of infection, the *C. burnetii* strain, and the inoculum size (186). The first experiments were performed using intraperitoneal inoculation of *C. burnetii*, which led to a short incubation time and diffuse infection (230). Using intranasal injection and aerosolization routes, organ lesions were observed 7 days after infection and involved mainly the lungs, with lower systemic spread of bacteria. Since then, aerosolization of *C. burnetii*, which better mimics the natural route of infection, has become the reference method to induce *C. burnetii* infection in animals for pathogenicity studies (231–233).

The bacterium's virulence includes its capacity to induce clinical symptoms such as fever, splenomegaly, and death. Early studies have compared the pathogenicity of the Nine Mile phase I and the Priscilla phase I strains of *C. burnetii*, which present different phase I lipopolysaccharides (LPSs) (217). Intraperitoneal inoculation of *C. burnetii* in guinea pigs showed that an inoculum of fewer than 4 organisms of the Nine Mile phase I was sufficient to induce seroconversion and fever, whereas more than 10^5 bacteria of the Priscilla strain were necessary to induce fever (234). The lower virulence of the Priscilla strain in comparison to the Nine Mile strain was then confirmed by Kazar et al. in BALB/c mice and guinea pigs infected via the intraperitoneal route (235). Using aerosolization in guinea pigs and SCID mice to compare the pathogenicities of 8 *C. burnetii* strains from four phylogenetic groups, Russel-Lodrigue et al. confirmed that the Priscilla strain was less virulent than the Nine Mile strain, as evidenced by a delayed onset of symptoms, a slower progression of the disease, and a lower bacterial load in the spleens in animals infected with the former strain (223). In contrast to data obtained in humans, the Priscilla strain was not associated with a higher risk of endocarditis, probably because host factors such as preexisting valvulopathy were not considered in this work. However, these last results correlated with previous studies of Q fever endocarditis in rabbits, showing that the Priscilla strain did not induce more cardiac lesions than the Nine Mile strain (217, 236). Comparing the Nine Mile RSA493 strain to 8 other isolates (African RSA334, Ohio 314RSA270, MSU Goat Q177, P Q172, G Q212, S Q217, and Dugway 5J108-111), Russel-Lodrigue et al. showed that the Nine Mile strain was the most virulent (223). Nine Mile strain pathogenicity has been widely investigated in mouse models, but none have compared its virulence with those of *C. burnetii* epidemic strains. We recently compared the virulence of the *C. burnetii* French Guiana and Netherlands-like strains to the referent Nine Mile strain using SCID and BALB/c mice. The Netherlands-like strain was isolated in 1992 in Germany from a ewe placenta and displayed the same MST33 genotype as the one responsible for the Netherlands outbreaks (143). We first noted that SCID mice infected with the French Guiana strain presented more severe signs of infection, with up to 20% weight loss at day 28 postinfection, whereas symptoms appeared later with the Netherlands-like and the Nine Mile strains (237; C. Melenotte et al., presented at the ESCCAR International

Congress on Rickettsia and Other Intracellular Bacteria, 13 to 16 June 2015, Lausanne, Switzerland). Spleen weights and bacterial loads were significantly higher and histological lesions appeared earlier in mice infected with the French Guiana and the Netherlands-like strains than in those infected with the Nine Mile strain. Finally, the serological response was exacerbated with the Guiana and the Netherlands-like strains, which corroborated data obtained in humans (238). This *in vivo* model highlights the higher virulence of the two epidemic strains, Guiana and Netherlands-like, than of the Nine Mile strain. The French Guiana strain was found to be the most virulent, followed by the Netherlands-like strain, which displayed intermediate virulence potential between those of the French Guinea and the Nine Mile strains.

(ii) Role of secretion systems in virulence. The sequenced genome of *C. burnetii* (Nine Mile RSA493) revealed the presence of several genes encoding adhesion, invasion, detoxification, and secretion system proteins (9). Interestingly, genes encoding components (IcmT, IcmS, and IcmK) of a type IV secretion system (T4SS), which are mechanistically related to the *Legionella* Dot/Icm apparatus (239), may contribute to the formation of the *C. burnetii*-containing vacuole (240, 241). The propagation of in axenic (host cell-free) culture (38, 41) has opened a technique for the genetic manipulation of this bacterium. The manipulation of an avirulent strain of *C. burnetii* and screening of the *C. burnetii* DNA library for genes encoding protein effectors have highlighted the fact that the *C. burnetii* Dot/Icm T4SS is involved in the establishment of a mature *C. burnetii*-containing vacuole and thus in intracellular multiplication of this pathogen (240, 241). However, such results could not be extrapolated to virulent strains. More recently, the importance of secretion systems in *C. burnetii* virulence was unraveled using the virulent *C. burnetii* 175 strain causing Q fever cases in Cayenne, French Guiana. The authors suggested that the virulence of the *C. burnetii* 175 strain is related to the loss of the type 1 secretion system (T1SS) encoded by the *hlyCABD* operon and thus to a genome reduction event (144).

Persistent infection. (i) LPS. The genetic diversity and virulence potential of *C. burnetii* strains are related to the expression of the lipopolysaccharide (LPS). *C. burnetii* displays LPS antigenic variations. The phase I infective form of *C. burnetii* is isolated from patients with Q fever. The avirulent form, known as phase II bacteria, is obtained following long-term *in vitro* propagation of *C. burnetii* in cell cultures (86). This leads to an irreversible modification of the LPS, with a progressive decrease in its molecular weight, up to a severely truncated LPS (rough form), owing to a genomic deletion (86). Lipid A of the LPSs from virulent and avirulent *C. burnetii* strains displays the same ionic species and fragmentation profiles, suggesting that it has a very similar structure in both types of strains (242). The major difference between the LPSs of virulent and avirulent *C. burnetii* strains resides in the core sugar, since the O antigen is missing in the LPS of the latter strains. The LPS of virulent strains (but not that of avirulent strains) contains sugars such as virenose, dihydrohydroxystreptose, and galactosamine uronyl-(1,6)-glucosamine (243). Interestingly, an intermediate-length LPS has been characterized at the surface of the Nile Mile Crazy strain (244), due to a large chromosomal deletion eliminating open reading frames involved in the biosynthesis of O-antigen sugars, including the rare sugar virenose (245).

(ii) Other virulence factors. Adhesion genes encode an RGD motif or proteins containing ankyrin repeats (9). Interestingly, the eukaryotic cell receptor for *C. burnetii* is an integrin $\alpha v\beta 3$, which interacts with the RGD motif (246). Genes encoding invasion proteins are similar to those engaged in cytoskeleton reorganization and uptake of *L. pneumophila* and enteropathogenic *Escherichia coli* by host cells. These genes probably play a role in the different levels of uptake of virulent and avirulent *C. burnetii* strains (246) and cytoskeleton reorganization observed during cell infection by *C. burnetii* (247). Detoxification genes encode superoxide dismutase, catalase, and acid phosphatase. These enzymes have been described as allowing *C. burnetii* to escape from the microbicidal activity of macrophages due to the detoxification of reactive oxygen intermediates produced by the host cells (248, 249). In addition, in the *C. burnetii* genome, a gene coding for a peptidyl-poly-*cis-trans*-isomerase has been identified,

similar to those expressed by *L. pneumophila*. It has been suggested that peptidyl-poly-*cis-trans*-isomerase may interfere with cytokine production and consequently with the replication of *C. burnetii* (250).

Role of the Host

Immunological response and phase variation. Humans develop primary *C. burnetii* infection that is symptomatic in fewer than half of cases. The primary infection almost always resolves without antibiotics, suggesting that the host immune response is sufficient to control *C. burnetii* infection (251). The host defense relies on systemic cell-mediated immunity involving innate and adaptive partners of the immune response. One of the main features of the immune response is the formation of a granuloma under the control of gamma interferon (IFN-γ). Granulomas are characterized by an accumulation of immune cells around a central open space and limited by a fibrin ring, which led to the definition of a doughnut granuloma. They are rich in macrophages with different levels of maturation, including epithelioid cells and multinucleated giant cells (252, 253). The presence of neutrophils in granulomas suggests that these cells are involved in the defense against *C. burnetii*, as recently demonstrated in neutrophil-depleted mice (254).

These granulomas have a microbicidal activity against *C. burnetii*, since they are paucibacillary during Q fever infection (251). The presence of neutrophils in Q fever granulomas suggests that these cells play a role in the defense against *C. burnetii*. Incubation of beads coated with *C. burnetii* phase I or II with mononuclear cells allowed us to investigate the mechanisms of granuloma formation in *C. burnetii* infection. First, phase II *C. burnetii* was less efficient than phase I microorganisms in inducing granuloma formation; second, monocytes were more critical than lymphocytes in granuloma formation; and third, granulomatous cells exhibited a transcriptional program that clustered with that of IFN-γ-stimulated macrophages, which are known to be a canonical model of microbicidal cells (255, 256).

The formation of granulomas in naive patients underlines the role of myeloid cells in the initial interaction of *C. burnetii* with innate immune cells. There is an exhaustive literature, including our contributions, which shows monocytes and macrophages to be major targets of *C. burnetii*. This bacterium binds to αvβ3 integrin expressed by myeloid cells and induces their activation, as assessed by remodeling of the cytoskeleton, signal signaling activation, and production of a large cytokine panel (246, 251). There is also a certain level of polarization of monocytes and macrophages after they have encountered *C. burnetii*. Resting monocytes that allow bacterial survival without replication exhibit an M1-type program similar to that induced by IFN-γ. *C. burnetii* replicates in macrophages and induces an M2-related program similar to that induced by IL-4 or IL-10 (257).

Dendritic cells (DCs) are specialized in antigen presentation to T cells and have been more recently recognized as targets of *C. burnetii*. Initial studies revealed that phase I *C. burnetii* infects and blocks the maturation of human dendritic cells, in contrast to phase II *C. burnetii* (258). Recently, we compared the responses of myeloid DCs (mDCs) to a panel of intracellular pathogens and demonstrated that the blockade of mDC maturation was partial, since they were still able to induce T-cell proliferation. The transcriptomic analysis of *C. burnetii*-stimulated mDCs revealed subtle alterations in type I IFN signaling (259). We also showed that *C. burnetii* is able to activate plasmacytoid DCs (pDCs), inducing their maturation and the release of type I IFN, a property of the host response to virus. The presence of *C. burnetii* within pDCs in Q fever lymphomas may be indicate an original role of pDCs during Q fever.

Besides αvβ3 integrin, the recognition of *C. burnetii* requires pattern recognition receptors, including Toll-like receptors (TLRs). Nevertheless, there are discordant data in the literature concerning their respective roles. We showed that TLR4 is involved in *C. burnetii*-stimulated cytoskeleton reorganization and the inflammatory response. However, it is not necessary for bacterial elimination *in vivo* (260). TLR2 plays a role in the type 1 immune response induced by phase II *C. burnetii*, and TL2-deficient macro-

phages are permissive to bacteria (242). We found that TLR2 is required for granuloma formation, as is TLR4 (261). The nature of the LPS from *C. burnetii* may explain the discrepancies about the role of TLR in bacterium-cell interactions. The lipid A of *C. burnetii* LPS is tetra-acylated, whereas LPS from *Escherichia coli* is hexa-acylated. Phase I LPS from *C. burnetii* would be a poor immunogenic stimulus and functions as an antagonist of TLR4-dependent signaling in macrophages, in contrast to LPS from *E. coli* (242). The use of a mouse model does not allow identification of this TLR4 antagonist property of *C. burnetii* LPS. Recently, it has been reported that humans with polymorphisms in TLR1 and NOD2 have reduced production of cytokines after infection with the *C. burnetii* Nine Mile and Dutch strains, and those with a polymorphism in TLR6 have an alteration of cytokine production only with the Dutch strain (262). Recently, it was reported that TLR10 exerts an inhibitory effect on cytokine production by *C. burnetii*-stimulated mononuclear cells; the polymorphisms of TLR10 described in Q fever patients were not associated with a specific clinical expression or prognosis (263).

The adaptive immune system is required to cure *C. burnetii* infection. The use of murine models such as SCID mice (264) and the follow-up of patients with immunosuppression (86) strengthen this statement. There is an unequal efficiency of the two arms of the adaptive immune response, T cells and antibodies.

T cells are necessary to control the infection, since nude and SCID mice are highly sensitive to *C. burnetii* infection (225, 264), and the reconstitution of SCID mice with $CD4^+$ or $CD8^+$ T cells is sufficient to restore protective immunity. It seems that $CD8^+$ T cells are more efficient in controlling *C. burnetii* infection than $CD4^+$ T cells (265). Recently, we found that central memory $CD8^+$ T cells are increased, whereas naive $CD8^+$ T cells are decreased, in patients with Q fever endocarditis (266). We also showed that the expression of PDL-1 is increased in patients with acute Q fever, and this would prevent the expansion of memory T cells (266). Such an imbalance of circulating lymphoid cells may play a major role in the chronic progression of Q fever.

It has been established for a long time that the polarization of the T-cell response toward the Th1 phenotype, i.e., the ability to produce IFN-γ, was the paradigm of the protective response against intracellular pathogens. Indeed, it has been shown that virulent *C. burnetii* induces a Th1 protective immune response, in contrast to avirulent variants (267). Some epitopes of *C. burnetii*, identified by bioinformatics, are able to stimulate Th1 T cells (268). There is converging evidence that IFN-γ makes macrophages resistant to *C. burnetii*. First, mice with knockouts (KO) in the IFN-γ gene are highly susceptible to *C. burnetii* infection (269). Second, we showed that IFN-γ induces a microbicidal response against *C. burnetii* in macrophages (270). The mechanisms used by IFN-γ to control *C. burnetii* infection are diverse and vary with the type of host. The microbicidal effect of IFN-γ does not depend on reactive oxygen intermediates, and Q fever is no more frequent in patients with chronic granulomatous disease (270, 271). The role of reactive nitrogen intermediates is debated, and if they contribute to the IFN-γ effect, this is restricted to murine models (242, 270, 272, 273). IFN-γ induces a microbicidal program directed at *C. burnetii* via oxygen-independent mechanisms, including phagosome maturation (274), apoptosis (270), production of cytokines such as tumor necrosis factor (TNF) (275), and regulation of nutrients such as iron (251). Taking the data together, a reductionist point of view about the role of Th1 cells and IFN-γ had prevailed in earlier years. The primary infection, with *C. burnetii* progressing to cure or the immune response to vaccination, was considered to correspond to a Th1 immune response with IFN-γ production, while both were considered defective in patients with persistent *C. burnetii* infection (276, 277). However, Dutch teams have demonstrated that, in contrast to what had been generally assumed, the production of IFN-γ is not defective in persistent *C. burnetii* infection (278, 279). The production of IFN-γ is used as a specific biomarker of chronic Q fever and, like IL-2, it is a good marker of antibiotic treatment efficacy (233, 280). In addition, these patients respond to IFN-γ by upregulating genes downstream of the IFN-γ-receptor and producing neopterin (T. Schoffelen, J. Textoris, C. P. Bleeker-Rovers, A. Ben Amara, J. W. M. van der Meer, M. G. Netea, J.-L. Mege, M. van Deuren, and E. van de Vosse, submitted for publication). The

fact that genetic polymorphisms in IL12B are associated with the development of Q fever (Schoffelen et al., submitted) emphasizes the fact that persistent *C. burnetii* infection does not result from a deficiency in Th1 polarization.

If the persistence of *C. burnetii* is not the consequence of a defective type 1 immune response, several hypotheses can be considered. There is an immunosuppressive context in the persistent progression of Q fever. There are changes in circulating immune cells, including depressed $CD4^+$ T cells (281), naive $CD8^+$ T cells (266), nonclassical monocyte subsets (282), $CD56^{dim}$ NK cells, and increased naive $CD8^+$ T cells (266) and regulatory T cells (283). There is also an exacerbated inflammatory response consisting of high levels of TNF, IL-1, IL-6, CD23, neopterin, and chemokines (251, 275, 284, 285). It is likely that this uncontrolled inflammatory reaction may contribute to the pathogenesis of Q fever. Also, the persistence of *C. burnetii* is associated with the upregulation of immunoregulatory mediators such as prostaglandin E_2 (276) and IL-10 (286, 287).

The second arm of the adaptive immune response, antibodies, has been considered dispensable for host protection against *C. burnetii* infection. Animal models and human infection are characterized by the production of specific antibodies. Initial studies showed that passive transfer of antibodies protected guinea pigs from a subsequent challenge with *C. burnetii* (288). Passive immunization of naive mice with antibodies isolated from vaccinated mice provided full protection (267). However, immune sera or B cells from *C. burnetii*-challenged mice do not confer protection against *C. burnetii* when transferred to SCID mice (267). High antibody titers directed to phase I and phase II antigens are found in persistent *C. burnetii* infection and are not effective for *C. burnetii* clearance. Moreover, it is likely that these specific antibodies have deleterious effects in Q fever patients. They can contribute to tissue lesions via the formation of immune complexes (289). They can also regulate the activity of cells expressing receptors for immunoglobulins, but that point remains debated. We showed that *C. burnetii* opsonized with specific immunoglobulins from Q fever patients is more readily internalized, reaches multibacillary vacuoles, and stimulates the production of IL-10 (290). Other groups did not find an immunosuppressive effect of opsonization but found rather that it could favor DC maturation and cytokine production (291). Finally, antibody formation during *C. burnetii* infection can target host components, and autoimmunity has been associated with acute Q fever (292–294). During primary *C. burnetii* infection, anticardiolipin antibodies are usually detected, and high levels of IgG anti-cardiolipin antibodies have been associated with rapid progression to *C. burnetii* endocarditis (295).

