Chapter 16
Antigenic Analysis for Vaccines and Diagnostics

Laura R. Hendrix and Chen Chen

Contents

L.R. Hendrix (✉) • C. Chen
Department of Microbial and Molecular Pathogenesis, College of Medicine,
Texas A&M Health Science Center, 3107 Medical Research and Education Building,
Bryan, TX 77807-3260, USA
e-mail: lhendrix@tamhsc.edu

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Abstract *Coxiella burnetii* infection is frequently unrecognized or misdiagnosed, as symptoms generally mimic an influenza-like illness. However, the disease (Q fever) may result in chronic infection, usually manifesting as potentially fatal endocarditis. The development of a chronic fatigue-like sequela may also occur. Infected ruminants are the major reservoir for infection in humans, primarily through exposure to birth products or aerosols that transmit the bacterium over wide regions. A vaccine against *C. burnetii* infection has been in use in Australia for abattoir and agricultural workers for many years. The possibility of adverse reactions in those with previous exposure to the agent has prevented its use elsewhere. Subunit vaccines, utilizing chemical extraction of components thought to cause adverse reactions, are in development, but none are yet licensed. Others have sought to combine immunogenic peptides with or without selected lipopolysaccharide components to produce a vaccine without the possibility of adverse reactions. Selected immunogenic proteins have been shown to induce both humoral and cellular immune responses. Although current diagnosis of infection relies on serological testing, the presentation of specific antibody occurs 7–15 days following the onset of symptoms, delaying treatment that may result in prolonged morbidity. PCR detection of DNA to specific *C. burnetii* antigens in the blood is possible early in infection, but PCR may become negative when PII IgG antibodies appear. PCR is useful for early diagnosis when Q fever is suspected, as in large epidemics, and shortens the delay in the identification of Q fever endocarditis. Others have combined PCR with ELISA or other methods to increase the ability to detect infection at any stage. The search for new diagnostic reagents and vaccines has utilized new methods for discovery of immunoreactive proteins. DNA analysis of the heterogeneity of *C. burnetii* isolates has led to a greater understanding of the diversity of isolates and a means to determine whether there is a correlation between strain and disease severity. 2-D SDS PAGE of immunogenic proteins reactive with human or animal infection sera and mass spectrometric analysis of specific secreted or outer membrane proteins have identified candidate antigens. Microarrays have allowed the analysis of peptide libraries of open reading frames to evaluate the immunogenicity of complete genomes.

Keywords *Coxiella burnetii* • Antigens • Diagnostics • Immunogenic proteins • Vaccines

16.1 *Coxiella burnetii* Infection

Coxiella burnetii causes a worldwide zoonotic disease known as Q fever, an occupational hazard for abattoir and agricultural workers with exposure to cattle, sheep and goats. While disease in animals is generally not apparent, infected animals represent a major reservoir for the dissemination of *C. burnetii* to humans, mainly via exposure to birth products for farm workers or through aerosols that may transmit the bacterium over wide regions. The bacterium exists as two infectious forms,

small spore-like cells and larger vegetative cells, with the small cell variants able to survive for long periods in the environment. In addition to domesticated animals, *C. burnetii* is known to infect wild vertebrates and arthropods. In humans, Q fever is generally a self-limiting, flu-like illness with characteristic high fever and headache, but may also present as pneumonia, hepatitis, meningoencephalitis, peripheral thrombosis, myopericarditis, or bone marrow aplasia (Ormsbee and Marmion 1990). The disease may persist after overt clinical symptoms have subsided and reactivate with appropriate stress (Baca and Paretsky 1983). Commonly, the disease is unrecognized or undiagnosed, and in many areas, a high percentage of the population has serological evidence of previous or latent infection (Luoto et al. 1965; Meiklejohn et al. 1981; Richardus et al. 1987; Wisniewski et al. 1969). Acute disease is readily treated with antibiotics, although the response to antibiotics may be slow. However, the disease may manifest as chronic infection in the form of endocarditis or hepatitis. These cases may have a poor prognosis and may be associated with suppression of cell-mediated immunity. Chronic infections have not responded well to antibiotic regimens, but the best outcome is seen following a combination of chloroquine and doxycycline administered over 1–2 years (Raoult et al. 1999; Million et al. 2010). A post-Q fever debility syndrome, with symptoms similar to chronic fatigue syndrome, is also recognized and may follow in up to 10% of acute disease cases (Marmion et al. 2005).

A vaccine to *C. burnetii* infection is licensed in Australia where large numbers of infections occurred annually before the implementation of a national vaccination program. However, the vaccine is not routinely used elsewhere due to adverse reactions that may occur in those with previous immunity, often from undiagnosed contact with the agent. While a variety of guidelines have been defined for specific sero-diagnostic strategies, diagnosis of disease is often hampered by serological evidence of past or latent infection. New methods for detection of acute cases of *C. burnetii* in blood using PCR (polymerase chain reaction) are currently restricted to the limited period before the appearance of IgG antibodies.

16.2 Early Vaccines

Vaccine development against Q fever, primarily prompted by the need to prevent disease in abattoir and agricultural workers, veterinarians and laboratory researchers, has been an active area of research for several decades. Early vaccines prepared from formalin-inactivated laboratory stocks passed many times in hen's eggs were found to be 100–300 times less effective than vaccines made from fresh isolates (Ormsbee et al. 1964). These findings were corroborated by observations that the bacterium exists as two antigenic phase variants, known as virulent phase I (PI) and avirulent phase II (PII), that differ in their ability to protect against infection in a guinea pig model (Stoker and Fiset 1956; Moos and Hackstadt 1987). Phase variation in the Nine Mile (NM) strain was found to relate to a large chromosomal deletion (Vodkin and Williams 1986; Hoover et al. 2002; Denison et al. 2007a) leading

to an irreversible smooth-to-rough mutation in lipopolysaccharide (LPS) O-side chain expression (Schramek and Mayer 1982) that occurs following passage of the organism in non-immunologically competent hosts, such as hen's eggs used for laboratory propagation (Lukacova et al. 2008).

Early research on Q fever was characterized by outbreaks of disease in laboratory workers. The ability to grow large numbers of organisms in chick embryo yolk sacs allowed the production of a formalin-inactivated whole cell vaccine to protect laboratory workers. A formalin-inactivated, ether-extracted 10% suspension of yolk sac (Smadel vaccine) from the Henzerling strain was highly immunogenic in man and animals (Smadel et al. 1948). This PI whole cell vaccine (WCV-PI) has been tested extensively for use in animals and humans, but its use is restricted in most countries due to the prevalence of local and systemic adverse reactions that can occur primarily in previously sensitized individuals. Nevertheless, studies have shown WCV-PI to be able to induce humoral and cellular immune responses to *C. burnetii* antigens and to prevent Q fever in humans and animals (Benenson 1959). These vaccines have also prevented shedding of *C. burnetii* into the environment by infected animals and offered protection against infertility, abortion and low birth weight in livestock (Bilberstein et al. 1977; Schmeer et al. 1987; Arricau-Bouvery et al. 2005). Vaccines utilizing killed PII organisms have not been shown to be effective to prevent natural infection in animals (Fishbein and Raoult 1992), but WCV-PI use in animals is not widespread since there is little economic incentive to eradicate what is generally a subclinical infection (Sawyer et al. 1987). Increased outbreaks in France, Germany, and the Netherlands have emphasized the importance of Q fever detection in livestock (Fishbein and Raoult 1992; Hellenbrand et al. 2001; Carrieri et al. 2002; Tissot-Dupont et al. 2007; Enserink 2010). Measures taken to curb infection of livestock in the Netherlands have included massive vaccinations of non-pregnant goats with an inactivated NM PI vaccine shown to reduce shedding and abortion in non-infected animals that unfortunately requires prior culling of pregnant ewes (CEVA, Libourne, France, Frankel et al. 2011).

An attenuated *C. burnetii* strain designated Grita M-44, thought to be in PII, was tested as a vaccine in the former Soviet Union (Genig 1968). It was later abandoned when studies showed this strain was able to persist in guinea pigs with mild lesions occurring in heart, spleen and liver, suggesting there could be a reactivation of infection in vaccinated individuals due to residual PI organisms (Johnson et al. 1976, 1977).

A new generation vaccine for Q fever is required to confer protection against infection without the necessity to screen for prior immunity before vaccination, as skin testing of potential vaccinees may result in adverse reactions and is a barrier to approval for use in the US and elsewhere. Chemical extraction methods were developed to reduce the adverse effects of whole killed cell vaccines while retaining good immunogenicity. Initial efforts focused on developing a vaccine from whole killed cells with the removal of components responsible for sensitization, thought to be due to PI-LPS. Extraction methods utilized included phenol-water mixtures, dimethylsulfoxide (DMSO), formamide, trichloroacetic acid (TCA) and chloroform-methanol (CM). Kazar et al. tested a TCA-extracted NM PI vaccine in workers at high risk for exposure to *C. burnetii* infection in the former Czechoslovakia.

While a significant antibody response occurred in vaccinated individuals, severe local and systemic reactions occurred in individuals who had been previously infected with *C. burnetii* (Kazar et al. 1982).

A CM-extraction residue vaccine (CMR) from PI Henzerling strain *C. burnetii* was developed through a joint effort between Rocky Mountain Laboratories and the US Army Research Institute for Infectious Disease (Williams and Cantrell 1982; Williams et al. 1986). This vaccine could be used at higher doses in mice than the WCV-PI without causing severe adverse reactions. Efficacy of the CMR vaccine has been shown so far in rodents, sheep and non-human primates (Brooks et al. 1986; Waag et al. 1997, 2002). Phase one human trials showed it could be safely administered to human volunteers unscreened for prior immunity at doses of 30 or 60 μg. However, the CMR vaccine was found to still contain some components responsible for adverse reactions, as higher doses in human volunteers caused reactions qualitatively similar to those seen with 30 μg doses of WCV-PI (Fries et al. 1993). A more recent study showed that a small priming dose given before vaccination with CMR vaccine is able to prime the immune system to mount an anamnestic response to *C. burnetii* (Waag et al. 2008). The production of specific antibody and the activation of peripheral blood cells by recall antigen in vitro indicated the CMR vaccine could offer protection to infection in humans.

16.3 Licensed *Coxiella* Vaccine

Since there are no *C. burnetii* vaccines that completely eliminate adverse reactions, none are currently licensed for use in the US. Australia, however, with large numbers of Q fever cases annually, has inoculated at-risk abattoir workers with formalin-inactivated WCV-PI made from PI Henzerling strain (Q-Vax, Commonwealth Serum Laboratories (CSL)) following testing in the 1980s. In 1994, Ackland et al. showed that the protective efficacy of Q-Vax is nearly 100%, with the duration of protection greater than 5 years (Ackland et al. 1994). However, at the end of 2005, production of the vaccine halted as new regulations for biocontainment facilities were put into effect. With government support, CSL was able to restart production and extend the program to include at-risk rural communities. Results indicate that broadening the population offered the vaccine has resulted in a significant decline in case numbers (Marmion 2007).