In conclusion, the control of *C. burnetii* primary infection is obtained via the induction of a full cell-mediated immune response, in which the Th1 response and IFN-γ production are the most important, while antibodies are dispensable. This strong Th1 response may cause autoimmune disorders. The progression to *C. burnetii* persistent infection reflects failure of the Th1 response and results from a combination of intrinsic and extrinsic parameters, in which IL-10 plays a significant role.

Determinants of the intracellular persistence of *C. burnetii*. In contrast to the case for human mononuclear phagocytes, cumulative evidence suggests that *C. burnetii* vacuoles mature through the endolysosomal cascade to a phagolysosome in nonprofessional phagocytes. However, this is overly simplified, because *C. burnetii* modifies the phagosome to create a vacuole with fusion with other endolysosomal and autophagic compartments and cargo from secretory pathways (296).

In human mononuclear phagocytes, the intracellular trafficking of *C. burnetii* has been well described (274). After phagocytosis, virulent and avirulent *C. burnetii* organisms are localized within early phagosomes harboring early endosome antigen 1 (EEA-1) and the small GTPase rab5. The early phagosome is then converted into a late phagosome, characterized by lysosome-associated membrane protein 1 (LAMP-1), LAMP-2, and LAMP-3, the mannose-6-phosphate receptor (M6PR), and vacuolar proton ATPases responsible for the acidic pH (pH 4.5) of the compartment containing virulent or avirulent *C. burnetii*. The phagosome hiding avirulent *C. burnetii* acquires the small

rab7, which is required for phagolysosome transition (297–299), and the lysosomal enzyme cathepsin D (274). In this compartment, avirulent bacteria are destroyed. Interestingly, virulent *C. burnetii* blocks the conversion of the late phagosome into a phagolysosome by inhibiting the recruitment of rab7 and cathepsin D (274, 300), avoiding its degradation in the phagolysosome. Part of the mechanisms used by virulent *C. burnetii* to hijack the phagosome maturation has been elucidated (301, 302). Indeed, it has been demonstrated that variations in LPS determine the intracellular localization of *C. burnetii*. Avirulent *C. burnetii* LPS stimulates host p38α–mitogen-activated protein kinase (MAPK) signaling, which is required for the trafficking of bacterium-containing vacuoles to phagolysosomes for their destruction. In contrast, *C. burnetii* LPS does not. The defect in *C. burnetii* targeting to degradative phagolysosomes involves an antagonistic engagement of Toll-like receptor 4 (TLR4) by *C. burnetii* LPS, lack of p38α-MAPK-driven phosphorylation, and blockade of recruitment of the homotypic fusion and protein-sorting (HOPS) complex component vacuolar protein sorting 41 (Vps41) to *C. burnetii* LPS-containing vesicles. Thus, p38α-MAPK and its cross talk with Vps41 play a central role in trafficking *C. burnetii* to phagolysosomes (301). In addition, it has been recently demonstrated that *C. burnetii* avoids p38α-MAPK activation by the disruption of the association of TLR2 and TLR4 via an actin cytoskeleton reorganization induced by LPS (302). Several other factors, such as opsonization (290) and cytokine production (303), may greatly contribute to the establishment of the *C. burnetii* replicative vacuole, but the mechanisms involved remain unclear. Opsonization of *C. burnetii* with specific antibodies produced during persistent Q fever seems to prevent phagosome conversion, because large parasitophorous vacuoles containing *C. burnetii* but not expressing cathepsin D are formed (290). Cytokines have been shown to modulate phagosome maturation (303). In case of *C. burnetii* infection, several data suggest that IL-10 could contribute to the establishment of *C. burnetii* replicative phagosomes. The neutralization of IL-10 from monocytes of patients with persistent *C. burnetii* infection increases the killing of *C. burnetii* and rescues phagolysosome fusion similarly to what is observed in cured patients. In contrast, adding IL-10 to monocytes from patients with cured *C. burnetii* endocarditis avoids the killing of *C. burnetii* and inhibits phagolysosome biogenesis (300).

Host susceptibility factors. Epidemiological studies have shown that Q fever symptoms are more frequent in men than in women (male/female ratio, 2.5), while both are similarly exposed to this pathogen, as evidenced by similar seroprevalence rates (304, 305). Recently, the follow-up of patients vaccinated during the Q fever outbreak in the Netherlands revealed higher rates of adverse effects in women than in men (306). The use of murine models of infection revealed the role of sex hormones in the predisposition for infection in men. Hence, female C57/BL6 mice are more resistant than males to *C. burnetii* infection; this property is lost after ovariectomy and is restored by the administration of 17β-estradiol (307). The study of gene expression signatures in mice infected with *C. burnetii* with and without castration shows the importance of sex-related genes (86% of genes differentially modulated in males and females) and the role of sex hormones, which account for 60% of modulated genes (308). The functional annotation of modulated genes enabled the identification of different clusters in males and females. The enrichment in clusters containing genes associated with the inflammatory response may account for the inflammatory profile of men with Q fever. Among the clusters enriched in infected females, the circadian rhythm pathway (consisting of positive molecules such as Clock and Arntl and negative molecules such as Per) is still not understood and may reveal some features of a microbicidal response to *C. burnetii*. Data for humans are rare. It has been shown that the expression of Per2 is increased in men with acute Q fever (309).

(i) Age. Age is a risk factor for *C. burnetii* infection. Symptomatic Q fever is more frequent in individuals older than 15 years (310). The immunity of adults to *C. burnetii* accounts for the adult response to vaccine. During the vaccination campaign with Q-vax vaccine in the Netherlands, the best cellular and humoral responses to the

antigen as well as protection against the infection were reported in young adults, who also suffered most frequently from local adverse effects (306).

In 14-month-old mice (corresponding to adult humans), the bacterial burden and the number of granulomas in tissues were higher than those in 1-month-old mice (307). Elderly patients develop an immune response to *C. burnetii* that reflects the level of immunosenescence. Patients at risk of persistent Q fever who are older than 75 years are lower producers of IFN-γ and specific antibodies than younger patients (311). The use of immunosuppressive drugs is likely a confounding factor.

(ii) Pregnancy. The pathophysiology of Q fever during pregnancy is poorly understood, especially in humans. The infection of goats with *C. burnetii* provided some mechanistic hypotheses. After inoculation of pregnant goats, the primary targets are the trophoblasts of the allantochorion, while trophoblasts covering cotyledonary villi are not infected. The infection leads to the production of specific antibodies that persist for several months after delivery. There is an inflammatory reaction varying from mild infiltration of mononuclear cells to necrotic lesions (90). There is also a cellular response during and after parturition. IL-10 is upregulated during pregnancy, whereas TNF and IL-1 are increased after parturition (312). In women, there is some evidence that the immune response is silenced and antibodies are produced in response to infection (313). This might be related to IL-10 production, which may account for the increased risk of endocarditis after delivery. In human trophoblasts infected *in vitro* with *C. burnetii*, the bacteria replicate within vacuoles that express lysosomal markers, in contrast to myeloid cells. In addition, the interaction of *C. burnetii* with trophoblasts does not induce an inflammatory program, accounting for the relative immune silence observed during Q fever infection in pregnant women (314). This is strengthened by a recent study in which we showed that *C. burnetii* is unable to activate decidual DCs (315). It is likely that alterations of the immune response during pregnancy correspond mainly to the production of IL-10 and the silencing of DCs, which favors bacterial growth (312, 314).

(iii) Genetic factors. Preexisting valvulopathy enhances the risk of endocarditis in patients with *C. burnetii* infection. The lack of vegetation and inflammation suggests a mechanism distinct from the colonization of cardiac valves found in usual infectious endocarditis. This suggests that factors related to the cardiac valve context, including the immune context, are involved. The study of cardiac valves and circulating mononuclear cells from patients with degenerative valvulopathy using microarray technology revealed the enrichment of the inflammatory program (316, 317); this creates the condition for the binding of inflammatory leukocytes, infected or not with *C. burnetii*. These findings are supported by the detection of activated lymphocytes and macrophages infiltrating bicuspid and tricuspid calcified stenosis (318). The study of lymphocytic populations revealed the important role of $CD8^+$ T cells with a memory-effector phenotype and oligoclonal repertoire, while the role of memory-effector $CD4^+$ T cells was less significant (319). *C. burnetii* endocarditis is more frequently observed in patients with bicuspid aortic valves (BAV) (320). BAV is the most frequent congenital heart disorder and has a sizeable heritable component, with some genes specifically associated with this entity (321). The analysis of genes expressed in BAV compared with tricuspid aortic valve controls showed the enrichment of the latter, with genes associated with inflammation and the immune response, compared with the former, in which genes involved in the development are overexpressed. This poor inflammatory context of BAV may favor the colonization of valves with infected cells. The mechanism of Q fever endocarditis in patients with BAV remains hypothetical. Although mechanical conditions such as increased flow turbulence due to inappropriate opening are likely, we hypothesize that apoptosis may be involved. Indeed, BAV is associated with increased apoptosis of vascular smooth muscle cells (322).

Role of IL-10

IL-10 properties. Interleukin 10 (IL-10) is a class 2 cytokine produced by macrophages, monocytes, dendritic cells (DCs), lymphocytes, B cells, mast cells, eosinophils,

and CD4$^+$ T cells. The main functions of IL-10 are immunosuppressive and anti-inflammatory, influencing both innate and adaptive responses. Hence, IL-10 contributes to maintaining appropriate conditions for microbe survival and persistent infection. During the innate immunity response to microbes, IL-10 downmodulates the production of inflammatory mediators and promotes the expression of anti-inflammatory mediators, including regulatory receptors such as the IL-10 receptor by myeloid cells. IL-10 has a strong potential for inhibiting the microbicidal activity of macrophages and the antigen-presenting activity of dendritic cells. On the other hand, IL-10 enhances its own production by CD4$^+$ regulatory T cells. The anti-inflammatory properties of IL-10 were first demonstrated in IL-10-deficient mice, which developed chronic bowel disease, probably secondary to an inappropriate and aberrant immune response to bacterial antigens. During the adaptive response, IL-10 downregulates the production of Th1 cytokines, such as IL-2, IL-3, IFN-γ, and major histocompatibility complex class II (MHC II), and costimulatory molecule expression and interferes with T-cell polarization. Thus, IL-10 promotes the proliferation and differentiation of B cells (323, 324). Animal models have been used to exhaustively describe the influence of IL-10 on host susceptibility during primary infection (323). The reduction of IL-10 increases the resistance to infection, whereas IL-10 overproduction increases host susceptibility to bacteria and parasites (323). Interleukin-10 levels are determinant in the clearance or the persistence of pathogens infecting humans (324, 325). Hence, IL-10 is associated with tuberculosis (TB) reactivation, *Bartonella henselae* persistent infection, and *Bartonella quintana* chronic asymptomatic bacteremia (326, 327). In Q fever patients, *C. burnetii* is able to persist in macrophages, to escape the immune system, and to induce persistent infection; this persistence has been associated with sustained production of IL-10 (284, 300) (see below).

Role of IL-10 in endocarditis. *C. burnetii* endocarditis lesions exhibit fibrosis, calcification, slight inflammation and vascularization, and small or absent cardiac vegetation, probably due to the intracellular nature of the bacterium (328). The persistence of *C. burnetii* in the infected host is characteristic of the progression to endocarditis. This statement is the consequence of several observations during the past years. First, IL-10 is secreted by mononuclear cells in patients with *C. burnetii* primary infection and valvulopathy at risk of developing endocarditis (286, 287). Second, IL-10 is important for the persistence of *C. burnetii*, since it interferes with the microbicidal program of host cells via the downmodulation of TNF production (300). The effect of IL-10 is not a result of nonspecific immunosuppression of macrophages, since other immunoregulatory cytokines failed to induce *C. burnetii* replication (227). Third, the impairment of macrophage microbicidal activity in patients with Q fever endocarditis is under the control of IL-10 (300). Fourth, the use of mice overexpressing IL-10 in the myeloid compartment reproduces most of the features of Q fever endocarditis, including bacterial persistence, lack of granuloma formation, and overproduction of specific antibodies (226). However, IL-10 by itself is not sufficient to cause endocarditis. Fifth, IL-10 is produced during Q fever by monocytes, by dendritic cells, and in response to anti-*C. burnetii* antibodies. When the latter are used as opsonizing antibodies, they favor the formation of multibacillary vacuoles within the macrophages and the secretion of IL-10 (290). This amplification loop may be critical in the pathophysiology of *C. burnetii* endocarditis, since IL-10 favors the production of antibodies, and specific antibodies via the recognition of phospholipid determinants are likely involved in the progression to endocarditis. The presence of IL-10 may also explain the poor inflammatory phenotype of *C. burnetii* endocarditis. We hypothesized that the phagocytosis of apoptotic leukocytes known to lead to IL-10 release is involved in the formation of endocarditis. Valvulopathy is associated with altered fluid shear stress and increased circulation of cells, including leukocytes (257). The uptake of leukocytes by monocytes and macrophages stimulates the replication of *C. burnetii* and the production of IL-10. It is likely that the immune context will determine the development of endocarditis: if patients with valvulopathy are able to exhibit a Th1-like immune response, the effects of apoptosis and Il-10 are prevented; in contrast, patients unable to develop a Th1-like response would have an

increased risk of *C. burnetii* endocarditis (251). This hypothesis will require confirmation in an animal model.

Role of IL-10 in lymphoma. IL-10 is known to play a role in B-cell lymphoma genesis by promoting B-cell proliferation and by compromising the function of immune cells, which then become unable to kill tumor cells (329, 330). High levels of IL-10 are observed in patients with non-Hodgkin lymphoma and are associated with a poor prognosis (331, 332). IL-10 was also used as a prognostic and therapeutic marker in patients with diffuse large B-cell lymphoma (DLBCL) (330, 331, 333). It was also proposed as a new therapeutic target in DLBCL (333). *C. burnetii* has recently been reported as being linked to B-cell non-Hodgkin lymphoma (334). Patients with Q fever presented an excess risk of DLBCL and follicular lymphoma (FL) compared to the general population. The direct evidence for the role of *C. burnetii* in lymphomas was provided by the study of tumoral biopsy specimens using fluorescence *in situ* hybridization; viable *C. burnetii* cells were present within macrophages and plasmacytoid dendritic cells (pDCs) but not in B cells. Furthermore, patients with Q fever and lymphoma presented an overproduction of IL-10 compared to that in patients with Q fever lymphadenitis or with acute Q fever but no valvulopathy. High levels of IL-10 were also observed in patients with *C. burnetii* lymphadenitis, in which *C. burnetii* was identified in lymph node macrophages but not in pDCs (334). By infecting macrophages and pDCs and by inducing overexpression of IL-10, *C. burnetii* might be responsible for an immune impairment that promotes both *C. burnetii* replication within the tumoral microenvironment and tumoral growth (334, 335).

CURRENT TOOLS FOR LABORATORY DIAGNOSIS

Because *C. burnetii* does not grow with the use of standard routine laboratory culture techniques, specific indirect diagnostic tools have been mainly used for diagnosis. Consequently, serology is still the most common method for testing for *C. burnetii* infection. Currently, detection of *C. burnetii* DNA by qPCR in various clinical samples (including blood, cardiac valves, or other surgical tissue biopsy specimens) is also available. It has the advantage of detecting *C. burnetii* before seroconversion in patients with primary infection. Culture can be performed by reference laboratories on the same clinical samples, but this requires a biosafety level 3 (BSL3) laboratory. Finally, pathological analysis of infected tissue samples, after immunohistochemistry staining, is an interesting tool for diagnosis when these samples are available. Improvement of the sensitivity of the main diagnostic techniques has been the principal objective in recent years.

Serology

General principles. In the presence of symptoms suggestive of *C. burnetii* infection, serology is the first-line diagnostic technique. The immune response induces the production of anti-phase II and anti-phase I antibodies (42). *C. burnetii* phase II antigen is obtained after several passages in cell cultures or eggs, and anti-phase II antibodies are predominant during primary infection (336). *C. burnetii* phase I antigen is obtained from the spleens of infected mice, and anti-phase I antibodies are associated with persistent infection (336). The phase II antibodies are detectable 7 to 15 days after the onset of clinical symptoms and decrease thereafter within 3 to 6 months (42). The diagnosis of primary infection can be made by detection of a 4-fold increase in phase II IgG or IgM antibodies between two serum samples taken 3 to 6 weeks apart (42). Antibodies are detectable by the third week after infection in 90% of patients (336). For that reason, two serum samples (one from the acute phase and one from the convalescent phase) should be analyzed. Cutoffs for a positive serological titer can vary between countries. Generally, titers of phase II IgG of $\geq$200 and/or IgM of $\geq$50 are considered significant for the diagnosis of primary Q fever infection (336, 337), and phase II IgG titers tend to be higher than phase I IgG titers during primary infection (42, 86). Independently of the symptomatology, residual IgG antibody titers may be detectable for years and even for life (338). Wielders et al. have shown that early treatment

of primary infection (before an antibody response) did not influence the subsequent IgG II response (339).