Marmion (2007) described the components of Q-Vax that may allow it to be an effective vaccine, suggesting these components would likely be required for new non-cellular vaccines to be effective. First, the highly immunogenic PI LPS or a component of it may be required for the resulting protective qualities of the vaccine. Second, *C. burnetii* proteins that interact with monocytes-macrophages stimulate the cellular immune system to produce interferon-γ and other cytokines, resulting in restriction of *C. burnetii* intracellular growth. Marmion postulated that antibody to PI LPS blocks the occurrence of other interactions of the bacterium with monocytes-macrophages that might down-regulate the cellular immune response, including

down-regulation of interferon-γ formation. The third component required for vaccine efficacy may be the small-cell variants of *C. burnetii.* Due to their slow biodegradability, they may display both PI LPS and protein antigens as a continuing antigenic stimulation.

16.4 Diagnostics

The natural antibody response in early stages of *C. burnetii* infection is primarily to protein components. In late stage acute infection or in chronic disease, the antibody response is primarily to the LPS O-antigen. This is detected clinically through the differential response to fixed whole-cell PI and PII antigen at different stages of infection. Patient serum from early acute disease reacts initially with PII IgM antibody followed by PII IgG (Geddes 1983). Previous infections are characterized by the presence of PII and low PI IgG antibody levels. High IgG titers to PI antigen, originally set at >800 but now revised to >1,600, signal possible chronic infection (Dupont et al. 1994; Fournier et al. 1996; Frankel et al. 2011). While current platforms for serological testing include indirect immunofluorescence, complement fixation, enzyme-linked immunosorbent assay (ELISA), and microagglutination (La Scola 2002), commercially available products all currently require the use of whole-cell antigen.

LPS antigen was shown to have interstrain heterogeneity in silver-stained SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) at the 10–17 kDa size range among *C. burnetii* isolates from acute and chronic infection. LPS antigenic types were separated into three distinct groups – two groups from chronic disease isolates and one from acute disease isolates (Hackstadt 1986). These results suggest that heterogeneity of LPS may affect PI titers, depending on the infecting strain, as organisms used for testing have generally been from one strain (Nine Mile).

Likewise, protein heterogeneity between strains may affect PII titers, as strains have been shown to vary by DNA banding patterns, by PAGE patterns of stained or immunoblotted proteins and other methods, with protein heterogeneity between strains confirmed by genomic sequencing data (Beare et al. 2009). These results suggest testing patient sera using NM PI and PII cells may not always give an accurate picture of the possibility and stage of disease in patients having symptoms compatible to *C. burnetii* infection. In practice, this is not considered to be an issue. For example, tests of the response to past infection or vaccination with Q-Vax show equivalent protection to Priscilla, Henzerling and NM strains as measured by proliferation of blood mononuclear lymphocytes on antigen challenge, with T lymphocytes being the major responders (Izzo et al. 1991). However, eight PI *C. burnetii* isolates from four genomic groups were shown in mouse and guinea pig models of acute Q fever to cause genogroup-specific disease manifestations while vaccine challenge between isolate groups were cross-protective (Russell-Lodrigue et al. 2009). Also, a study of the antigenic variation of polypeptides from 18 strains of *C. burnetii* showed a 28 kDa protein was immunodominant in acute disease isolates but not in strains

from human cases of chronic Q fever, suggesting this polypeptide could be a marker for acute disease (To et al. 1998; Zhang et al. 2005). Nevertheless, discovery of new diagnostic reagents have focused on proteins unique to *C. burnetii* that are conserved among all isolates.

16.4.1 Commercially Available Diagnostics

The lack of characteristic symptoms for Q fever infection often leads to a misdiagnosis of an influenza-like illness. Identification of the infecting organism from clinical specimens is not advised as *C. burnetii* is highly infectious and requires a biosafety level three laboratory for cultivation. Since a firm diagnosis of the disease relies on evidence of a four-fold rise in serum antibody titer approximately 14 days after the first serum sample was taken (Derrick 1937; Wisniewski et al. 1969; Brown 1973), serologic testing is a necessary supplement to clinical history. In 1937, Burnet and Freeman demonstrated macroscopic agglutination reactions between patient sera and spleen cell emulsions from infected mice (Burnet and Freeman 1937). The microagglutination test (MAA) developed from this early observation that whole bacterial cells clump or agglutinate in the presence of positive sera. Since PII cells spontaneously auto-agglutinate in normal serum, the creation of particulate PII antigens from TCA treatment of PI cells is required.

Commercially available assays to detect Q fever include the complement fixation test (CFT) and immunofluorescence assay (IFA). The CFT was first developed in 1941 (Bengtson 1941) and entails the addition of *C. burnetii* antigen to patient sera from which any naturally occurring complement has been depleted. A standard amount of complement is added back, followed by the addition of sheep erythrocytes pre-bound to anti-erythrocyte antibodies. If antibodies to *C. burnetii* are present in the patient sera, they will bind to *C. burnetii* antigen and complement will be depleted from the assay due to its binding to the antigen-antibody complexes. The assay is made quantitative by diluting patient sera until antibody levels are low enough for complement to bind to and lyse the erythrocytes. The CFT has been shown to be less sensitive than other assays and has largely been replaced by newer assays, including IFA.

The most common serological test currently used is the indirect IFA (Fenollar et al. 2004). This test is a primary binding assay, similar to the ELISA, and utilizes phase variation of *C. burnetii* LPS to distinguish the acute convalescent response from chronic infection responses. Dilutions of sera are reacted with PI and PII organisms in each slide well and labeled with an immunoglobulin class-specific fluorescent conjugate. Results are viewed using fluorescence microscopy and compared to positive and negative control wells. Positive sera are rerun to determine the end-point titer (the reciprocal of the antibody dilution) to compare to earlier or later samples from the same patient. In acute cases, titers to PI are accompanied by equal or higher titers to PII. This is reversed in chronic disease. PII titers over 1:256 are evidence of active or recent infection. PI IgG is often below detectable levels until

convalescence or in the presence of chronic disease. Chronic hepatitis may have high and equal PI and PII titers, while endocarditis frequently has higher PI titers. The IFA is the current reference method for Q fever serodiagnosis (La Scola 2002).

The enzyme-linked immunosorbent assay (ELISA) is a sensitive and reproducible colorimetric assay that can easily process large numbers of serum samples determined by optical density (OD) units on a microplate reader (spectrophotometer). PI or PII whole killed cells, LPS, or individual purified proteins may serve as antigens absorbed to wells of a microtiter plate. Dilutions of patient sera are added to wells and anti-human class specific antisera conjugated to an enzyme, usually peroxidase or alkaline phosphatase, is used to detect sera containing *C. burnetii* antibodies following the addition of the enzyme substrate. Titers are determined by comparison of OD differences between antigen wells and non-antigen control wells. The ELISA has been shown to be more sensitive than CFT, IFA or MAA with fewer false positives (Peter et al. 1987; Waag et al. 1991). However, all current serological tests may require the determination of cutoff values for selected locations or test samples. A commercial ELISA detecting *C. burnetii*-specific IgG was evaluated in 2002 for pre-vaccination screening and diagnosis in Australia, but results suggested negative reactions by ELISA be confirmed by IFA or CFT (Field et al. 2002). A comparison of four commercially available assays to detect IgM PII antibodies to *C. burnetii* included three IFA tests and one ELISA. Sera from 23 patients with clinical symptoms of acute Q fever were tested along with 88 control sera from blood donors. The specificity of results varied from 97.7% to 100% for the IFAs and 98.9% for the ELISA. Sensitivity was 95.65% for the ELISA and 100% for all IFAs (Frangoulidis et al. 2006).

The ELISA may be modified to a fluorescence assay (ELIFA), as was done by Schmeer et al., resulting in a 50-fold higher sensitivity for the analysis of humoral immune responses in vaccinated and naturally infected cattle to several *C. burnetii* proteins (Schmeer et al. 1988). Interestingly, the results showed that the early humoral response in cattle immunized with either chloroform-methanol extracted cell residues or a 27 kDa protein (Com1) was directed against the 27 kDa protein. They were also able to distinguish vaccinated from naturally infected cattle by measuring IgG1 and IgG2 subclass responses with ELIFA.

16.4.2 Diagnostics in Development

16.4.2.1 Sero-Diagnostics

Due to its sensitivity and ability to screen large numbers of samples, investigators have modified the Q fever ELISA for specific situations. Guatteo et al. applied the test to compare the detection of antibodies to an ovine *C. burnetii* strain in milk or serum from six infected dairy cattle herds. Of the 264 positive serum samples, 249 were also positive in milk. Of the 184 serum negative samples, 176 were negative in milk.

These results showed that a more convenient milk-ELISA could be comparable to serum-ELISA in lactating dairy cattle (Guatteo et al. 2007).

Many investigators have looked for specific antigens to replace whole killed cells for use in the ELISA platform. Sekeyova et al. identified candidate *C. burnetii* protein antigens through the creation of three monoclonal antibodies to NM strain and one to Priscilla strain (Sekeyova et al. 2010). They tested the monoclonal antibodies using one- and two-dimensional SDS-PAGE, immunoblot analysis and mass spectrometry and found they identified three specific *C. burnetii* proteins, CBU_0937 (unknown function), CBU_1910 (Com1), and CBU_0236 (elongation factor Tu). They purified Com1 and CBU_0937 proteins through the expression of PCR amplified clones and used them in an ELISA to screen sera from Q fever endocarditis and acute disease patients. Their results showed CBU_0937 was a better diagnostic tool for Q fever ELISA than Com1 protein.

A monoclonal antibody to virenose, an unusual sugar in *C. burnetii* PI LPS O antigen, was generated using a virenose-rich O-polysaccharide LPS fraction conjugated to tetanus toxoid. Testing of the monoclonal antibody at a dilution of 1:1,000 in an ELISA and by immunoblot analysis showed it to be a sensitive and specific method for the detection of virulent *C. burnetii* (Palkovicova et al. 2009).

An IgG ELISA microplate assay using *C. burnetii* heat shock protein, HspB (GroEL), was developed and tested in goats. Sera from naturally and experimentally infected goats were tested. The purified rHspB was recognized by 18 days post-infection (dpi) and was positive at 39–60 dpi in 80–90% of the animals tested. Also, animals presenting with reactivation of infection had statistically higher titers than those in latent infection (Fernandes et al. 2009).

16.4.2.2 DNA-Based Diagnostics

PCR assays detect genes encoding bacterial proteins or non-transcribed regions independent of viability, although non-viable forms are likely to be more readily degraded over time. A real-time PCR assay for the detection of *C. burnetii* DNA in serum samples targeting IS*1111* transposase sequences (also known as htpAB-associated repetitive element (Hoover et al. 1992)) was used in the Netherlands to diagnose acute Q fever early in infection to avoid delay in treatment that can lead to increased hospital admission rates and prolonged morbidity. Researchers found the latest time point after onset of disease in which *C. burnetii* could be detected by PCR of serum samples was day 17. They found 98% of seronegative sera, 90% of sera with IgM-PII antibodies, 23% with IgM-PII/IgG-PII antibodies, 5% with IgM-PII/IgG-PII/IgM-PI antibodies, and 0% with IgG-PI antibodies in combination with any other antibody types were positive by PCR (Schneeberger et al. 2010).

Previously, Fournier and Raoult used a similar IS*1111* probe to compare LightCycler nested-PCR (LCN-PCR) to serology by immunofluorescence for early diagnosis of acute Q fever. While the time line for detection of *C. burnetii* by PCR was similar to Schneeberger et al., the sensitivity in their assay was 26% for seronegative samples, and 5% for seropositive patients (Fournier and Raoult 2003).