Elevated phase I IgG titers (IgG I titer of ≥1:800) are associated with persistent Q fever. Higher phase I IgG titers correlate with a higher positive predictive value (PPV) for the diagnosis of *C. burnetii* endocarditis: a PPV of 37% was found for IgG I titers of ≥1:800, and this reached 75% for IgG I titers of ≥1:6,400 in a study from our reference center (340). For that reason, investigation for persistent infection should be performed in the case of persistent high levels of phase I antibodies 6 months after completion of treatment (see Management Strategy for Patients with *C. burnetii* Infection section and Clinical Aspects section below). However, cases of Q fever endocarditis with low antibody titers have been reported. In a series of 125 patients with cardiovascular infection who underwent surgery, we found 4 patients with a definite cardiovascular infection with positive culture and/or PCR on valvular biopsy, three of whom had a phase I IgG titer at 1:400 and one of whom had a phase I IgG titer at 1:200 (341).

Serology methods. Indirect immunofluorescence assay (IFA) is the reference method, but the complement fixation test (CFT) and ELISA are also used. Others techniques exist, such as Western blotting, dot immunoblotting, radioimmunoassay, microagglutination, and the indirect hemolysis test, but they remain anecdotal. To date, only IFA, CFT, and ELISA are commercially available. The advantage of ELISA is that it is easy to perform, interpretation is less subjective than for IFA and CFT, and automation is possible. This method is mentioned in the CDC case definition of acute and chronic Q fever (42). The specificity, sensitivity, and positive predictive value vary according to the technique and the antigen used. Wegdam-Blans et al. recently compared the performance of commercially available ELISA, CFT, and IFA. For the diagnosis of primary infection, the authors used serum samples from patients with a positive qPCR on blood (342). The most sensitive technique was IFA for detecting IgM antibodies at an early phase of infection and after 12 months of follow-up. Regarding IgG, IFA was more frequently positive than ELISA and CFT (100%, 95.2%, and 96.8%, respectively), but the difference was not statistically significant (342). Regarding phase I antibodies in patients with persistent *C. burnetii* infection at the time of diagnosis, the sensitivities of CFT and ELISA were 83% and 93.9%, respectively, but the difference was not statistically significant (343). Overall, using CFT, 16.3% of patients with phase I IgG were undetected. Also, a poor correlation was observed between antibody kinetics in ELISA, CFT, and IFA. Therefore, the sensitivity of CFT appears to be too low to be recommended for diagnosis and follow-up of *C. burnetii* persistent infection.

Most reference laboratories have developed their own in-house immunofluorescence assay. In our center, screening is performed with phase II antigen on serum diluted at 1:50 and 1:100 to detect total immunoglobulins (IgT) directed against *C. burnetii* antigens (344). For all positive screenings with IgT titers of ≥1:100, quantification detection of antibodies for the subclasses IgG, IgM, and IgA for both phase I and phase II is performed. The titration of IgM and IgA is performed after removal of IgG using a rheumatoid factor absorbent to eliminate false-positive results due to interference with this protein. Moreover, the sera are diluted in phosphate-buffered saline with 3% nonfat powdered milk to saturate the antigenic site and avoid a nonspecific fixation of antibodies. Sensitivity was assessed at 58.4% and specificity at 100% (344). For sera with titers inferior to these cutoffs, the serology should be repeated within 10 to 15 days to confirm or rule out the diagnosis.

One study compared Q fever serological results from different reference centers from three countries (United Kingdom, France, and Australia) (345). The concordance between the three centers in microimmunofluorescence interpretation was only 35%. France and the UK had the lowest concordance, and the UK and Australia had the highest. This reflects the fact that serology is not the most perfect tool, because of high subjective variability of interpretation. Cross-reactions with *Legionella micdadei* and *Bartonella* have been described with IFA (346, 347). Musso and Raoult found that 34.5% of patients with primary Q fever presented significant antibody titers for *L. micdadei* (346). La Scola and Raoult observed that sera from 50% of patients with Q fever

displayed cross-reactivity with *Bartonella* (347). However, in most cases, a low level of cross-reactions was observed, and antibody titers were higher for *C. burnetii*, so that generally there was no problem in the interpretation of results.

Recently, a new automated epifluorescence assay was developed by InoDiag (Signes, France) for Q fever serological diagnosis. Except for the deposition of the serum, all subsequent steps have been automated. The performance of this technique was recently compared with that of a gold standard microimmunofluorescence technique. It showed heterogenous performances, with a low sensitivity for primary infection due to low reactivity of phase II antigens but an excellent performance for persistent infection, with 100% sensitivity in the detection of phase I antigens (348).

Molecular Detection

Several PCR-based assays have been developed for the detection of *C. burnetii* in clinical samples. The first standard PCR systems targeted sequences of different types of plasmids (349), the 16S-23S RNA, the superoxide dismutase gene, the *com1* gene or the IS*1111* repetitive elements in human or animal samples (56, 350–352). The detection limits of these different methods ranged from 10 to 10^2 bacteria. Also, nested PCR systems have been proposed, but these methods lack specificity (353, 354). Real-time PCR or quantitative PCR (qPCR) is a less time-consuming technique than PCR and has the advantage of quantifying the amount of bacteria in clinical samples. Thus, this method has become the most frequently used PCR system for diagnosis. The qPCR system targeting IS*1111* (a repetitive element which is present in about 20 copies in the *C. burnetii* Nine Mile genome) is the most sensitive (124, 355, 356). This qPCR can detect the bacterium in the sera of patients within the first 2 weeks of infection, when serology is not yet positive. It also allows detection of *C. burnetii* DNA in the blood of patients with persistent *C. burnetii* infection (357). In the Netherlands, Schneeberger et al. found *C. burnetii* DNA in 10% of seronegative samples from patients with signs of primary infection, confirming the usefulness of this method in the first 2 weeks of infection (358). In another study from the Netherlands, this assay displayed sensitivity, specificity, PPV, and negative predictive value (NPV) of 92.2%, 98.9%, 99.2%, and 89.8%, respectively, during the outbreak (359). In that study, a high DNA load during primary infection was associated with progression to persistent infection. Tilburg et al. assessed the interlaboratory concordance of IS*1111* qPCR according to the assay and a DNA extraction method used in seven laboratories across the Netherlands (360). They found that multiple combinations of DNA extraction kits and qPCR assays gave equivalent results for Q fever diagnosis. In Switzerland, a qPCR system targeting the *ompA* gene has been used for 7 years for detection of *C. burnetii* in clinical samples (361). The sensitivity was 88% for valvular samples, 69% for blood samples, and 50% for urine samples. In our laboratory, another qPCR system targeting IS*30A* repetitive elements displayed a lower sensitivity than IS*1111* qPCR (124).

Recently, we improved the sensitivity of the qPCR test targeting the *IS1111* gene by concentrating DNA extracted from clinical samples by lyophilization (362). The detection limit of *C. burnetii* DNA was 100-fold lower in lyophilized sera (1 bacterium/ml) than in nonlyophilized sera (10^2 bacteria/ml). This strategy was tested in 73 sera from patients with primary *C. burnetii* infection and 10 sera from endocarditis patients, in whom the IS*1111* qPCR performed under the usual conditions remained negative. In patients presenting with primary Q fever, we observed qPCR sensitivity gains of 44% for the seronegative sera and 30% for early seropositive sera after lyophilization. The sensitivity of qPCR was also higher in sera from patients with endocarditis, of whom 8/10 (80%) were positive after lyophilization.

Culture

The isolation of *C. burnetii* can be achieved from a wide range of clinical samples, including old samples if they have been stored at −80°C before cultivation. The shell vial technique is still the most frequently used method (363). A sample of 1 ml of the clinical specimen is inoculated on HEL cell monolayers in shell vials. The shell vials are

then centrifuged (700 × *g* at 20°C) for 1 h. Centrifugation allows better attachment and penetration of *C. burnetii* inside cells. Infected cells are then incubated at 37°C in a 5% CO_2-enriched atmosphere for 5 to 7 days. Gimenez or immunofluorescence staining is used for detection of the bacterium inside cells. Lockhart et al. have compared four different cell lines for the isolation of *C. burnetii* using two different isolates, the Henzerling and Arandale strains (364). For the Henzerling strain, DH82 cells were the most sensitive, while for the Arandale isolate, Vero cells showed the highest sensitivity. The L929 and XTC cell lines were less suitable for culture of *C. burnetii*.

Recently, the first isolation of *C. burnetii* in axenic medium from clinical samples was reported using ACCM2 (170). A sample of the heart valve from a patient with *C. burnetii* endocarditis was incubated in 20 ml of ACCM2, and growth was observed after 6 to 8 days of incubation. Inoculation of an ACCM2 agar plate with a sample of the culture-positive liquid ACCM2 yielded several colonies at day 5 (170). This new possibility could significantly facilitate the routine cultivation of *C. burnetii* from clinical samples.

Pathology and Immunohistochemistry

The immune reaction provoked in various organs by *C. burnetii* can be unraveled by pathological analysis of tissue samples after fixation and paraffin embedding. During primary infection, a typical fibrin-ring granuloma with a "doughnut" aspect can be observed on hepatic biopsy specimens (365). These granulomas have also been observed in the bone marrow (366–369). During persistent infection, pathological analysis of cardiac valves and vascular tissue can also be informative. In patients with *C. burnetii* endocarditis, histological analysis can reveal significant fibrosis, calcifications, slight inflammation and vascularization, and little or no vegetation (328). These features illustrate a slow "degenerative-like" infectious process. Immunohistochemical (IHC) detection is a more specific tool that can aid detection of *C. burnetii* in tissues. It uses a monoclonal antibody with an immunoperoxidase-based method (328). Lepidi et al. also developed a method called "autoimmunochemistry," using antibodies produced from the patient's own serum (370). IHC was used for detection of *C. burnetii* in aortic graft samples and hepatic and valvular biopsy specimens (328, 371, 372).

New Tools

Immuno-PCR. Immuno-PCR is an interesting method, combining the amplification power of PCR with the specificity and versatility of ELISA, allowing an improvement in sensitivity. We tested this method on a collection of serum samples from Q fever patients (373). Immuno-PCR had significantly better sensitivity than ELISA and IFA (90% versus 35% and 25%, respectively) in sera collected during the first 2 weeks after the onset of symptoms (373). Its specificity was evaluated at 92%.

IFN-γ and IL-2 detection. The detection of *C. burnetii*-specific gamma interferon (IFN-γ) production has been proposed as a new diagnostic tool. Schoffelen et al. have compared the performance of this method with those of classic IFA and skin tests for Q fever (278). The IFN-γ production assay is performed after *in vitro* stimulation of whole blood with antigens from the Q-vax vaccine or the inactivated Nine Mile strain. The measurement of IFN-γ production is then performed using ELISA. IFN-γ production was higher in seropositive and skin test-positive patients than in negative ones. The sensitivity and specificity (87% and 90.2%, respectively) were similar to those for the association of serology and skin test. In another study, the same authors evaluated the usefulness of this method in differentiating between past and persistent *C. burnetii* infection. IFN-γ production was significantly higher in patients with persistent infection than in controls, but overlapping values existed. Assay of a second cytokine, IL-2, revealed significantly higher values in controls than in patients with persistent infection. As a consequence, an IFN-γ/IL-2 ratio of >11 had a sensitivity and specificity of 79% and 97%, respectively, for the diagnosis of persistent infection (233). In a recent study, this ratio was proposed for monitoring treatment of persistent *C. burnetii* infection (233). It decreased significantly when patients had a successful

treatment outcome (233). Thus, an IFN-γ/IL-2 ratio of >11 may be an interesting additional marker to monitor the progress of patients with persistent infection.

CLINICAL ASPECTS

Chronic Q Fever: from Historical Background to Recent Controversy

Historical background. In 1937, Edward Holbrook Derrick reported the first clinical description of a *C. burnetii* infection when investigating an outbreak of febrile illnesses among slaughterhouse workers in Brisbane, Queensland, Australia (374). He proposed the name of "Q fever" for "query fever," until further knowledge could allow a better name. In the following years, the first U.S. case of human Q fever, in a member of the NIH staff who was cultivating an infectious agent isolated by Cox in Guinea pigs inoculated with a tick sample, was reported (375). Several outbreaks in different parts of the world were then reported, including in the Americas in the 1940s (at the NIH, in California, and in Panama) (376–378) and in Europe among allied troops during World War II (in northern Italy and the Balkans) (379). In these early reports, "Q fever" was considered to be an acute disease causing outbreaks of fever and pneumonia.

In 1949, Beck and Bell were the first to note that: "several persons have been found with a chronic febrile illness dating back to a proved attack of Q fever" (378). Some years later, Marmion et al. described what they called "a subacute Rickettsial endocarditis," which was a negative blood culture endocarditis with high levels of Q fever phase I antibodies; isolation of the bacterium was obtained in guinea pigs from an aortic valve sample (380, 381). In these reports, the term "chronic Q fever" was coined for the first time. In 1962, Powell and Stallman found that detection of IgG I was associated with protracted convalescence from Q fever in the elderly. However, the authors stated that the presence of phase I antibodies was an indication of "past persistent infection" but that it was not necessarily proof of present persistent infection in the absence of clinical signs (382). One year later, the WHO study group on rickettsial diseases established a serological cutoff for phase I antibody titers at 1:200 for the diagnosis of "chronic Q fever" (383). Over the following years, both terms, "Q fever endocarditis" and "chronic Q fever," were used synonymously to describe one and the same entity (383–385), as stressed by an editorial in the Lancet in 1976 entitled "Chronic Q fever or Q fever endocarditis?" (386). It was previously observed that these cases of endocarditis affected mainly the aortic valve (387) and had a very poor prognosis, with frequent relapses after surgical valve replacement and treatment with tetracyclines alone (388). In 1978, Turck discussed the accuracy of the serological criteria for Q fever endocarditis developed by the WHO study group (389). This author pointed out that some cases of proven Q fever endocarditis (i.e., with the organism being isolated from valves postmortem) were reported with only slightly elevated levels of phase I antibodies (389). Also in 1978, Spicer suggested that the significance of phase I antibodies should be interpreted carefully, in conjunction with the medical history (383). In 1983, Ellis et al. reported a series of 16 cases of "chronic" Q fever, among which eight were not endocarditis but one of the following conditions: aortic prosthetic graft infections, spondylodiscitis with psoas abscess, infection of a ventricular aneurysm, infection during pregnancy, and unexpected death of a 9-month-old infant (390). that paper suggested early that *C. burnetii* infectious foci could be very diverse and that a wide range of clinical settings were artificially grouped under the generic term "chronic Q fever."

However, some years later, Peacock et al. reinforced the idea that IgG I serological cutoffs could differentiate between two restricted patterns of the disease: "primary Q fever" and "chronic Q fever," which could manifest as granulomatous hepatitis or endocarditis (391). That study included 15 patients (5 with primary infection, 5 with endocarditis, and 5 with "granulomatous hepatitis") and found that granulomatous hepatitis was associated with persistent elevated phase II IgG and that endocarditis was associated with both phase I and II elevated antibodies and high levels of IgA (391). A second work by the same group with a larger sample of patients confirmed the association of phase I IgG with endocarditis (392). However, in this seminal study, the

entity of "chronic granulomatous hepatitis" was not clearly defined. Actually, granulomas were more frequently described in previous reports in acute Q fever hepatitis, while chronic liver abnormalities were described mainly in the context of endocarditis. Moreover, early serological studies in countries where the disease was endemic failed to demonstrate that Q fever was a cause of chronic liver disease (383, 390). Until now, this entity of isolated "chronic granulomatous Q fever hepatitis" has been extremely rarely reported, and most of the reports employing this term actually deal with either acute granulomatous hepatitis or chronic hepatitis associated with endocarditis (372, 393–400). This early example illustrates that cautious definitions are mandatory in order to avoid considering artificial clinical entities.

Nevertheless, the accumulation of restricted serological criteria coupled with the lack of other powerful microbiological diagnostic tools induced a dissociation between "serological chronic Q fever" and possible clinical manifestations of the infection. This is illustrated by a report in 1985 dealing with "subclinical chronic Q fever" by Fergusson et al. In that paper, the authors described seven patients with high IgG I levels but without a detectable localized infection. In fact, the serological definition of "chronic Q fever" prevented the authors from diagnosing what can easily be considered today, with current classifications, as four possible cases of endocarditis, two possible infections of cardiac ventricular aneurysms, and one possible abdominal aortic aneurysm infection (401). Unfortunately, the damage from this misleading term has continued until very recently, discouraging clinicians from investigating *C. burnetii* infectious foci. In a case report from 2006, de Silva et al. (688) described two patients with "atypical chronic Q fever" considered to be without a focus of infection, which using current diagnostic criteria could be considered possible endocarditis and/or osteoarticular infection and thus should have led to more thorough investigations.

Our team, like all specialists working on Q fever worldwide, also used the consensual "chronic Q fever" term in the past. In particular, we confirmed early on that endocarditis was associated with high levels of IgG1 antibodies (402), with titers of $>$800, and defined by a spontaneous progression longer than 6 months (337, 396). Also, we contributed to the modification of the Duke criteria, showing that the inclusion of this serological cutoff as a major criterion would improve the diagnosis of *C. burnetii* endocarditis (403, 404). Some years later we revaluated the PPV of this cutoff in the French situation of endemicity and proposed a new serological IgG1 cutoff at 1,600 to allow better detection of these cases of endocarditis (340). We also contributed to the early description of *C. burnetii* vascular graft, joint, and bone infections (394, 405), which were all grouped at that time under the generic "chronic Q fever" term. However, the reanalysis of the literature by a different team, leading to a corpus of controversial hypotheses, has helped us to clarify the existing nosology of *C. burnetii* infection and to abandon the inaccurate "chronic Q fever" term.