The sensitivity of the assays of Schneeberger et al. was greater, possibly due to (1) an increase in numbers of IS*1111* in those isolates, (2) the use of a smaller sized amplification product or (3) a larger initial serum sample. These results indicate that PCR detection of *C. burnetii* can be a useful tool for early diagnosis when Q fever is suspected, such as in large epidemics that have occurred since 2007 in the Netherlands. PCR detection overcomes the lag in antibody response of 7–15 days following onset of symptoms that hampers the standard serological diagnosis (Schneeberger et al. 2010).

Fenollar et al. have also used LCN-PCR with an IS*1111* probe to shorten the diagnostic delay in the identification of Q fever endocarditis and vascular infections. They retrospectively and prospectively tested samples from 48 Q fever endocarditis or vascular infection patients and compared results to 100 controls with endocarditis caused by other agents. They prospectively used the same technique to test 30 patients treated for Q fever endocarditis and 13 patients convalescent for acute Q fever. Results showed that sera stored more than 1 month exhibited 16.2% positive results, while 64% of fresh samples analyzed were positive. High IgG PI titers decreased the sensitivity of the PCR assay. None of 15 serum samples with a PI IgG titer equal to or greater than 1:25,600 were PCR positive, compared to 39.4% positive when the PI IgG titer was less than 1:25,600. If only the fresh sera were counted in samples that had titers less than 1:25,600, 100% were positive. All serum samples from patients treated for Q fever endocarditis or who were diagnosed with acute Q fever and whose IgG PI titers were 1:800 or greater, were negative by PCR. All control patients with endocarditis from other organisms were negative. For 12 patients the first serum sample was positive, but after 1 month of treatment, all subsequent samples were PCR negative. Positivity of all PCR products was confirmed by sequencing (Fenollar et al. 2004).

A high throughput real-time PCR assay with an internal control system and automated DNA preparation was developed as a diagnostic tool to be used during biological weapons attacks or local epidemics (Panning et al. 2008). Use of the IS*1111* transposase results in a probe with a high specificity for *Coxiella*, although individual strains vary as to the number of IS*1111* elements in their genomes, and strains lacking the transposase have been described (Denison et al. 2007b).

A recent study evaluated real-time PCR assays and DNA extraction methods for Q fever diagnosis in use in seven diagnostic or reference laboratories in the Netherlands. All laboratories in the study used real-time PCR assays targeting the IS*1111a* insertion element using different DNA extraction methods. While a low degree of variation in the sensitivities of most of the developed assays was observed, PCR assays that amplified short DNA fragments (70–87 bp) yielded better results than those using larger fragments (202 bp). It was postulated that a shorter sequence would present a better target, due to the likelihood of bacterial DNA degradation in serum. Sequence polymorphisms at target sequences in isolates tested may also affect PCR performance. DNA extraction methods using certain commercial kits yielded better results than others, despite similar chemistries involved (Tilburg et al. 2010).

The detection of *C. burnetii* in tissues has been demonstrated using commercial human *C. burnetii* positive control serum as the primary antibody source (PanBio, Inc, Columbia, MD, USA) and stained using a peroxidase immunohistochemistry

method (Vectastain Elite ABC Kit, Vector Laboratories, Inc., Burlingame, CA, USA). Results were compared with those obtained from an experimental fluorescent *in situ* hybridization test targeting the 16 S ribosomal RNA to detect *C. burnetii* in placentas from ruminant abortions. Both methods performed equally well, giving equivalent results (Jensen et al. 2007). Immunohistochemistry alone had been used earlier to detect *C. burnetii* in bovine placentas (Dilbeck and McElwain 1994; Bildfell et al. 2000).

16.5 Searches for Immunogenic and Protective Antigens

The likelihood that Q-Vax would not be approved for licensing in other countries has led to a search for specific immunogenic and protective proteins for the development of a protein subunit vaccine and improved diagnostics. Several early studies suggested that subunit protein vaccines could provide protection. Williams *et al.* showed the 29 kDa *C. burnetii* porin protein P1 conferred protection from lethal challenge in mice (Williams et al. 1990). A protein of similar size was extracted from outer membrane preparations with the detergent Empigen BB and found to be immunogenic and protective in mice against *C. burnetii* challenge. The Empigen-extract was shown to also contain *C. burnetii* LPS (Lukacova et al. 1994). A partially purified 67 kDa GroEL protein conferred full protection in guinea pigs and mice (Zhang et al. 1994). However, because these proteins were not homogeneous, the vaccines may have contained other components not fully identified.

Further studies focused on the identification of immunogenic proteins defined by strong reactivity to infection-derived sera. Muller et al. identified an immunogenic 27 kDa *C. burnetii* antigen on SDS-PAGE gels purified from infected cell cultures by guanidinium hydrochloride treatment and chloroform/methanol extraction (Muller et al. 1987). Techniques including immunoprecipitation of surface-iodinated *C. burnetii*, Western blot analysis of bacterial outer membrane fractions separated on SDS-PAGE, or bacterial products released into acidic media were used to identify surface-exposed candidate antigens (Hendrix et al. 1993; Redd and Thompson 1995). With these techniques, protein isolation and purification was required in order to test immunogenicity, which was time-consuming and, in most cases, not easily done before the advent of PCR and genome sequencing. Methods using immune sera to select immunoreactive antigens from *E. coli* gene banks of surface-exposed *C. burnetii* proteins or N-terminal and internal sequencing of proteins from detergent fractions of infected cells on SDS-PAGE gels were possible when DNA sequencing techniques were available. These methods identified several predominant antigens that were similar in size, including Com1 and P1 (Hendrix et al. 1993; Varghees et al. 2002). Cloned antigens allowed DNA sequencing to be done to positively identify selected antigens and to express and purify them from *E. coli*. In vitro transcription and translation protocols also made possible the conversion of cloned DNA into protein.

More recently, four candidate antigens (Com1, P1, P28 and Cb-Mip) have been cloned and characterized through testing in a sublethal challenge model in BALB/c

mice using protection from the development of severe splenomegaly as an indicator of vaccinogenic activity. None of the identified antigens individually conferred significant protection in this model, suggesting multiple antigens may be required to generate protection or that another delivery system may be more appropriate (Zhang and Samuel 2003). A comprehensive search that made use of antisera obtained from an experimental infection at various times post infection resulted in the identification of these proteins and an increased number of immunoreactive antigens by SDS-PAGE, providing a time course of proteins involved in immunity (Zhang et al. 2004).

In a study that was able to generate protection, a purified recombinant protein containing a fusion of *C. burnetii* antigens P1 and HspB (GroEL) was used to immunize mice. Detection of antibodies, T-cell proliferation and cytokine secretion indicated animals immunized with the fusion protein exhibited stronger humoral and cellular immune responses than those immunized with either protein alone. Spleens removed from animals challenged with *C. burnetii* showed those mice that received the fusion protein had smaller spleens that contained significantly lower bacterial loads (Li et al. 2005). These results suggested a promising method for generating a subunit vaccine may reside in the protein-folding properties of the chaperonin protein.

Projects to produce a vaccine through the combined cloning of portions of the O antigen of the LPS molecule heterologously with immunogenic proteins are currently in development. A prerequisite to the development of such a strategy is the determination of all genes and their enzymatic activities required for the assembly of PI *C. burnetii* O antigen. This is made more difficult since the *C. burnetii* O antigen contains unique sugar components and the pathways to their synthesis are not fully characterized (Narasaki et al. 2011).

16.6 New Methods to Identify Immunogenic Proteins

16.6.1 Strain Diversity

In order to successfully detect infections of *C. burnetii*, it is important to have some knowledge of the strain diversity of specific antigens to be used in diagnostics and vaccines. Several typing schemes have been used to classify *C. burnetii* isolates to determine their antigenic diversity over time and geographic locations. Such studies may pinpoint areas having increased diversity of isolates that may require polyvalent vaccines or diagnostics specific to the area or indicate antigens specific only to certain isolates are not appropriate for vaccines or diagnostics for common use. Early studies utilized restriction enzyme-digested DNA and agarose or polyacrylamide gel electrophoresis to detect restriction fragment length polymorphisms (RFLP) to compare isolates, with some studies utilizing pulsed field gels (Vodkin et al. 1986; Hendrix et al. 1991; Jager et al. 1998). These early studies separated the available isolates into distinct genomic groups based on DNA restriction fingerprints. Plasmid types correlated with the genomic groups and in some studies, isolates causing

chronic infection were grouped together. Isolates that varied only by passage history, including those that differed by LPS phase, showed no apparent differences in restriction enzyme banding patterns when EcoR1 or BamH1 enzymes were used (Hendrix and Williams 1991). NM LPS phase variants were shown to differ if DNA was digested with Hae III (Vodkin et al. 1986). Differentiation of *Coxiella* isolates was also carried out through a repetitive element PCR genotyping method to detect the presence of insertion sequences of the IS*1111* transposase. Differences between isolates in the number of elements per genome and in sequence alterations within and near IS element coding regions allowed them to be divided into five genomic groups that corresponded to groups determined by restriction enzyme banding patterns (Denison et al. 2007b).

Later genotyping schemes were developed to have greater discriminatory ability to distinguish between non-clonal isolates, such as may be required in epidemiological investigations in order to follow outbreaks. Unlike other genera, 16S ribosomal RNA genes and the generally hypervariable 16S–23S ITS (internal transcribed spacer) region are conserved throughout *C. burnetii* isolates (Stein et al. 1997; Thiele et al. 1994). This has led to the development of more powerful assays utilizing regions that are not transcribed and thus likely to be more variable. Multispacer sequence typing, based on intergenic region sequencing, was used to classify 173 *C. burnetii* isolates. Using 10 variable spacers, the authors were able to establish 30 different genotypes characterized into 3 monophyletic groups by phylogenetic analysis inferred from the sequences (Glazunova et al. 2005). Svraka et al. used Tandem Repeats Finder Software (Benson 1999) to search the *C. burnetii* RSA493 genome sequence for tandem repeats and for the development of primer sets for multiple locus variable number of tandem repeat (VNTR) analysis (MLVA) typing. Twenty-one isolates, including five passage variants, were characterized using seven tandem repeat loci. Results indicated that the number of repeats per VNTR locus varied between 2 and 18 and the number of variant alleles per locus varied between 3 and 6. Nine unique marker allele size combinations or MLVA types were observed among the 21 *C. burnetii* samples. Cluster analysis indicated the MLVA types fell into five major clusters of closely related isolates. Analysis of passage history variants was used to determine the stability of the chosen markers. All five NM passage variants, 2S and two Priscilla variants were stable over time and geographic location (Svraka et al. 2006).

Arricau-Bouvery et al. employed infrequent restriction site-PCR (IRS-PCR) and MLVA typing to characterize 42 *C. burnetii* isolates from livestock and ticks. The IRS-PCR typing of 14 isolates by 4 different restriction assays resulted in 6 genotype groups. MLVA typing of the 14 isolates and 28 additional isolates resulted in 22 additional genotypes using 10 minisatellites from NM RSA493 having repeat units greater than 9 base pairs. The authors proposed two separate panels of markers, one using minisatellite markers which can be typed in any laboratory on agarose gels and a second panel of microsatellites which can be typed using capillary electrophoresis. The Microorganisms Tandem Repeats Database at http://minisatellites.u-psud.fr was used to establish the panel of markers. With the availability of additional *Coxiella* genome sequences, additional tandem repeats will be included in typing schemes that may not be present in RSA493 (Arricau-Bouvery et al. 2006).