The controversy raised by Dutch Q fever consensus group guidelines. The recent widespread outbreak of Q fever in the Netherlands allowed us to reexamine the existing nosology of the infection, leading to continuing controversy on this topic. The total number of reported human cases of primary Q fever during this epidemic reached 4,108 between 2007 and 2011 (406). Moreover, because *C. burnetii* primary infection is frequently asymptomatic, these notifications reflected only part of the real magnitude of the outbreak (407). A seroprevalence study in blood donors by Van der Hoek et al. in a high-prevalence area estimated that 3,522 reported infections between 2007 and 2009 corresponded to approximately 44,000 primary *C. burnetii* infections (408). Consequently, the early detection of long-term complications of the infection was soon considered a central public health issue. With this in mind, in 2012 the Dutch consensus Q fever group published new criteria for "chronic Q fever" (409). The new guidelines distinguished three categories of possible, probable and proven cases of chronic Q fever (Table 2). Possible chronic Q fever was defined by an isolated serological criterion (IgG I greater than 1:1,024), and proven chronic Q fever was defined by a positive PCR on blood or tissue or by serological criteria associated with evidence of endocarditis (according to the modified Duke criteria) or vascular infections. Probable chronic Q

TABLE 2 The Dutch consensus guidelines criteria for chronic Q fever[a]

Category and criterion (criteria)[b]
Proven chronic Q fever
Positive *C. burnetii* PCR in blood or tissue **or**
IFA titer of ≥1:1,024 for *C. burnetii* phase I IgG **and**
Definite endocarditis according to the modified Duke criteria **or**
Proven large-vessel or prosthetic infection by imaging studies (18 F-FDG PET, CT, MRI, or AUS)
Probable chronic Q fever
IFA titer of ≥1:1,024 for *C. burnetii* phase I IgG **and** one or more of the following criteria:
Valvulopathy not meeting the major criteria of the modified Duke criteria
Known aneurysm and/or vascular or cardiac valve prosthesis without signs of infection by means of TEE/TTE, 18 F-FDG PET, CT, MRI, or abdominal Doppler ultrasound
Suspected osteomyelitis or hepatitis as manifestation of chronic Q fever
Pregnancy
Symptoms and signs of chronic infection such as fever, wt loss, and night sweats, hepatosplenomegaly, persistent elevated ESR and CRP
Granulomatous tissue inflammation, proven by histological examination
Immunocompromised state
Possible chronic Q fever
IFA titer of ≥1:1,024 for *C. burnetii* phase I IgG without manifestations meeting the criteria for proven or probable chronic Q fever

[a]Adapted from reference 409 with permission of Elsevier.
[b]MRI, magnetic resonance imaging; AUS, abdominal ultrasonography; ESR, erythrocyte sedimentation rate.

fever consisted of the association of serological criteria with a myriad of clinical situations from pregnancy to the immunocompromised state or evidence of granulomatous lesions (Table 2). Actually, several important issues were raised by these new criteria and were pointed out by our team (410).

(i) Isolated serological criteria for diagnosis are simplistic. In the Dutch consensus guidelines, the "possible chronic Q fever" category is based solely on a serological criterion without clinical manifestation or notion of follow-up duration. The problem with such a definition is that IgG I titers can vary widely, depending on the clinical situation and *C. burnetii* strain involved (238). For example, the MST 17 *C. burnetii* strain from Cayenne, French Guiana, is responsible for high levels of phase I IgG in acute Q fever pneumonia patients. We showed in a previous work that 36% of patients from Cayenne, French Guiana, with acute Q fever had phase I IgG titers of >1:1,600 (238), so these patients could have been falsely classified as having "possible chronic Q fever" with the Dutch definition. The other risk of serological cutoffs when they are considered alone is that cases of "possible chronic Q fever" are not thoroughly investigated to find a focus of infection, because the recommended management in this situation is only "follow-up." On the contrary, investigations are necessary to identify a nosologic entity that allows determination of the most appropriate treatment (including the need for dual antibiotherapy or surgery) and its duration. Moreover, today new diagnostic tools such as 18-fluoro-2-deoxyglucose positron emission tomography/computed tomography (18 F-FDG PET/CT) are available and frequently allow localization of the infection (411, 412). Thus, we consider that this "possible chronic Q fever" category is not only useless but deleterious, because classifying a patient under this imprecise term can lead to neglecting a real persistent focus of infection.

(ii) Mixing different clinical entities under a generic term neglects the natural history and determinants of the disease. The term "chronic Q fever" is misleading, because it mixes very different clinical entities that warrant different strategies for prophylaxis, diagnosis, and treatment. this gave us the opportunity to reanalyze how we consider the natural history of *C. burnetii* infection (Fig. 4). We think that this natural history is quite similar to what happens in tuberculosis (TB). In TB, the primary infection can be symptomatic or not. In the absence of treatment and depending on host susceptibilities, persistent infection can develop, with various possible localizations (lymphadenitis, miliary, meningitis, or Pott's disease) that have to be detected in order

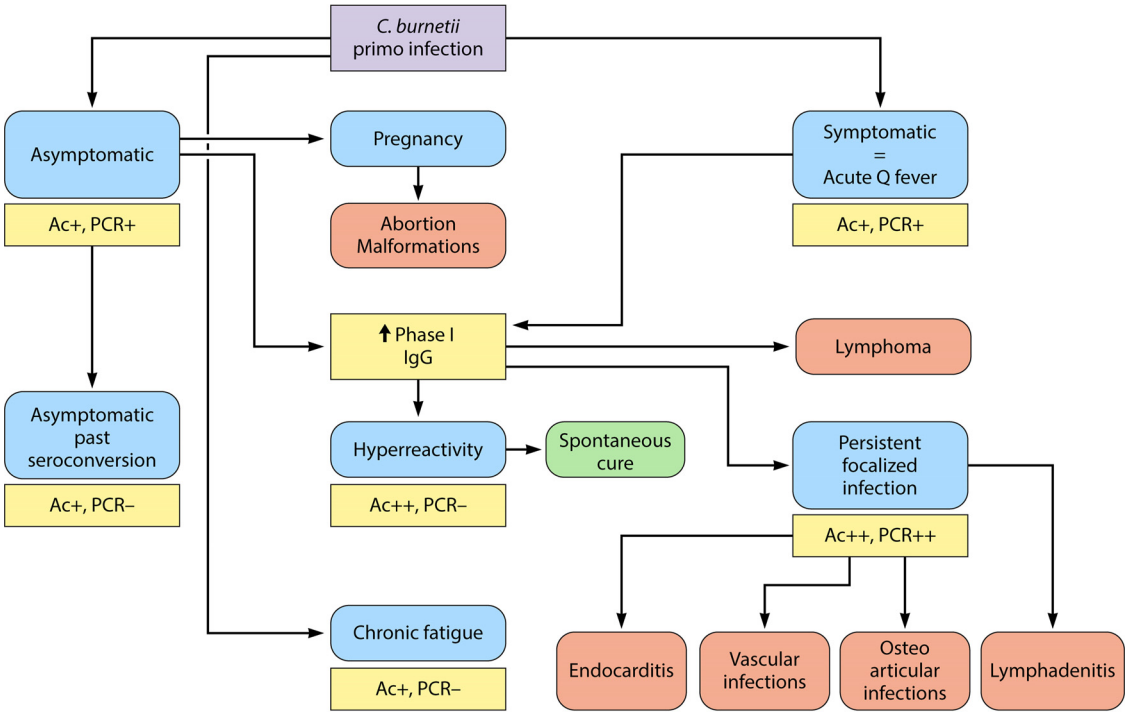

FIG 4 Natural history of *C. burnetii* infection.

to determine a specific treatment strategy. Regarding *C. burnetii* infection, the primary infection can also be symptomatic (acute Q fever) or not, depending on the strain involved (144, 165) and on the patient's susceptibilities (due to age, sex, immunosuppression, or pregnancy) (413). If left untreated, diverse persistent focalized infections can develop, depending mainly on host susceptibilities.

C. burnetii endocarditis occurs in patients with preexisting valvulopathy (mainly bicuspid aortic valve) (414) and is initially associated with high levels of anticardiolipin antibody (320). Its prognosis has significantly improved, due to a strategy of systematic screening for valvulopathy in cases of primary *C. burnetii* infection and initiation of prophylaxis (415). Another argument against the acute/chronic dichotomy is the recent description of "acute" endocarditis caused by *C. burnetii* (416). This is a new clinical entity, due to a probable autoimmune mechanism during *C. burnetii* primary infection.

Vascular infections occur in patients with preexisting aneurysms of a vascular graft and remain very severe diseases (with mortality rates up to 25%), for which surgery appears to be mandatory (417, 418). In this group, *C. burnetii* infections of preexisting aneurysms or vascular grafts have to be distinguished from mycotic aneurysms resulting from septic emboli in *C. burnetii* endocarditis. Osteoarticular infections are an emerging clinical entity for which no deaths have been reported to date (419). Joint prosthesis may be a predisposing factor, but further studies are needed to confirm this (420). Persistent lymphadenitis is another recognized focus of infection (412).

Also, we recently showed that *C. burnetii* persistent infections could lead to lymphomagenesis (334). *C. burnetii* infection in pregnant women is a particular entity, most often with an asymptomatic primary infection that can lead to severe obstetrical complications and fetal malformations (148) (Fig. 4). Its outcome may also depend on the involved strain (164).

Given the polymorphic manifestations of *C. burnetii* infection, we proposed alternative definitions for all *C. burnetii* persistent infectious foci (Table 3) (410, 412, 420).

For each localization of persistent infection, we defined minor, major, and definite criteria, and association with these criteria leads to definite or possible diagnosis. Definite criteria for Q fever include isolation of *C. burnetii* in culture or detection by

TABLE 3 Definition criteria for *C. burnetii* persistent focalized infections

Criterion or diagnosis type	Definition for *C. burnetii*: Endocarditis	Vascular infection	Prosthetic joint arthritis	Osteoarticular infection (without prosthesis)	Lymphadenitis
Criteria					
Definite	Positive culture, PCR, or immunochemistry of a cardiac valve	Positive culture, PCR, or immunochemistry of an arterial sample (prosthesis or aneurysm) or a periarterial abscess or a spondylodiscitis linked to aorta	Positive culture, PCR, or immunochemistry of a periprosthetic biopsy specimen or joint aspirate	Positive culture, PCR, or immunochemistry of bone or synovial biopsy specimen or joint aspirate	Positive culture, PCR, immunohistochemistry, or fluorescence *in situ* hybridization of lymphadenitis
Major	Microbiology—positive culture or PCR of the blood, an embolus or serology with IgG1 antibody titer of ≥6,400 mg/dl. Evidence of endocardial involvement—(i) Echocardiogram positive for infective endocarditis: oscillating intracardiac mass on valve or supporting structures, in the path of regurgitant jets, or on implanted material in the absence of an alternative anatomic explanation; abscess; new partial dehiscence of a prosthetic valve; or new valvular regurgitation (worsening or changing of preexisting murmur is not sufficient). (ii) PET scan displaying a specific valve fixation and mycotic aneurism.	Microbiology—positive culture, PCR of the blood or emboli, or serology with IgG1 antibody titer of ≥6,400 mg/dl. Evidence of vascular involvement—(i) CT scan: aneurysm or vascular prosthesis + periarterial abscess, fistula, or spondylodiscitis. (ii) PET scan specific fixation on an aneurism or vascular prosthesis.	Microbiology—(i) Positive culture or PCR of the blood. (ii) Positive *Coxiella burnetii* serology with IgG1 antibody titer of ≥6,400 mg/dl. Evidence of prosthetic involvement—(i) CT scan or MRI positive for prosthetic infection: collection or pseudotumor of the prosthesis. (ii) PET scan or indium leukocyte scan showing a specific prosthetic hypermetabolism consistent with infection.	Microbiology—(i) Positive culture or positive PCR of the blood. (ii) Positive serology with IgG1 antibody titer of ≥800 mg/dl. Evidence of bone or joint involvement—(i) Clinical arthritis, osteitis, or tenosynovitis. (ii) CT scan or ultrasonography (for joint) or MRI: osteo-articular destruction, joint effusion, intra-articular collection, spondylodiscitis, synovitis, acromio-clavicular localization. (iii) PET scan or indium leukocyte scan showing a specific osteo-articular uptake.	Microbiology—(i) Positive culture or positive PCR of the blood. (ii) Positive serology with IgG antibody titer of ≥800 mg/dl. Evidence of lymph node involvement—(i) Clinical lymphadenitis. (ii) CT scan or ultrasonography (for joint) or MRI: lymphadenitis of >1 cm. (iii) PET scan showing specific lymph node uptake.
Minor	Predisposing heart condition (known or found on ultrasound). Fever, temp of >38°C. Vascular phenomena, major arterial emboli, septic pulmonary infarcts, mycotic aneurysm (observed during PET scan), intracranial hemorrhage, conjunctival hemorrhages, and Janeway lesions. Immunologic phenomena: glomerulonephritis, Osler's nodes, Roth spots, or rheumatoid factor. Serological evidence: IgG1 antibody titer of ≥800 and <6,400 mg/dl.	Serological IgG1 antibody titer of ≥800 and <6,400 mg/dl. Fever, temp of ≥38°C. Emboli. Underlying vascular predisposition (aneurysm or vascular prosthesis).	Presence of a joint prosthesis (indispensable criterion). Fever, temp of >38°C. Joint pain. Serological evidence: positive *C. burnetii* serology with IgG1 antibody titer of >800 and <6,400 mg/dl.	Serological IgG1 antibody titer of ≥400 and <800 mg/dl. Fever, temp of ≥38°C. Mono- or polyarthralgia.	Serological IgG1 antibody titer of ≥400 and <800mg/dl. Fever, temp of ≥38°C.
Diagnoses					
Definite	(i) 1 definite criterion, (ii) 2 major criteria, or (iii) 1 major criterion and 3 minor criteria (including 1 microbiological characteristic and a cardiac predisposition)	(i) 1 definite criterion, (ii) 2 major criteria, or (iii) 1 major criterion and 2 minor criteria (including 1 microbiological characteristic and a vascular predisposition)	(i) 1 definite criterion, (ii) 2 major criteria, or (iii) 1 major criterion and 3 minor criteria (including 1 piece of microbiology evidence and presence of a joint prosthesis)	(i) 1 definite criterion, (ii) 2 major criteria, or (iii) 1 major criterion and 3 minor criteria (including 1 microbiological characteristic)	(i) 1 definite criterion, (ii) 2 major criteria, or (iii) 1 major criterion and 2 minor criteria (including 1 microbiological characteristic)
Possible	(i) 1 major criterion and 2 minor criteria (including 1 microbiological characteristic and a cardiac predisposition) or (ii) 3 minor criteria (including 1 microbiological characteristic and a cardiac predisposition)	Vascular predisposition, serological evidence, and fever or emboli	(i) 1 major criterion and 2 minor criteria (including 1 piece of microbiology evidence and presence of a joint prosthesis) or (ii) 3 minor criteria (including positive serology and presence of a joint prosthesis)	(i) 1 major criterion and 2 minor criteria or (ii) 3 minor criteria	(i) 1 major criterion and minor criterion or (ii) 2 minor criteria

molecular biology or immunochemistry of *C. burnetii* in the affected organs (Table 3). Major criteria include indirect microbiological evidence of infection (positive serology or positive PCR on blood) associated with compatible morphological abnormalities,

with 18 F-FDG PET/CT playing a central role in this setting. Minor criteria associate serological evidence, unspecific clinical signs of infection, and predisposition to the suspected focus of infection (e.g., known vascular aneurysm or graft in the case of *C. burnetii* vascular infection).

Apart from this corpus of persistent focalized infections with evidence of multiplying *C. burnetii*, a long-term complication of the infection can manifest as "chronic fatigue syndrome" (CFS) (Fig. 4).

(iii) Prevention strategies and prognosis for *C. burnetii* infection depend on the definition and understanding of the natural history of the disease. In a recent publication, the Dutch Q fever consensus group argued against the definition of persistent *C. burnetii* infections proposed by our team (endocarditis and vascular infections) by comparing definition criteria applied to the patients from the Dutch National Chronic Q fever database (421). They observed that some patients diagnosed with "proven chronic Q fever" in their cohort would have been missed by the criteria proposed by our team. Particularly, six patients from their cohort (4 with endocarditis and 2 with vascular infections) died of "chronic Q fever" and would not have been diagnosed by our alternative criteria. First, this study suffers from a bias, which is the use of our criteria *a posteriori*, because the definition of patients had already been made from their criteria. Consequently, our criteria could only show lower performances. Moreover, different definitions lead to different management of the infections. In the cases they presented, even if patients had been diagnosed with "chronic Q fever" from their criteria, they died of *C. burnetii* endocarditis and vascular infections. Indeed, regarding *C. burnetii* endocarditis, our strategy of systematic echocardiography to detect significant silent valvulopathy and initiating 12 months of prophylactic treatment with doxycycline plus hydroxychloroquine has proven effective (320). In 31 patients followed in our center who were diagnosed with significant valvulopathy during primary infection, 18 patients completed a 12-month course of antibiotic prophylaxis and 13 patients did not. We observed no endocarditis in the group with antibiotic prophylaxis, while all 13 patients who did not receive antibiotic prophylaxis progressed to endocarditis (320). The efficacy of such antibiotic prophylaxis is also illustrated by the fact that we observed a reduction in the incidence of *C. burnetii* endocarditis in our center over a 27-year period, despite a concomitant increase in the number of diagnosed primary infections (422). We believe that our management strategy would have allowed a lower mortality rate in this cohort of patients, because death due to endocarditis would have been prevented.

Conversely, in the Netherlands, echocardiographic screening of patients with primary *C. burnetii* infection was abandoned early, due to a 1-year follow-up study by Limonard et al. showing no cases of endocarditis despite detection of valvulopathy in 59% of patients (423). One of the arguments advanced was the unfavorable cost-effectiveness ratio of echocardiography in the context of their epidemic. We consider that such a decision was very premature, given the potential long-term consequences of *C. burnetii* endocarditis for public health. In another Dutch study, 62% of patients classified as having "proven" or "probable" chronic Q fever had a symptomatic primary infection, with 28% of them having a predisposing valvulopathy and 62% of them having a minor echocardiographic criterion (424). Unfortunately, none of these patients benefited from antibiotic prophylaxis because they were not screened during the primary infection. Recently, Keijmel et al. performed a retrospective case-control study on the diagnosis of acute Q fever in the Netherlands. That study found that 50% of patients with acute Q fever and risk factors (significant valvulopathy) developed endocarditis if no prophylaxis was given (425). This is a worrisome result, since a large number of patients with indication for prophylaxis may have been missed, due to insufficient screening of valvulopathy from the beginning of the epidemic.

We recommend close serological follow-up of all patients with *C. burnetii* primary infection at 3 and 6 months for early detection of an increase in antibody titers and patient investigation using 18 F-FDG PET-CT. This strategy has been abandoned in

some centers in the Netherlands, with a serological control performed only 9 months after primary Q fever (426).

The same controversy exists about the management of *C. burnetii* infection during pregnancy. We previously described that infection during pregnancy could lead to severe obstetrical and neonatal complications (427, 428). We recently performed a meta-analysis confirming that these infections, although frequently asymptomatic, were associated with miscarriage, fetal death, malformations, and prematurity (148). To avoid these severe complications, we recommend treatment with co-trimoxazole until the eighth month of pregnancy (164). A recent Danish study confirmed such associations, with 47% of pregnant women seropositive for *C. burnetii* presenting obstetrical complications (miscarriage, preterm delivery, small infant for gestational age, oligohydramnios, fetal growth restriction, or perinatal death) (313). Interestingly, previous large case-control studies in the same country using serological markers of past *C. burnetii* infection in pregnant women failed to detect an association with obstetrical complications (429, 430). In the Netherlands, a randomized controlled study of serological screening in pregnant women in high-prevalence areas was performed in 2010 and found no advantage of screening in terms of prevention of obstetrical complications (431). As a consequence, systematic screening for *C. burnetii* infection in pregnant women in high-risk areas was abandoned in the Netherlands. As in the case of endocarditis, this decision seems quite premature, given the available data from the literature. Also, a comment from E. Leshem in 2012 about this strategy noted that this decision could expose unprotected medical personnel (especially obstetrical staff) to infection by inhalation of high concentrations of *C. burnetii* from the placentas of infected women (432). To date, the consequences of such a strategy are unknown.

Finally, regarding the particular severity of *C. burnetii* vascular infections, we recently proposed systematic screening for vascular aneurysm in men older than 65 years presenting with primary Q fever. We proposed that a CT scan or abdominal ultrasound (in case of renal contraindication) be performed for these patients in order to initiate prophylactic treatment, similar to what has been done for endocarditis (418).

In conclusion, this controversy illustrates the importance of nosology in the management of infectious diseases. In the particular setting of *C. burnetii* infection, which has long been considered a rare and mysterious disease, it is necessary to consider updated data resulting from clinical observations in the field. Definitions must play the role of practical tools, assisting clinicians' decisions, and must not consist of misleading terms lacking a microbiological substratum. In the following section we provide a detailed description of each clinical entity caused by *C. burnetii* infection.

The other current definition criteria for "chronic Q fever." As a consequence of this controversy, several different definitions for "chronic" or "persistent focalized" infection can be found in the recent literature. In addition to the definition from the Dutch consensus guidelines and the one proposed by our team, there is a third definition, which was proposed by the CDC in 2013 (Table 4). This definition uses the terms "probable Q fever" and "confirmed chronic Q fever." "Probable Q fever" is defined by the existence of a clinical presentation compatible with this diagnosis, such as culture-negative endocarditis or vascular aneurysm or graft infection (Table 4), combined with an IgG I titer of $<$800. "Confirmed Q fever" is defined by the same clinical picture combined with laboratory confirmation with serological titers of $>$800 or detection of *C. burnetii* by culture, PCR, or IHC in a clinical sample. Compared to the Dutch definition, it has the advantage of taking into account the suspected localization of the infection as an important criterion. However, it continues to use the term "chronic Q fever," which we consider to be confusing, now that *C. burnetii* foci of infection are well described.

Clinical Manifestations

Primary infection. *C. burnetii* primary infection can manifest itself through a wide diversity of clinical symptoms. The incubation period for the primary infection before the onset of symptoms can last from 2 to 3 weeks, and it depends on the size of the

TABLE 4 Definition criteria for "chronic Q fever" from the CDC[a]

Category	Description
Indications	Newly recognized culture-negative endocarditis (particularly in a patient with previous valvulopathy or compromised immune system), suspected infection of a vascular aneurysm or vascular prosthesis, or chronic hepatitis, osteomyelitis, osteoarthritis, or pneumonitis in the absence of other known etiology
Laboratory confirmed	One or more of the following criteria: IgG titer of ≥1:800 to *C. burnetii* phase I antigen by IFA, detection of *C. burnetii* DNA in a clinical specimen by PCR, demonstration of *C. burnetii* in a clinical specimen by IHC, isolation of *C. burnetii* from a clinical specimen by culture
Laboratory supportive	IFA IgG titer of ≥1:128 and <1:800 to *C. burnetii* phase I antigen
Confirmed chronic Q fever	Clinical evidence of infection with laboratory confirmation
Probable chronic Q fever	Clinical evidence of infection with laboratory supportive results

[a]Adapted from reference 40.

inoculum. In a large proportion of patients, the primary infection can be asymptomatic (433). In other cases, pneumonia, hepatitis, or flu-like syndrome can be observed.

However, these extremely polymorphic features of *C. burnetii* primary infection are not predictive of the development of long-term complications, such as persistent focalized infection or chronic fatigue syndrome (Fig. 4).

This issue has been raised by a recent study in the Netherlands. The European Union case definition for reporting Q fever used during the outbreak prevented the reporting of asymptomatic cases, leading to an underestimation of the true burden of the disease (407, 434) and to a probable significant lack of prevention of long-term complications for at-risk patients.

The determinants of the symptomatology in *C. burnetii* primary infection depend on host factors and on the strain involved. Tissot-Dupont et al. long ago showed the role of age and sex in the clinical expression, with older men being more frequently symptomatic than young women and pregnant women (413, 435). This was recently illustrated during the Netherlands epidemic, with symptomatic patients being significantly older and more often men than asymptomatic patients (407). Children are also less frequently symptomatic than adults (310). The role of immunosuppression in the severity of *C. burnetii* primary infection is not well defined. An old report of an outbreak in a residential facility for drug users in Italy suggested that respiratory signs were more frequent in HIV-positive patients and that the incidence of infection was significantly higher in this population (436). A study in Marseilles also suggested a more frequently symptomatic primary infection in HIV patients than in the general population (437). However, other studies have shown a similar seroprevalence of primary *C. burnetii* infection in HIV and non-HIV patients, with no significant difference in terms of symptomatology (438–440). In Cayenne, French Guiana, patients with *C. burnetii* community-acquired pneumonia were not more frequently diabetic, HIV positive, or on corticosteroid treatment than patients with community-acquired pneumonia of other etiologies (80). A recent study among patients with rheumatoid arthritis in the Netherlands found no difference in seroprevalence or symptomatology in patients on anti-TNF-α therapy (441).

The strain of *C. burnetii* involved is the second determinant of the clinical manifestations of primary infection. This phenomenon can be illustrated by the example of the MST 17 clone, the unique genotype responsible for *C. burnetii* infection in Cayenne, French Guiana. This clone causes higher rates of symptomatic cases than clones from metropolitan France. In a recent outbreak in a military camp, it was shown that 100% of contaminated patients were symptomatic, illustrating the particular virulence of this genotype (unpublished data).

(i) Asymptomatic and pauci-symptomatic primary infection. Classically, it is stated that approximately 60% of individuals are asymptomatic during *C. burnetii* primary

infection (433). Mild symptomatic forms resemble a common cold and are often diagnosed retrospectively or during outbreaks through systematic testing. This phenomenon was soon illustrated by the discrepancy between seroprevalence studies and rates of symptomatic cases of Q fever during early outbreaks in Montana and Switzerland (442, 443). A more recent study performed in Denmark showed a rate of 64% of patients with asymptomatic primary infection (444). In the Netherlands, Hogema et al. found a rate of 12.2% of blood donors positive for anti-*Coxiella* IgG, resulting in an incidence rate of seroconversion of 5.7% per year, which is more than 10 times higher than the local number of symptomatic diagnosed cases per year in this region (445). This issue is of particular concern in these settings of epidemicity, since it has been demonstrated that *C. burnetii* can survive and remain infectious for several weeks in stored human blood samples (132).

(ii) Acute Q fever: isolated febrile syndrome or flu-like illness. In acute Q fever with isolated febrile syndrome or flu-like illness, the abrupt onset of high fever (often up to 40°C) is the predominant sign, which can last for more than 15 days and is frequently associated with myalgia and headache, mostly retro-orbital (433, 446). It was the most frequent clinical presentation in reported acute Q fever patients during the outbreak in the Netherlands (423). This nonspecific presentation is misleading and supports the fact that clinicians should include screening for *C. burnetii* primary infection in the presence of an isolated fever of unknown origin. Specific examination is crucial in this context, searching for epidemiological risk factors (rural setting, occupation, and contact with ruminants or parturient mammals).

(iii) Acute Q fever: pneumonia. The prevalence of pneumonia during primary infection is highly variable. In Cayenne, French Guiana, *C. burnetii* MST 17 is responsible for the highest rate of community-acquired pneumonia in the world (around 40%) (238). It is also the predominant presentation in the Maritime provinces of Canada (447), northern Spain (448), Croatia (449), and the Netherlands (72). In Cameroon, *C. burnetii* was responsible for up to 10% of community-acquired pneumonia (60, 450, 451). During the Netherlands epidemic, pneumonia was present in up to 86% of hospitalized patients with acute Q fever (452).

Q fever pneumonia usually presents in middle-aged men, often with no comorbid conditions (446). Acute pneumonia typically combines fever, cough, dyspnea, and auscultation abnormalities. It is frequently associated with extrapulmonary signs, such as myalgia, arthralgia, relative bradycardia, sore throat, chills, vomiting, abdominal pain, nausea, diarrhea, or constipation (446, 453, 454). Patients are also more likely to complain of headache (80, 452), with some reports mentioning the headache as "the most severe pain they ever had" (446). This sign has been reported in up to 40.5% of patients with *C. burnetii* primary infection (455). Less frequently, a skin rash or neurological signs can be observed (confusion, prostration, or Guillain-Barré syndrome) (446, 452).

Regarding laboratory findings, leukocyte counts are generally normal or low compared with those in pneumonia caused by other microorganisms (80, 446, 452, 456). Conversely, C-reactive protein (CRP) levels are typically high (452) and significantly higher than in other pneumonias (80, 425). In a report from the Netherlands, elevated liver enzymes were reported in 32.3% of patients (452), and this was also reported in 60% of patients with acute Q fever in Croatia (449).

Radiological findings are highly polymorphic. Several reports found rounded opacities (446, 457, 458) with a halo sign suggestive of an angio-invasive process, but classical interstitial or segmental opacities involving the lower lobes are also common (459). CT scanning has shown lobar, segmental, multilobar, or patchy involvement, sometimes associated with lymph node enlargement (446). More severe forms, such as necrotizing pneumonia, have been described in the immunocompromised patient (458). Pleural effusions are also described (80, 458) and have been reported in 9.9% of patients with *C. burnetii* pneumonia from Europe and North America (460). Exceptional cases of lung pseudotumor have been described (394, 461, 462), with a good response to doxycycline.

The prognosis of *C. burnetii* pneumonia is usually favorable, with resolution of symptoms within 30 days (446). In the Netherlands, patients hospitalized for *C. burnetii* pneumonia had significantly lower CURB-65 scores than patients with other community-acquired pneumonias (452). However, respiratory distress syndrome leading to death can occur and has been described in Cayenne, French Guiana (80). The mortality rate reported in the Netherlands was approximately 1% in two studies (452, 463). Death was more frequent in patients with comorbidities.

(iv) Acute Q fever: hepatitis. Isolated hepatitis is a frequent presentation of acute Q fever. It is more frequent than pneumonia in countries where the disease is endemic, such as France (238), Spain (464), Israel (453), Portugal (459), and Taiwan (454, 465). Elevated liver enzymes are associated in almost all cases with fever, chills, and headache (465). Headache was even proposed in early reports for differentiating *C. burnetii* hepatitis from viral hepatitis (466). Other accompanying findings are anorexia, vomiting, and sometimes diarrhea and painful hepatomegaly (238, 453, 459, 464). Jaundice is rare, but it has been reported in severe hepatitis, especially in Taiwan, where hyperbilirubinemia was found in more than one-third (37%) of cases (465). In that study, patients with jaundice presented a significant delay in reduction of fever after initiation of antibiotic therapy compared to patients without jaundice (11.5 versus 5.0 days; $P = 0.002$) (465). Globally, the mean duration of fever decrease after initiation of adequate antimicrobial therapy in Taiwanese patients was 10 days, and 8 patients experienced a fever lasting more than 28 days (465). This particular feature may explain the confusion in early reports between what could be considered acute or chronic Q fever hepatitis (467). In developing countries where coinfection with viral hepatitis is high, clinical manifestations of *C. burnetii* hepatitis do not seem to be more severe, but further studies are needed to evaluate this feature, since only one retrospective study in Taiwan has been performed on this presentation (468). When performed, positron emission tomography can reveal an intense diffuse uptake of the whole hepatic parenchyma (469, 470), helping to establish the diagnosis in the case of fever of unknown origin.

When liver biopsy is performed, granulomatous hepatitis and typical "doughnut" granulomas are found (86). However, atypical pathological aspects have been reported recently, such as like epithelioid granuloma with eosinophilic infiltration, extensive extravasated fibrin without ring granuloma and acute cholangitis without granuloma (365). The prognosis of *C. burnetii* acute hepatitis is good. Fatal cases due to hepatic insufficiency are very rare and have been reported in a child (471), or in patients with cancer or alcoholism (472, 473).

(v) Acute Q fever: cardiac involvement. *(a) Pericarditis.* Acute pericarditis accounted for approximately 1% of Q fever cases diagnosed in our center (474) in the 1980s. This presentation has been reported as mimicking a lupus-like syndrome (475). The diagnosis of *C. burnetii* pericarditis has been improved with the use of a systematic prescription kit in our center (476), revealing that Q fever was involved in 24% of 81 cases of pericarditis investigated over an 8-year period (477). That study suggested that *C. burnetii* pericarditis is not as rare as previously postulated. The severity of these cases of pericarditis is variable, with reported cases of life-threatening tamponade (478) and some constrictive cases (478, 479).

(b) Myocarditis. Acute myocarditis is a life-threatening and fortunately rare form of primary infection. A total of 23 cases of acute myocarditis are reported in the English literature, (459, 474, 480–484), of which one involved a child (485). It represents 0.5% to 1% of cases diagnosed in our laboratory (305, 474). Among these 23 cases, 7 deaths (30%) were reported, illustrating the poor prognosis of this focalized infection. Although it is a rare manifestation, acute Q fever should be considered in the diagnosis of acute myocarditis because appropriate treatment may significantly improve the prognosis of this potentially fatal localization.

(c) Acute endocarditis. *C. burnetii* endocarditis is still considered the archetype of "chronic" Q fever. However, we recently reported cases of "acute endocarditis" (416). We observed a transient 10-mm aortic vegetation in a patient with primary Q fever,

associated with high levels of IgG anticardiolipin (aCL) 1 week after fever onset. This patient had no past medical history of valvular heart disease (VHD). This case prompted us to investigate this clinical presentation in patients followed in our National Referral Center and to compare this syndrome with classical primary Q fever in patients with or without preexisting VHD but without vegetation. We found 9 patients presenting features of "acute endocarditis" (416). All vegetations were localized to the aortic valve. Patients presented various associated clinical features such as isolated fever, pneumonia, or hepatitis. Regarding the prognosis, one patient died after 15 months from mesenteric infarction (long after successful treatment of primary Q fever), six patients progressed to *C. burnetii* persistent endocarditis, and two patients never fulfilled the criteria for persistent endocarditis and were treated for 12 months. The comparison between "acute endocarditis" and the other categories of primary infections found that predictors for this condition were immunosuppression and very high levels of IgG aCL (>100 IgG phospholipid units [GPLU]). The pathophysiological scenario for this new entity would be that *C. burnetii* primary infection causes an explosive secretion of autoantibodies, including IgG aCL, causing autoimmune valvular lesions. Further studies are needed to confirm and further characterize the features of this new clinical entity.