MLVA typing has also been applied to isolates from five patients and six sheep in the 2007 outbreak in the Netherlands using six microsatellites from NM RSA493. Results indicated at least four *C. burnetii* genotypes were involved in the outbreak (Klaassen et al. 2009).

Beare et al. used comparative genome hybridization (CGH) on DNA microarrays containing all open reading frames of the NM PI reference isolate to assess intrastrain/species whole genome sequence variation to analyze the diversity of 24 *C. burnetii* isolates. The analysis confirmed earlier RFLP groupings (Hendrix et al. 1991) and showed that two previously ungrouped isolates represent distinct genomic groups. Results showed that both small and large genetic changes were responsible for attenuated virulence due to truncated LPS expression and truncation or loss of other genes may similarly affect virulence. CGH assessment of genome content can be useful to identify cross-protective subunit vaccine candidates and aid in the development of new diagnostic reagents (Beare et al. 2006).

16.6.2 2-D SDS PAGE Western Analysis

Early analysis of antigens utilized one-dimensional SDS-PAGE and immunoblotting with infection-derived sera to detect reactive antigens. With the sequencing of the *C. burnetii* genome and advances in mass spectrometry that allowed protein identification from smaller sample sizes, the ability to select and identify antigens on two-dimensional SDS-PAGE was made easier, although 2-D gels remain a labor intensive and technically demanding method. Studies comparing antigens of large and small cell replication variants of *C. burnetii* determined that two highly antigenic proteins of 12.5 and 15 kDa are strong candidates for diagnostics. They were seen in vaccinated and infected animals throughout infection and were associated with LPS, predominately in SCVs (small cell variant), late in infection (Coleman et al. 2007). Deringer et al. identified nine novel immunogenic proteins by mass spectrometry on Western blots of 2-D SDS-PAGE using guinea pig immune sera and 11 additional proteins which were also previously shown to react to human sera in *C. burnetii* whole cell lysates (Deringer et al. 2011). Sekeyova et al. utilized acute stage versus chronic stage infection sera to select antigens on 2-D SDS-PAGE of *C. burnetii* membrane extracts. Results of these studies showed that 29 *C. burnetii* antigens reacted with acute or chronic sera, with two proteins recognized only by chronic patient sera. These proteins were considered possible candidates for serodiagnostic assays to detect Q fever endocarditis (Sekeyova et al. 2009).

16.6.3 Mass Spectrometric Analysis

As *C. burnetii* do not grow on bacteriologic media in common use and require increased containment for growth, rapid methods to identify it as the biological

agent responsible during outbreaks are needed. Several different strategies for the rapid identification of intact bacteria using mass spectrometric approaches have been applied to *C. burnetii* isolates. In 2002, Domingues et al. analyzed phospholipids from *C. burnetii* using fast atom bombardment mass spectrometry (FAB-MS). They acquired constant neutral loss scanning mass spectra (CNL) from NM PI and PII cells to identify various phospholipids within phospholipid classes. Results showed that phospholipids from PII *C. burnetii* were less complex than PI phospholipids and lacked the phosphoinositol class of phospholipids. They postulated that this rapid method could be used to differentiate virulent *C. burnetii* from low virulent strains (Domingues et al. 2002).

Shaw et al. used whole PI *C. burnetii* organisms grown and purified at different times and places to identify unique and reproducible biomarkers by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. Small cell variant protein A (ScvA) was one of three consistently identified proteins that would be expected to be present in environmental or intentionally released samples (Shaw et al. 2004). Pierce et al. expanded the use of mass spectrometry to identify *C. burnetii* strains and phase. This approach involved the use of MALDI-TOF mass spectrometry and supervised pattern recognition using Partial Least Squares-Discriminant Analysis (PLS-DA) on seven *C. burnetii* isolates grouped into six strain types. The method was validated by the prediction of unknown *C. burnetii* samples with 100% sensitivity and specificity for five out of the six strain classes (Pierce et al. 2007).

Hernychova et al. employed MALDI-TOF MS analyses on acetonitrile and TCA extractions of inactivated *C. burnetii* to obtain specific fingerprints of *C. burnetii* surface-exposed molecules to be used for detection and identification of *C. burnetii*. They also used electrospray tandem mass spectrometry coupled with nanoscale ultra performance liquid chromatography (LC-MS/MS) to identify 20 acetonitrile-extracted *C. burnetii* proteins (Hernychova et al. 2008).

These techniques have also been used for the discovery of vaccine candidates through identification of specific secreted or outer membrane products. Samoilis et al. used mass spectrometry techniques to identify proteins of *C. burnetii* secreted into the cytosol of infected Vero cells (Samoilis et al. 2010). Similarly, Flores-Ramirez et al. used mass spectrometry and bioinformatics techniques to identify 21 predicted outer membrane proteins and nine lipoproteins in NM PI lysates as possible vaccine candidates (Flores-Ramirez et al. 2009).

16.6.4 Microarray Analysis

Knowledge of the genome sequence has made possible the construction of libraries containing all open reading frames to evaluate the vaccinogenic activity of the complete genome using microarray technology. Various studies have used either animal or patient sera to select and analyze immunoreactive proteins.

Beare et al. generated 1,988 *C. burnetii* open reading frames as transcriptionally active PCR products from which full-length proteins were synthesized for 75% of

the ORFs using an *E. coli* based in vitro transcription-translation (IVTT) system. Crude IVTT lysates were used to spot nitrocellulose microarrays that were probed with acute Q fever patient sera and sera from Q-Vax vaccinees. Approximately 50 *C. burnetii* proteins reacted strongly with immune sera. Immunoreactivity to IVTT lysates was confirmed by ELISA using recombinant proteins corresponding to selected array-reactive antigens. Positive proteins included previously reactive immunogens, an ankyrin-repeat containing protein and many hypothetical proteins (Beare et al. 2008).