(vi) Acute Q fever: neurological signs. Neurological involvement is rare and can be observed alone or combined with other organ involvement. A total of 14 references concerning this presentation are found in the English literature, consisting mainly of case reports or small series (304, 474, 486–497). Apart from headache, which is a common sign in acute Q fever, revealing a possible neurological tropism of the bacterium (455), more severe manifestations such as meningitis and meningoencephalitis have been reported. Among 1,383 Q fever infections diagnosed in our reference center between 1985 and 1998, 1% had meningoencephalitis and 0.7% had meningitis alone (474). In a Greek report of 121 patients with Q fever consisting mainly of acute pneumonia, 4.1% presented with confusion and 0.8% had meningitis (455). Meningoencephalitis appears to be the most frequent acute severe neurological complication and can sometimes be the only manifestation of the disease, followed by meningitis and peripheral myelitis (491). Cerebrospinal fluid (CSF) findings show lymphocytic meningitis. Postinfectious neurological signs probably related to immunological disorders have been described, such as peripheral sensory neuropathy or Guillain-Barré syndrome (488, 490), with a good response to steroid therapy. The use of fluoroquinolones, which have good cerebrospinal penetration, has been proposed for treatment of *C. burnetii* meningitis (495).

(vii) Acute Q fever: rare clinical manifestations. *(a) Dermatological signs.* Cutaneous involvement was present in 4% of patients diagnosed with acute Q fever in our laboratory between 1985 and 1998 (474), and recent acute Q fever series have reported a prevalence ranging from 1% in the Netherlands, (452) to 9% in Israel (449, 453, 454, 498). These manifestations consist mainly of maculopapular or vesicular exanthema and sometimes purpuric lesions (474). Cases of granulomatous panniculitis (499) and erythema nodosum have also been reported (366, 500, 501).

(b) Bone marrow involvement. Although rarely reported, bone marrow lesions were already described in very old reports. The pathological analysis of bone marrow biopsy specimens shows a typical "doughnut" or "fibrin ring" granuloma during *C. burnetii* primary infection (253, 367–369, 469, 502–508). Also, a single case of bone marrow necrosis following acute Q fever was reported in 1980 (509). Hemophagocytic syndrome can be a consequence of bone marrow involvement. This clinical presentation was reported for the first time in 1984 by Estrov et al. (510), and since then fewer than 10 cases have been reported (511–513), with the most recent involving an asplenic patient (514). 18 F-FDG PET/CT, which is less invasive than bone marrow biopsy, can detect bone marrow involvement. A case of diffuse bone marrow increased uptake in the context of primary *C. burnetii* infection has been reported recently (515), and we found 11 patients with bone marrow hypermetabolism in the context of acute Q fever (412).

(c) Acute lymphadenitis. Thirteen cases of acute Q fever lymphadenitis have been described, involving cervical, axillary, mediastinal, abdominal, or inguinal lymph nodes and associated with fever, headache, pneumonia, or hepatitis (458, 516–521). Also, in a recent study concerning the clinical relevance of 18 F-FDG PET/CT in *C. burnetii* infection, we observed seven cases of lymphadenitis in the context of primary Q fever, associated with bone marrow, lung, or splenic hypermetabolism. One patient with acute lymphadenitis was subsequently diagnosed with lymphoma (334).

(d) Cholecystitis. Cases of acute acalculous cholecystitis have been reported (522–527). A total of nine cases are reported in the literature. Patients present with right upper quadrant pain and fever. Abdominal CT scanning shows a diffuse symmetrically thickened and hypodense gallbladder. The diagnosis was made by serology in all cases. Six of the patients underwent cholecystectomy, and the pathology review showed associated fibrin ring granulomas in the liver for three of them. In one case, *C. burnetii* was detected by qPCR in the gallbladder (524).

(e) Autoimmunity. Biological markers of autoimmunity are frequently present in acute Q fever. Anti-smooth muscle antibodies, antineutrophil cytoplasmic antibodies (ANCA), and antinuclear and antiphospholipid antibodies have been detected during acute Q fever (292, 475, 475, 528–531). Lefebvre et al. have reported a series of seven cases of *C. burnetii* infection that could have been confused at initial presentation with Goodpasture's syndrome, Crohn's disease, Still's disease, polymyalgia rheumatica, polyarteritis nodosa, essential type II cryoglobulinemia, and giant-cell arteritis (531). Other authors have reported two cases mimicking exacerbations of systemic lupus erythematosus (475, 532).

Cases with positive antiphospholipid antibodies complicated by thrombophlebitis have been reported. This suggests that most clinical features of the antiphospholipid syndrome can be found in acute Q fever with laboratory classification criteria for the antiphospholipid syndrome (lupus anticoagulant and anticardiolipin antibody of IgG and/or IgM isotype) (686, 687; M. Million, N. Bardin, S. Bessis, N. Nouiakh, C. Douliery, S. Edouard, E. Angelakis, K. Griffiths, A. Bosseray, O. Epaulard, S. Branger, D. Chaudier, K. Blanc-Laserre, N. Ferreira-Maldent, E. Demonchy, F. Roblot, J. Reynes, F. Djossou, C. Protopopescu, P. Carrieri, H. Lepidi, L. Camoin-Jau, J.-L. Mege, and D. Raoult, unpublished data). Also, in the primary *C. burnetii* infection, high levels of IgG aCL antibodies and VHD have been associated with progression to persistent endocarditis (295). Rare cases of Q fever associated with amyloidosis (533) or mixed cryoglobulinemia (531, 534) are reported in the literature. Two case of Jarisch-Herxheimer reactions following the treatment of a *C. burnetii* pneumonia or endocarditis have been reported (535, 536).

***C. burnetii* persistent focalized infections. (i) Endocarditis.** Q fever endocarditis is the most frequently reported form of persistent *C. burnetii* infection in the literature. It is the most frequent persistent form in France (340) and the second most frequent one in the Netherlands (78). In Brazil, *C. burnetii* was found to be the etiological agent in approximately 10% of blood culture-negative endocarditis cases (685). In Israel, 9.6% of patients undergoing valve replacement for endocarditis had a *C. burnetii* infection (537). *C. burnetii* endocarditis has also been reported in 8.3% of 60 patients diagnosed with bacterial endocarditis in Thailand (538). In Africa, it represents from 1% to 3% of infective endocarditis in cohort studies (450). However, its prevalence is probably underestimated in most developing countries, where microbiological tools for diagnosis are lacking.

Factors associated with progression to endocarditis after primary infection are male sex, age above 40 years, and, most importantly, underlying valvular heart disease, even if clinically silent at diagnosis (320). The incidence of endocarditis after acute Q fever in patients with valvulopathy has been estimated to be 39% (539). Having a valvular prosthesis (mechanical or bioprosthesis) represents the most important risk factor, as initially reported by Fenollar et al. (539). Also, even minor valvulopathies are at risk, with the highest risk for aortic bicuspidy, followed by mitral valve prolapse and minimal valvular leaks (414, 540). Given the fact that the prevalence of bicuspidy has been estimated to be 1% to 2% in the general population, screening for this valvulopathy is

of particular importance in patients with primary Q fever (541). Regarding more severe valvulopathy, aortic regurgitation is at higher risk than mitral regurgitation and aortic stenosis (320). Also, three cases of *C. burnetii* infection of cardiovascular implantable electronic devices and a single case of *C. burnetii* infection of an intracardiac thrombus mimicking atrial myxoma have been reported to date (412, 542, 543).

The clinical presentation of *C. burnetii* endocarditis is nonspecific, and patients can present symptoms such as isolated relapsing fever, chills, night sweats, weight loss, and hepatosplenomegaly (433). Sudden cardiac insufficiency, stroke, or other embolic signs are also presentations of the disease (415). Mycotic aneurysms resulting from endocarditis can occur and must be distinguished from isolated vascular infection of a preexisting aneurysm (418, 544). Laboratory signs can consist of a persistent inflammatory syndrome and hyperleukocytosis or, on the contrary, pancytopenia (539, 545). Cases of coinfection with *Enterococcus faecalis*, *Streptococcus mitis*, and *Streptococcus gallolyticus* have been reported (415, 546–548).

Million et al. have reported presentations with valvular vegetations in only 30% of cases and discovery or worsening of a valvular insufficiency in 75% of cases, illustrating the difficulty in the diagnosis of Q fever endocarditis (415). This is illustrated by a study in Marseilles, where systematic screening of 6,401 patients undergoing valve surgery yielded an unexpected diagnosis of *C. burnetii* endocarditis in 14 patients (0.2%) (549). Also, Tyler et al. recently reported a case of *C. burnetii* endocarditis incidentally discovered during routine valve replacement in a patient with unexplained pancytopenia and splenomegaly (545), and similar cases were reported in the Netherlands by Kampschreur et al. (550). In most patients, the infection can be latent for years, while *C. burnetii* progressively destroys heart valves, causing irreversible damage (549). Million et al. have shown that high levels of IgG aCL during acute Q fever were associated with the presence of a valvulopathy and a predictive biomarker of progression to persistent endocarditis (295). In particular, IgG aCL levels higher than 90 IU were strongly associated with endocarditis.

Serological IgG1 titers of $>$1,600 are associated with endocarditis (340), and this cutoff has been added in the modified Duke criteria for endocarditis. However, some cases of endocarditis with IgG1 titers of $<$800 have been described (341), so that this criterion alone is not sufficiently accurate for diagnosis. Specific qPCR on blood or serum is positive in about 30% of cases, and blood culture is positive in 14% (415). The analysis of resected valves by culture, qPCR, immunohistochemistry, and pathology shows variable performances (from 0% to 87% positivity), depending on the time the resections are performed in relation to treatment.

Pathological examination of cardiac valves may reveal fibrosis and calcification, slight inflammation and vascularization, and minimal or absent vegetation (328). These signs can be confused with noninfectious cardiac valve damage, so immunohistochemical analysis is helpful to confirm the diagnosis (328).

18 F-FDG PET/CT scanning has been used as a new tool for detecting infected valves in *C. burnetii* endocarditis, and it seems to be particularly useful when no vegetation is present and in cases of prosthetic valve infection. Barten et al. have reported four cases of *C. burnetii* endocarditis, all with hypermetabolism in a prosthetic valve (551). A case report from Australia describes the diagnosis and follow-up of *C. burnetii* endocarditis in a patient with aortic bicuspidy with an 18 F-FDG PET/CT-scan (552). Also, the diagnosis of *C. burnetii* infection of a Bentall graft or prosthetic mitral or aortic valve with 18 F-FDG PET/CT scanning has been reported (553, 554). In a recent retrospective study from our center of 99 patients with *C. burnetii* infection and a positive 18 F-FDG PET/CT scan, we observed 21 patients with hypermetabolism of a cardiac valve, with a majority of them involving a prosthetic valve (66%) (412).

Because there is no pathognomonic sign or accurate paraclinical tool for the diagnosis of *C. burnetii* endocarditis, new criteria have recently been proposed, inspired by the modified Duke criteria (Table 3), incorporating PCR and culture on blood samples, echocardiography, and 18 F-FDG PET/CT scanning as major diagnostic criteria (410).

The prognosis of *C. burnetii* endocarditis has considerably improved because of earlier diagnosis and appropriate dual-antibiotic therapy. In the Netherlands, Kampschreur et al. found mortality rates of 9.3% for *C. burnetii* endocarditis, (78) with acute presentation (severe endocarditis) being associated with a poor outcome. In our center, we found mortality rates of about 5% at 3 years, and factors independently associated with death were age, stroke, and prosthetic valve at diagnosis (415). At 1 year of follow-up, the absence of a 4-fold decrease of IgG1 and IgGA and persistence of IgM were also associated with death (415). We recommend performing systematic echocardiography in patients diagnosed with primary Q fever to detect any predisposing valvular lesions and, if such lesions are detected, providing regular monitoring and prophylactic treatment with doxycycline and hydroxychloroquine (540). We have demonstrated retrospectively in our cohort from the National Referral Center that this strategy is effective in preventing progression to endocarditis in all patients with valvulopathy who underwent complete treatment (320). Conversely, patients diagnosed with primary Q fever and valvulopathy who did not follow this prophylaxis systematically progressed to endocarditis (320).

(ii) Vascular infections. An increasing number of reports of *C. burnetii* vascular infections have been published in the last decade, mainly due to the outbreak in the Netherlands (78, 371, 405, 417, 551, 555–575). At present, it is the most frequent form of persistent infection reported in the Netherlands (371, 405, 417, 418, 553, 557–577). In that country, a seroprevalence study among patients with abdominal aortic/iliac aneurysm detected *C. burnetii* antibodies in 16.7% of them, of which 30% had serological titers suggesting persistent vascular infection (562). In France, it is the second most prevalent site of persistent infection after endocarditis (417, 474). In our center, we observed an increase in the incidence of vascular infections in the last 5 years compared to the 22 preceding years (418). This may be due to several factors: systematic screening of patients with aneurysm and vascular graft for whom biological samples are available in our laboratory, elaboration of a diagnostic score (Table 2), and new diagnostic tools such as the 18 F-FDG PET/CT scan (412). No data exist regarding prevalence in other countries, because the literature consists mainly of case reports.

These infections develop after *C. burnetii* primary infection when a preexisting lesion is present on a vessel, such as an aneurysm or vascular graft. The most frequent localization is the abdominal and thoracic aortas (417). Because *C. burnetii* vascular infections are latent and initially present with unspecific symptoms (weight loss or unexplained fever in a patient with an aneurysm or vascular graft), in the majority of the reported cases the diagnosis was made when complications occurred. The main complications are aortoduodenal fistulas (555, 558, 568) leading to catastrophic hemorrhage, spondylodiscitis (417, 565, 575) often associated with psoas abscesses (418), graft or aneurysm rupture (417), (78) and embolic complications (78, 417, 557). A single case of coinfection with *Yersinia enterocolitica* has been reported (411). The overall prognosis is poor, with mortality rates between 18% and 26% (78, 417, 418). In a recent Dutch study, the presence of vascular Q fever infection was significantly associated with mortality (78) in a cohort of patients with "chronic" Q fever.

As is the case for endocarditis, the diagnosis of vascular infection is challenging. IgG1 titers of $>$6,400 were observed in 45% of patients from our center, and the median IgG1 level was 3,200 (418). Specific qPCR on blood samples was positive in only 14% of patients. Culture and qPCR on vascular samples were positive in 58% and 91% of cases, respectively, sometimes after several months of antibiotic treatment (418). Pathological analysis of the vascular wall can show necrotizing granulomas (564). 18 F-FDG PET/CT scans have also been used on this indication in a total of 36 patients (of which 19 patients had a vascular graft) (411, 551). This exam has the advantage of detecting other sites of infection that are frequently associated, such as spondylodiscitis and psoas abscess.

To improve the early diagnosis of these infections, proposed diagnostic criteria with PCR, culture, serology, CT scanning showing an aneurysm or vascular prosthesis, and 18

F-FDG PET/CT scanning showing aneurysm or graft hypermetabolism were included as major criteria (410) (Table 3).

(iii) Osteoarticular infections. Bone and joint *C. burnetii* infections were considered a rare entity, occurring in 2% of Q fever cases in initial series (474). However, they have been increasingly reported in the last decade. Osteomyelitis, often multifocal, seems to be more a frequent presentation in children, with a total of 11 cases reported in the literature (577–583). In adults, the clinical presentation is more variable. Isolated osteomyelitis seems to be much less frequent, with only 2 cases in the literature, one involving the cheek (584) (but this case would not fulfill current criteria for *C. burnetii* osteoarticular infection because of the absence of microbiological proof and low IgG1 titers) and one bilateral tibio-femoral osteomyelitis in a 49-year-old man with a doughnut granuloma and serological titers compatible with persistent infection (585). Six cases of isolated spondylodiscitis (not associated with endocarditis or vascular infection) have been reported (412, 474, 586, 587). A total of eight cases of culture-negative prosthetic joint arthritis have been reported, seven of them involving the hip and one involving the knee (412, 419, 420).

Other localizations include tenosynovitis of the wrist (2 cases) (586) and of the tibia (1 case) (412), subacromial bursitis (3 cases) (412, 419), and coxitis, sacroiliitis, arthritis of the ankle and shoulder (412, 419, 474). The use of an 18 F-FDG PET/CT scan has been reported for *C. burnetii* osteoarticular infections, and we have proposed diagnostic criteria for prosthetic joint arthritis and other osteoarticular infections (412, 419, 420) (Table 3).

(iv) Persistent lymphadenitis. A total of 18 cases of persistent focalized lymphadenitis have been reported (334, 412, 517). One case was associated with a diagnosis of lymphoma. Four cases of isolated persistent lymphadenitis were diagnosed with 18 F-FDG PET/CT scanning. Other cases were isolated (2 cases) or associated with endocarditis (1 case), vascular infections (4 cases), or osteoarticular infections (6 cases) (412). We recently developed a diagnostic score for *C. burnetii* persistent lymphadenitis (412) (Table 3).

C. burnetii Infection in Special Populations: Pregnant Women and Children

***C. burnetii* infection during pregnancy.** Primary infection in pregnant women is most often asymptomatic (413). However, poor obstetrical outcomes have been described, mainly when *C. burnetii* infection occurs during the first trimester (428, 588). Seroprevalence studies in pregnant women show very variable rates in areas of endemicity: 0.15% in southeastern France (589), 3.8% in Canada (590), and 4.6% in London, United Kingdom (591). In Denmark, a seroprevalence rate of up to 47% was reported in pregnant women who were occupationally exposed to livestock, versus 4.8% in unexposed women (592). During the outbreak in the Netherlands, seroprevalence rates between 3.4% (593) and 9% were reported in high-prevalence areas, with a statistically significant correlation with proximity to an infected dairy goat farm (594).

Various obstetrical complications have been reported in pregnant women infected with *C. burnetii*, such as miscarriage, fetal death, malformations (omphalocele, hypospadias, Potter syndrome, congenital hydronephrosis, and syndactyly), growth retardation, oligohydramnios, and premature delivery (148, 164, 428). A case of nosocomial transmission between 2 women in an obstetrical ward has been reported (131), probably due to dissemination of spores from birth products. Prevention of nosocomial transmission consists of precautions such as wearing masks and gloves during placental manipulation and isolation in a single room for infected pregnant women (428, 432). The mechanism of early abortion involves placental abruption due to placentitis (428, 595), and *C. burnetii* can be isolated from the infected placenta (164).

Obstetrical complications have been reported mainly in case series from France, Spain, Canada, and Australia (148, 590, 595–599), while recent large-scale population-based serological studies from Germany (600), Denmark (429, 430), and the Netherlands (431, 593) have shown no increased risk of adverse obstetrical outcomes in seropositive pregnant women (601). A recent meta-analysis from our team of 136 cases and 7

population-based studies confirmed some key points: seropositivity and untreated Q fever during pregnancy are associated with fetal death, and antibiotic treatment prevents this complication (148). The discrepancy in previous studies can be explained by several factors. First, Angelakis et al. found that *C. burnetii* strains harboring the QpDV plasmid were associated with an increased risk of abortion, suggesting that genotypes from different geographical areas could induce different rates of obstetrical complications (164). Second, contradictory results in studies from the Netherlands and Denmark may be due to bias in study design. For example, in Denmark, the same authors observed poor obstetrical outcomes in a case series (313, 602) and no increased risk of adverse pregnancy events in population-based serological studies (429, 430).

***C. burnetii* infection in children.** Specific studies of clinical manifestations of Q fever in children are few. Globally, children are more frequently asymptomatic than adults and tend to present less symptomatic forms of the primary infection. Seroprevalence studies in the general child populations from different geographical areas are available and show very variable rates of seropositivity (603). In Queensland, Australia (413), a seropositivity rate of 2.5% has been found in children <15 years (604). In West Africa, a recent study found a seroprevalence rate for Q fever of 8.3% among 796 children (605). An early Japanese study found 32.7% seropositivity among 55 school children with an influenza-like syndrome (606).

Regarding clinical manifestations, a recent study in the Netherlands of 49 children revealed that the top five clinical symptoms of primary infection were influenza-like syndrome, lower and upper respiratory tract infection, malaise, and digestive signs (gastroenteritis-like symptoms). The outcome was favorable in all cases. To date, no case of persistent *C. burnetii* infection has been reported in children in the Netherlands, but based on the prevalence of congenital heart disease, at least 13 children in the highest-prevalence area may be at risk of developing endocarditis (603). As is the case in adults, the clinical presentation of primary infection is not specific and can mimic other classical childhood infections, and testing for Q fever is rarely performed by pediatricians (607).

However, cases of hepatitis, meningoencephalitis, pericarditis, myocarditis, lymphadenitis, skin rash, rhabdomyolysis, and hemolytic-uremic syndrome in children caused by *C. burnetii* primary infection have been reported (304, 310, 608–614).

The most frequently reported persistent infection in children is osteomyelitis, which is frequently multifocal, with evidence of granulomatous bone lesions (577–583). Cases of endocarditis have been described mainly in children with congenital heart disease (187, 310, 615). One case of infection of a bovine jugular vein conduit graft (616) and one case of multiple recurrent abscesses (617) have also been described.

Other Related Clinical Syndromes

Ischemic stroke and atherosclerosis. Recently, a case-control study was conducted in Spain among patients aged >65 years presenting with ischemic stroke (618). Positive serology for persistent *C. burnetii* infection was found in 14.5% of cases and 6% of controls ($P < 0.004$). This association was conserved when adjusted for age, sex, and cardiovascular risk factors. Further studies are needed to investigate this association. The same authors found an association between past serological evidence of *C. burnetii* infection and cardiovascular atherosclerosis in the elderly (619). Before that, Lovey et al. had already found an association in a Swiss cohort between primary *C. burnetii* infection and the risk of developing a cerebrovascular accident and cardiac ischemia (620).

Lymphoma. Our team has recently demonstrated that *C. burnetii* infection is associated with an increased risk of lymphoma (334). Before that study, 22 cases of lymphoproliferative disease in the context of Q fever were available in the literature (621–624). A patient who was followed in our center for a *C. burnetii* vascular infection was diagnosed with B-cell lymphoma, and *C. burnetii* was detected in the lymphoma tissue. Therefore, we performed a retrospective study among all patients diagnosed in the French national reference center with Q fever to search for other cases of *C. burnetii*-associated lymphoma. A total of seven cases were found, presenting mature

B-cell lymphoma (6 diffuse and one low-grade B-cell lymphoma). An excess risk of diffuse B-cell lymphoma and follicular lymphoma was found in Q fever patients compared to the general population (standardized incidence ratios [95% confidence intervals {CI}], 25.4 [11.4 to 56.4] and 6.7 [0.9 to 47.9], respectively). Moreover, patients with *C. burnetii* persistent focalized infections were more at risk of lymphadenitis and progression to lymphoma (odds ration [OR], 14.54; 95% CI, 2.14 to 337.7; $P = 0.007$). Consequently, *C. burnetii* may be a cofactor for lymphoma, and the possible mechanism could be the infection of monocytes and dendritic cells, causing impairment of the immune system leading to lymphoma.

CFS. Chronic fatigue syndrome (CFS) is usually described several years after primary infection with *C. burnetii*. The symptoms are characterized by persistent fatigue following a primary infection with no sign of persistent infection, and it has been described as Q fever fatigue syndrome (QFS). Nevertheless, cautious analysis of the literature is necessary regarding QFS, because the definition of this syndrome has been variable through the years. In particular, the first case-control studies from Australia and the UK showed a wide range of clinical signs, such as persistent fatigue, increased sweating, blurred vision, arthralgia, alcohol intolerance, breathlessness, or enlarged painful lymph nodes (625, 626). Moreover, no details are given about serological follow-up, so that symptoms attributed to QFS could in fact be related to undiagnosed persistent *C. burnetii* infection. This is illustrated by the study by Wildman et al. that took place 10 years after an outbreak in the UK. Among the 108 patients followed, some died of aneurysm rupture and endocarditis with cardiac failure, suggesting possible undiagnosed persistent infection, but no data were given about serological titers for these patients (627). In some studies, confusion between "chronic Q fever" and QFS is really tangible, because a proportion of the patients have a positive PCR for *C. burnetii* on blood samples or high IgA1 levels, so that no conclusion, in particular about the efficacy of antibiotics in this syndrome, can be drawn from them (628–631).

However, even if these studies suffer from bias, all have shown a significantly higher frequency of patients reporting fatigue after a primary *C. burnetii* infection than that found in healthy controls. Moreover, two studies have reported significant rates of *C. burnetii* seropositivity in cohorts of patients with CFS (27% and 17%) (632, 633). In these first studies, patients were tested with questionnaires inspired from the CFS criteria of the CDC (1994) (634). CFS was defined as a fatigue lasting for 6 months or more with elimination of somatic disease (hypothyroidism, narcolepsy, sleep apnea, drugs, chronic viral hepatitis, alcoholism, psychiatric trouble, or obesity) (634).

More recent studies have used a battery of different validated tests for evaluation of health status (SF-36, Chalder fatigue scale score, SF-12, MFI, SOMS, CDC-SI, Whiteley index, F-Sozu, and OQ-45) with duration of follow-up from 27 months to 6 years. They found a higher frequency (from 32% to 54%) of fatigue symptoms and impaired quality of life in patients who had experienced an episode of primary Q fever than in healthy controls (635–637). Strauss et al. also found that patients with fatigue symptoms following Q fever in Germany were more frequently subject to hypochondriacal worries and beliefs and somatization (637). However, Thomas et al. found no significant association between *C. burnetii* seropositivity and psychiatric morbidity in a retrospective cohort of farmers from the UK (638).

Regarding the pathophysiological explanation for this syndrome, Helbig et al. found a significantly increased frequency of HLA-DRB1*11 in patients with QFS, suggesting an immunological mechanism, depending on the host susceptibility (639, 640). Similarly, Pentilla et al. found that peripheral blood mononuclear cells (PBMCs) from patients with CFS exhibited higher levels of IL-6 secretion than those from controls when stimulated with *C. burnetii* antigens (285). Other studies failed to identify specific patterns of immune gene expression in QFS (641, 642). Some studies from the UK have tried to identify antigenic residual particles in patients with QFS. However, the results are doubtful, since some of them are based on the detection of *C. burnetii* by PCR (643, 644), suggesting rather persistent *C. burnetii* infection, and this hypothesis was not confirmed in the follow-up of patients in the UK (636).

A more recent body of literature concerning QFS has been available since the Netherlands outbreak. Interest in this pathology was increased during and after the outbreak, not only in the scientific world. Thus, the real prevalence of this syndrome in this situation is difficult to evaluate. In an outbreak situation such as the one experienced in the Netherlands, sudden media coverage about a new disease can be stressful, and various information and rumors can easily circulate within social networks. As a consequence, and because primary Q fever can be asymptomatic, a higher proportion of patients having a great diversity of subjective symptoms could attribute their malaise to Q fever. For that reason, efforts have been made to use the most standardized tools possible for the diagnosis of QFS. The authors of the first studies used the Nijmegen Clinical Screening Instrument (NCSI) for assessment of the patient's health status. This is an empirically validated battery of tests evaluating functional impairment, subjective symptoms, and quality of life (645). In these studies, patients with possible, probable, or proven "chronic Q fever" were excluded. Limonard et al. investigated the health status of 82 patients with a past primary infection at 1 and 4 years of follow-up and found a higher frequency of severely impaired general quality of life and undue fatigue (50% and 46%, respectively) than in an adjusted control group (645, 646). These proportions were stable through the years. Morroy et al. identified 58.9% of patients with abnormal fatigue at 26 months of follow-up among 515 reported Q fever patients (647). Van Loenhout et al. performed a cohort study over a 24-month follow-up, using NCSI and SF-36 scores (648) among reported Q fever patients. They found rates of severe fatigue of 73% at 3 months, and this proportion decreased to 37% at 24 months but was still significantly higher than that in a healthy control group (2.5%). Baseline characteristics that were associated with long-term impaired health status were young women and preexisting health problems. In 2015, Keijmel et al. compared the characteristics of QFS patients and patients with CFS of other etiologies (649). QFS patients were significantly older, had a higher body mass index (BMI), and were more frequently men than patients with CFS. They also had received treatment for depression less often before the onset of symptoms. However, the two preceding studies suffer from a bias, which is the inclusion only of patients "reported" as having acute Q fever following the European guidelines (407), i.e., only patients with symptomatic primary infection. This bias was highlighted by Van Loenhout et al., who showed that long-term health status was altered at comparable levels for 193 reported and 448 nonreported cases at 4 years of follow-up (407).

MANAGEMENT STRATEGY FOR PATIENTS WITH *C. BURNETII* INFECTION

Given the clinical polymorphism of *C. burnetii* infection, there is no single management strategy. Recent studies have revealed that each situation requires specific treatment and follow-up. Regarding primary infection, the main issues after diagnosis are screening for potential risk factors for complications and choice of a prophylactic treatment with doxycycline (200 mg/day) and hydroxychloroquine (600 mg/day) to prevent progression to persistent focalized infection. Regarding endocarditis and vascular infections, early diagnosis should help to promptly initiate appropriate antibiotic therapy and rapidly decide if surgical treatment is needed. The special situation represented by pregnant women and children requires specific therapeutic options. In the case of other related complications such as chronic fatigue syndrome, no definite strategy is recommended to date, but cognitive behavioral therapy and doxycycline are under evaluation in a randomized controlled study in the Netherlands (650).

Treatment, Screening Strategy, and Follow-Up of Primary Infection

Treatment. When primary infection is symptomatic, it is recommended to initiate antibiotic treatment using doxycycline (200 mg per day). The first comparative nonrandomized study was performed in 1962 (651). Powell et al. compared two regimens of treatment and showed that duration of fever was shorter (1.7 days) in patients treated with doxycycline than in untreated patients (3.3 days) (651) This treatment seemed to be more effective when initiated within the first 3 days of symptoms.

Sobradillo et al. also observed that doxycycline treatment was associated with a more rapid decrease of fever (652). A recent retrospective study performed by Dijkstra et al. during the outbreak in the Netherlands confirmed that treatment with doxycycline, a fluoroquinolone, clarithromycin, or co-trimoxazole was associated with a reduced risk of hospitalization (653) compared with that for patients receiving beta-lactams or azithromycin. The same study found that a delay in treatment initiation of more than 7 days was associated with a higher rate of hospitalization. Side effects of doxycycline such as photosensitivity can be prevented by solar protection, and women should be on effective contraception for the duration of treatment. The standard duration of treatment is 14 days (654).

In case of doxycycline intolerance, minocycline, clarithromycin (500 mg twice daily), fluoroquinolones (ofloxacin 200 mg three times a day or pefloxacin 400 mg twice a day), and co-trimoxazole (160 mg trimethoprim and 800 mg sulfamethoxazole twice daily) are alternatives (17, 18). Azithromycin should not be a first choice, since *in vitro* studies have shown an elevated MIC (>8 mg/liter) and because of a higher risk of hospitalization with this treatment in the study from the Netherlands (21, 25, 653). For patients with neurological involvement during primary infection, fluoroquinolones are an interesting choice, since they have a good penetration in the cerebrospinal fluid (31). Glucocorticoids have been added to doxycycline in anecdotal reports, but we do not recommend using this treatment, since it can favor progression to persistent infection (292).

One study by Kampschreur et al. suggested that treatment of primary infection with doxycycline may prevent progression to persistent focalized infection (655). However, a significant bias of this result is that patients who have been treated for primary infection may have benefited from screening for risk factors and from closer follow-up than patients without treatment, who may have been actually undiagnosed during primary infection. To date, in the case of asymptomatic patients or when the diagnosis is made after resolution of symptoms, no treatment is recommended, except in the case of pregnancy (42).

Screening for risk factors of persistent focalized infection. After treatment of *C. burnetii* primary infection, the duration of treatment and follow-up are determined according to the results of the screening for risk factors of persistent infection. If these risk factors are detected, antibiotic prophylaxis with doxycycline and hydroxychloroquine should be initiated. This combination has proven to be effective in preventing endocarditis in a cohort study from our center (320). Since hydroxychloroquine may induce ocular side effects, ophthalmological examination before treatment and every 6 months during follow-up is recommended. Also, this drug is contraindicated in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency (654). When prophylaxis is initiated, we recommend serological follow-up 1 month after initiation of treatment and then every 3 months (422).

(i) Risk factors for endocarditis. As discussed in the preceding section, age and the presence of a valvulopathy are the most important risk factors for progression to *C. burnetii* endocarditis. Thus, we recommend performing systematic transthoracic echocardiography (TTE) in patients with primary infection. Antibiotic prophylaxis must be initiated in case of cardiac valve prosthesis, grade ≥2 valve stenosis or regurgitation, mitral valve prolapse, aortic bicuspidy, or remodeling or thickening of the valve (295) (Fig. 5).

Million et al. also demonstrated in a cohort study that high levels of IgG aCL were significantly more frequent in patients with valvulopathy (295). Therefore, in *C. burnetii* primary infection, IgG aCL may be a biomarker of valvulopathy, as it was highly elevated in patients for whom valvulopathy was diagnosed after two TTEs. Moreover, they also observed that greatly elevated IgG aCL during primary infection was an independent predictor of progression to endocarditis. For this reason, we recommend including an assay of IgG aCL in the systematic screening of patients with *C. burnetii* primary infection. Age greater than 40 and IgM II levels of >3,200 were also associated with progression to endocarditis. Consequently, in patients >40 years with an IgG aCL level

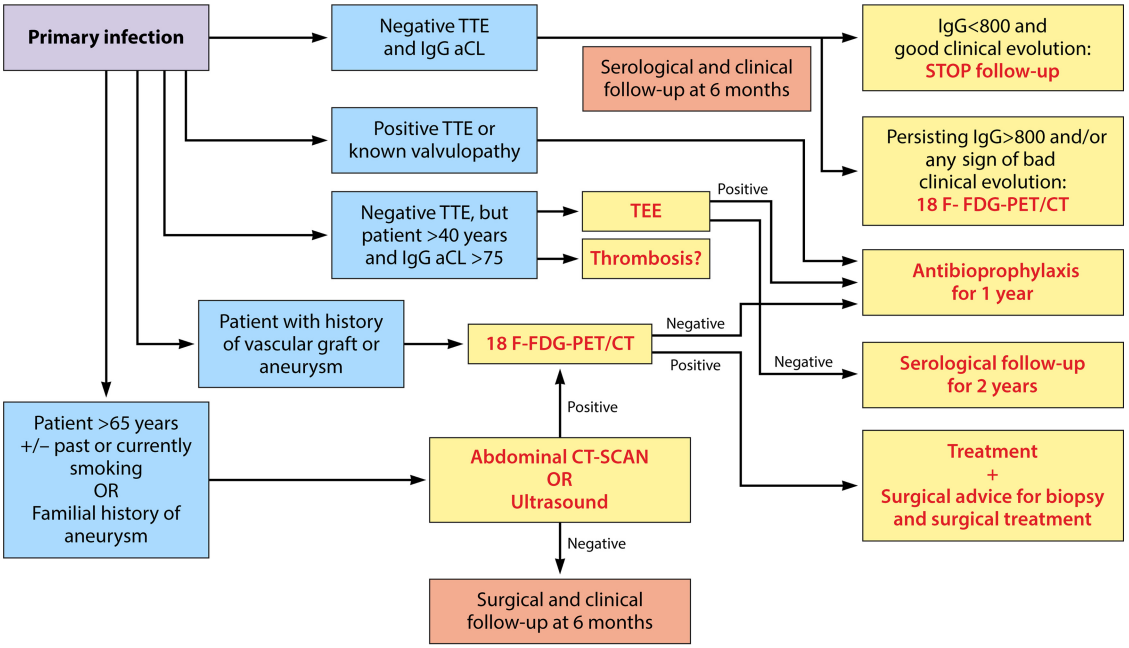

FIG 5 Management strategy for *C. burnetii* infection.

of >75 IU and a normal TTE, we recommend performing transesophageal echocardiography (TEE) to rule out any valvulopathy (295) (Fig. 5). If the TEE is negative, we recommend close serological and clinical follow-up for 2 years. Conversely, if the TTE is negative in a patient with a low IgG aCL level, we recommend routine serological and clinical follow-up at 6 months. Finally, high IgG aCL levels have been reported in patients presenting with thrombosis during *C. burnetii* primary infection (532, 533; Million et al., submitted). Consequently, we suggest screening patients for thrombosis in cases of high IgG aCL, in particular for thrombophlebitis of the leg and pulmonary embolism. (Fig. 5). In this context, in addition to doxycycline, patients with high IgG aCL levels (≥75 GPLU) may benefit from hydroxychloroquine treatment until the IgG aCL decreases to under this value (Million et al., submitted). Patients with thrombosis history, procoagulable state, or exceptionally high IgG aCL levels require prophylactic anticoagulation.

(ii) Risk factors for vascular infections. *C. burnetii* vascular infections are very severe, and no prophylactic strategy existed until recently. Major risk factors are vascular grafts and the presence of preexisting vascular aneurysms. The most frequent localization is the abdominal aorta (418). In a recent study, we proposed a screening strategy to detect patients with undiscovered abdominal aortic aneurysm (418). Since age of >65, tobacco use, and familial history of aneurysm are the main risk factors for vascular aneurysms, we recommend performing an abdominal CT scan or ultrasound (in case of renal contraindication) in these patients. If a vascular aneurysm is detected, we recommend performing 18 F-FDG PET/CT to screen for signs of early infection. In case of a negative 18 F-FDG PET/CT, initiation of doxycycline and hydroxychloroquine should be done for prophylaxis (Fig. 5).

(iii) Immunocompromised hosts. For immunocompromised patients, no data are available in the literature in favor of prophylaxis. However, we recommend close serological monitoring of these patients at 3, 6, 9, and 12 months after primary infection, because they may be at higher risk for developing focalized persistent infection (654).

(iv) Follow-up strategy when no risk factor is detected during primary infection. When all screening tests are negative, routine serological and clinical follow-up should be performed at 6 months (654). If serological titers persist at levels higher than 800 at 6 months of follow-up and if the patient shows signs of a poor clinical outcome,

(persistent fever, asthenia, weight loss, or persistent superficial adenopathy), an 18 F-FDG PET/CT scan should be performed. In a recent study, we showed that this exam discovered an unsuspected focus of *C. burnetii* infection in 38.7% of patients with persistent IgG I titers of >800 (412).

Treatment and Follow-Up of Persistent Focalized Infection

Treatment and follow-up of endocarditis. Antibiotic treatment for *C. burnetii* endocarditis combines doxycycline (200 mg/day) with hydroxychloroquine (200 mg 3 times/day). Hydroxychloroquine is necessary to raise the pH in the pseudolysosomal vacuole to restore doxycycline activity (36, 656). This combination has shown bactericidal activity *in vitro* (36). In 2010, Million et al. reported the results of a survey of 104 patients with *C. burnetii* endocarditis followed in our center (415). The main side effects reported were photosensitization in 23% of patients, digestive intolerance in 7%, ocular toxicity in 4%, and irreversible skin pigmentation in 3% (415). In that study, the rate of endocarditis-related mortality was 4% after 3 years of follow-up. Independent factors associated with death were age at diagnosis, stroke at diagnosis, prosthetic valve, and absence of a 4-fold decrease in IgG and IgA at 1 year of follow-up. The ideal time for serological cure (IgG1 level of <800) was 41 months, and 36% of patients had serological cure at 3 years. Independent factors associated with serological failure were male sex, the presence of IgG1 at diagnosis, and delay in treatment initiation. Forty-five percent of patients had surgical treatment, which was more frequently performed in cases of heart failure and cardiac abscess. However, surgery was not associated with a better survival, except in the group of patients with a valvular prosthesis. Clinical and serological cure is possible without valve replacement. Analyses of the excised valves (by culture and IHC) were all negative after 24 months of treatment. However, two prosthetic valves were positive after 18 months. In addition, serological relapse was associated with treatment of less than 18 months and prosthetic valve endocarditis. For these reasons, we recommend treating patients with native valve endocarditis for 18 months and patients with prosthetic valve endocarditis for 24 months. Longer treatment can be proposed in the case of absence of a 4-fold decrease in IgG and IgA and no disappearance of IgM II. Serological monitoring should be performed every 3 months during treatment. Rolain et al. have shown that serum doxycycline concentrations up to 5 μg/ml are correlated with good serological progression in patients with endocarditis (657). Thus, monitoring of doxycycline and hydroxychloroquine concentrations, with objectives of >5 μg/ml and 1 $\pm$ 0.2 g/ml, respectively, is useful. We observed 6% of patients with serological relapse at 5 years (415). For this reason, we recommend continuing serological monitoring until 5 years of follow-up.

Treatment and follow-up of vascular infections. For years no consensual guidelines were available concerning vascular infections, due to a too-small number of cases per center. However, a retrospective study of 32 patients by Botelho-Nevers et al. in 2007 suggested that surgical resection of the infected tissues was associated with recovery (417). Twenty-five percent of patients from this cohort had died at 3 years follow-up, and patients who died had a significantly shorter course of treatment (mean duration of treatment, 10 months), less frequent surgical treatment, and more frequent vascular rupture. Therefore, doxycycline and hydroxychloroquine treatment should last for a minimum of 24 months.

More recently, we performed a retrospective study of all patients with infections of vascular aneurysms or grafts who have been followed in our center between 1986 and 2015 (418). For 66 patients, a follow-up of 2.5 years was available, and the mortality rate was 18.5%. Mortality rates were significantly different for patients who were operated on and those who were not (6.5% versus 28.6%, respectively; $P = 0.02$), and surgical treatment was the only independent factor associated with survival (418). Surgical treatment was also a predictor of good serological progression. For three patients, qPCR on a vascular biopsy specimen was positive after 2 years of treatment, and for one patient, culture was also positive at that time. This illustrates that antibiotic treatment

alone is not sufficient to eradicate the infection in this setting and that resection of the infected tissue is mandatory, due to the high bacterial load.

In our study, patients with vascular graft infection were less frequently operated on than patients with vascular aneurysm infections, and in this subgroup of patients, surgery was associated with good serological progression, but correlation with survival was not statistically significant. This lack of correlation may be due either to a lack of statistical power or to the fact that postoperative mortality was higher in the group of patients with vascular grafts. In fact, these patients may have more cardiovascular comorbidities and higher anesthesia risks than patients with infection of vascular aneurysms.

The conclusion from this study is that surgical treatment should be performed in the case of *C. burnetii* infection of a vascular aneurysm. In the case of patients with vascular grafts, surgery should be performed after careful assessment of surgical and anesthesia risks. Semiconservative surgery, which was successful in a case report from Kloppenburg et al., may be a solution in patients presenting a very high surgical risk (658).

Serological monitoring should follow the same pattern as for endocarditis.

Treatment and follow-up of other persistent focalized infections. For other types of persistent focalized infections, such as osteoarticular infections and persistent lymphadenitis, no cohort study is available, so that treatment options rely on case reports and expert advice.

Regarding osteoarticular infection, administration of doxycycline and hydroxychloroquine for 18 months has been reported to be the best option (419, 420). However, one case of treatment failure was reported in a patient with prosthetic joint arthritis, but this patient had rheumatoid arthritis and was on immunosuppressive treatment. In this case, surgical removal of the prosthesis was necessary for cure. To date, no treatment recommendation exists for persistent lymphadenitis. We propose the same 18-month duration of antibiotic treatment when this diagnosis is definite according to our recent criteria. The same serological monitoring as for endocarditis and vascular infections should be performed in such patients.

Treatment and Follow-Up of Pregnant Women and Children

Pregnant women. A retrospective cohort study compared obstetrical outcomes in 16 pregnant women who received long-term co-trimoxazole (i.e., 160/800 twice daily for 5 weeks) versus 37 who did not receive this treatment. Obstetrical complications were observed in 81.1% of pregnant women without treatment versus 43.9% of pregnant women who received long-term co-trimoxazole ($P = 0.009$) (659). In particular, no intrauterine fetal deaths were observed in the latter case. Moreover, this treatment reduced the rate of placentitis and progression to a chronic serological profile. Angelakis et al. have confirmed this result, with 42% of treated pregnant women presenting obstetrical complications versus 100% of untreated pregnant women (164). Finally, a meta-analysis by Million et al. on this subject included 136 pregnancies (4 case histories and 7 population-based studies). This study revealed an increased risk of fetal death when *C. burnetii* serology was positive, but this risk was significantly reduced when pregnant women were treated (148). Because co-trimoxazole is a folic acid antagonist, supplementation with folic acid should be administered during the first trimester of pregnancy. After treatment, close serological follow-up should be performed at 3, 5, 9, 12, 18, and 24 months for early detection of progression to persistent focalized infection.

When persistent focalized infection is diagnosed during pregnancy, co-trimoxazole should be initiated and maintained until delivery. After delivery, a change to doxycycline and hydroxychloroquine should be made. In case of diagnosis in the peripartum period, breastfeeding is not recommended, due to possible transmission to the newborn (659).

Children. Doxycycline is used in the treatment of primary infection for children >8 years old (310, 609, 612). The pediatric dose for doxycycline is 2.2 mg/kg twice daily (42). The duration of treatment is 14 days, as for adults. For children with mild primary

infection who are younger than 8 years old, we recommend using co-trimoxazole because of the risk of dental staining. However, a recent review by Cross et al. concerning the risks and benefits of doxycycline suggests that, conversely to the case for tetracycline, no strong evidence of correlation with dental staining and teratogenic effects can be found in the literature (660). Therefore, in children <8 years old with severe primary infection, we recommend using doxycycline.

Regarding the treatment of persistent focalized infection in children, few data are available in the literature. Cases of endocarditis and infection of a bovine jugular vein conduit were treated with doxycycline plus hydroxychloroquine and surgery (187, 616). These cases involved children 11 and 13 years old. Osteomyelitis is treated with variable combinations of the following antibiotics: co-trimoxazole, ciprofloxacin, rifampin, doxycycline, clarithromycin, and azithromycin (578, 581, 582). The most frequent duration of treatment found in the literature is 6 months, and surgical drainage of multifocal osteomyelitis is often needed for cure (578). One reference laboratory described successful surgical treatment alone in a child with a single bone lesion (579). An anecdotal case of treatment with gamma interferon as salvage therapy in a 3-year-old child is reported, but it does not seem reasonable to recommend this treatment (661).

Treatment of Chronic Fatigue Syndrome

Regarding the treatment of QFS, a prospective randomized trial is currently in progress in the Netherlands to compare the efficacy of doxycycline and cognitive behavioral therapy versus placebo (650). Inclusion criteria for this study are based on the definition of Q fever fatigue syndrome (QFS) from the Dutch National Consensus. Cognitive behavioral therapies have proven to be effective in patients with chronic fatigue syndrome following other diseases and may therefore be an interesting option.

Prevention

In some situations, Q fever is an occupational disease. This has been illustrated by the first description of the disease, which occurred in a population of slaughterhouse workers. Also, a member of a laboratory team cultivating *C. burnetii* in the 1930s was infected (375). The main categories of people occupationally exposed are those working with animals (farmers, slaughterhouse workers, and veterinarians) and people working in laboratories cultivating the bacterium. Cases involving medical staff consist of people who participated in autopsies of patients with Q fever (662, 663) or an obstetrician who managed parturient women with Q fever (130). Cases in the military have also been reported (1, 664, 665). In this section we detail the available prophylactic measures in occupational settings.

Vaccination. A vaccine has been available in Australia since 1989 (Q-Vax; CSL Biotherapies, Parkville, Victoria, Australia) (666). It is a whole-cell formalin-inactivated vaccine produced and licensed in Australia. Its efficacy has been tested in one randomized control study among 200 slaughterhouse workers. During 15 months of follow-up, there were seven cases in the control group and no cases in the vaccinated group (667). However, this vaccine can induce local reactions, and patients should be evaluated with a cutaneous test (Q-Vax skin test) for Q fever before vaccination to avoid severe side effects. The Australian Veterinary Association (AVA) recommends vaccination for all veterinarians, veterinary students, and veterinary nurses. A recent survey in that country has shown that 74% of veterinarians and 29% of veterinary nurses sought vaccination (666). Also, a nationally funded vaccination program was initiated in the country in 2002 (668). Program adherence was 100% among slaughterhouse workers and 43% in farmers. After this campaign, reporting for Q fever decreased by 50%, and the number of hospitalizations also decreased (668). In 2011, in the southeast of the Netherlands, a vaccination campaign targeted people at risk for progression to endocarditis and vascular infections during the epidemic (669). However, coverage rates in high-risk people of only 11% to 18% were observed, illustrating poor efficacy from a public health perspective. No other country has tried to institute a large-scale vaccination program among occupationally exposed persons to date. In these areas, the

improvement of clinicians' awareness about the risk for *C. burnetii* infection in some categories of workers should help in the early diagnosis and treatment of the infection.

Isolation. Regarding the prevention of transmission in laboratory workers handling *C. burnetii* cultures, all manipulations have to be made in a biosafety level 3 (BSL3) laboratory with appropriate personal protective equipment (PPE). For health care personnel performing autopsies on patients suspected to have died from Q fever, wearing of an N95 respiratory protection mask is recommended. The same recommendation can be made for obstetrical staff who are in contact with parturient women diagnosed with *C. burnetii* infection.

Moreover, because *C. burnetii* can survive in the environment in a spore-like form, contaminated surfaces should be cleaned with a solution containing a dual quaternary ammonium-detergent compound, which completely inactivates the bacteria after a 30-min contact time (42). A 1:100 dilution of household bleach is also an effective solution.

PERSPECTIVES AND FUTURE CHALLENGES

A more accurate assessment of the risk factors in the progression to *C. burnetii* endocarditis will require particular studies. The understanding of individual susceptibility factors will allow better management through active treatment in order to prevent progression to this still-severe disease. The systematic detection of *C. burnetii* by multiplex PCR strategies in syndromes such as endocarditis, pneumonia, hepatitis, and fever during pregnancy should allow diagnosis and treatment of more cases in the world. Finally, new therapeutic strategies with shorter courses and better tolerance should be developed for endocarditis and vascular infections. *In vitro* testing, new antibiotic combinations, and randomized studies comparing new protocols to the therapeutic approach that has been used for the last 20 years are necessary and will be a major subject of future research.

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Max Maurin, M.D., Ph.D., is 54 years old and trained in Professor Didier Raoult's team from 1991 to 2002. He received his M.D. degree at the Medical Faculty of Marseille, Aix-Marseille II University, France, in 1991 and his Ph.D. degree at the Bichat Faculty, Paris VII University, France, in 1993. He was appointed as a professor of bacteriology at Grenoble University Hospital (Grenoble Alpes University) in 2002. He has been the head of this bacteriology laboratory since 2002 and of the French national reference Center for *Francisella* since 2006. Since 1991, he has been involved in the study of strict and facultative intracellular bacteria that are pathogenic for humans and animals. His main research topic has been focused on the characterization interactions between intracellular bacteria and antibiotics.

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Didier Raoult, M.D., Ph.D., specializes in infectious diseases and is a professor of microbiology at the Faculty of Medicine of Marseille, Aix Marseille University. In 1984, he created *ex nihilo* his research laboratory, the Rickettsia Unit. This unit has now become the Research Unit in Infectious and Tropical Emergent Diseases (URMITE), collaborating with the CNRS (National Center for the Scientific Research), the IRD (Institute of Research for Development), and INSERM (National Institute of Health and Medical Research). In 2011, he became the director of the University Hospital Institute Mediterranée Infection, which is a 600-person medical institute focused on infectious diseases. This facility includes the largest diagnostic and research microbiology laboratory in France. As of 2014, Professor Raoult has published more than 1,900 indexed publications. In the last 30 years, he has cultured approximately 16% of the bacteria isolated for the first time in humans, including *Tropheryma whipplei*, in his laboratory, and he developed a culturomics team in 2011.