Microarray analysis of 1,901 ORFs from *C. burnetii* using a plasmid-based expression system was used to evaluate humoral immune responses to Q fever patient sera from acute disease cases versus naïve controls for the discovery of specific seroreactive antigens. Positive responses indicated a bias for signal peptide-containing proteins and were validated using Western blot-style immunostrips. The investigators found 13 antigens that were significantly more reactive in Q fever cases among 21 seroreactive antigens. Sixty-four additional antigens were variably reactive in Q fever patients (Vigil et al. 2010).

In a separate protein microarray study, Vigil et al. compared humoral immune responses to acute and chronic disease sera accompanied by greater than 6 months of supportive sero-surveillance and clinical data. Results of the evaluation of IgM and IgG antibody responses at early and late time points of infection showed a limited number of proteins undergo increasing or decreasing seroreactivity. Serological markers able to differentiate between acute and chronic disease states were identified, along with numerous IgG biomarkers of acute infection and novel IgG biomarkers for acute and chronic infection. This study profiled for the first time the IgM antibody repertoire for both acute and chronic Q fever. These results provided a unique perspective on the development of the antibody response, insight into isotype switch, and identified novel seroreactive antigens for the development of recombinant protein-based diagnostics and potential subunit vaccine candidates (Vigil et al. 2011).

16.6.5 Dominant B and T Cell Antigens by Protein ELISA and ELISPOT Analysis

Only one study has examined both the humoral and cellular immune response to Q fever. This study used IFA positive sera from 55 convalescent Q fever patients, five chronic Q fever patients, and 32 IFA negative controls. Humoral immune responses were tested by IgG indirect-ELISA using six previously identified and five array-selected *C. burnetii* proteins expressed as His-tag fusion proteins in *E. coli*. Cellular immune responses were determined using T cells from infected mice, either with or without a humanized MHC locus (Chen et al. 2009), since previous studies showed murine and human MHCs recognize different epitopes (Engelhard 1994). Enzyme-linked immunosorbent spot (ELISPOT) assays were used to test antigen-specific interferon-γ recall of CD4 + T cells from vaccinated C57BL/6 or HLA-DR4

transgenic mice. Differences between the mice in antigen recognition confirmed the use of HLA transgenic mice is a more relevant model for screening human T cell antigens. This study showed that most strong antibody responses also induced interferon-γ recall responses in CD4+ T cells of vaccinated mice (Chen et al. 2009).

16.7 Future Developments

The development of safe and effective new vaccines can be guided by understanding the protective immune responses provided by existing vaccines. Zhang et al. showed that adoptive transfer of T cells from mice immunized with inactivated whole cell PI *C. burnetii* vaccine was protective against challenge for recipient mice (Zhang et al. 2007), confirming earlier studies that suggested T cell dependent immune responses are critical for protective immunity (Kazar et al. 1977, 1986). Additionally, low dose *C. burnetii* infection caused death in SCID and T cell deficient mice, but not B cell deficient mice, implying the essential requirement of T cells for host resistance to *C. burnetii* infection (Andoh et al. 2007). Microarray studies show a major portion of the vaccine-derived humoral responses consist of IgG antibodies to *C. burnetii* proteins (Vigil et al. 2010), indicating the relevance of CD4+ helper T cell responses to immunity. Antigen-specific CD4+ T cells direct secretion of cytokines, including IFN-γ and TNF-α. Both are critical for intracellular clearance of *C. burnetii* (Andoh et al. 2007). INF-γ stimulates production of nitric oxide and reactive oxygen molecules in macrophages, contributing to the control of infection, and may promote apoptosis in infected monocytes (Dellacasagrande et al. 2002; Howe et al. 2002; Brennan et al. 2004). IFN-γ was used successfully to treat previously intractable chronic Q fever and Q fever chronic multifocal osteomyelitis (Morisawa et al. 2001; Neth et al. 2011).

In experiments to determine the molecular targets of CD4+ T cell responses to *C. burnetii* PI-WCV for use in the development of a new subunit vaccine, Chen et al. focused on seven antigens previously shown to be targets of immunodominant B cell responses. Candidate peptides were selected from these antigens based on bioinformatic predictions of binding to murine MHC class II H-2 I-A^b. Peptides were screened for recognition by IFN-γ producing CD4+ T cells in PI-WCV vaccinated mice. Eight distinct H-2 I-A^b-restricted CD4+ T cell epitopes from four different proteins were identified, with the frequency of $CBU1910_{45–59}$ (Com1)-specific CD4+ T cells much higher than for other epitopes (Chen et al. 2011).

The search for *C. burnetii* protein antigens appropriate for use as diagnostic reagents and vaccines has been hampered by an inability to make mutants to identify specific virulence determinants in this obligate intracellular pathogen. New developments in the ability to grow *C. burnetii* in axenic media have now allowed for the creation of mutants, especially since the refinement of growth on plated media (Omsland et al. 2008, 2009). This should allow for the discovery of antigens unique to *C. burnetii* that are essential for virulence and are accessible to the immune system. Table 16.1 lists currently identified *C. burnetii* immunodominant protein antigens.

Table 16.1 Identified *Coxiella burnetii* immunodominant protein antigens

ID CBU No.	Gene	Size (AA)	Identification method	Sera reactive species	Annotation	References
CBU0008		62	Protein microarray analysis	Human	Tested for Q fever diagnostic	Beare et al. (2008)
CBU0092	ygbF	285	2D/mass analysis	Human		Sekeyova et al. (2009)
			Protein microarray analysis	Human		Vigil et al. (2010)
			2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU0098	nadC	274	Protein microarray analysis	Human		Beare et al. (2008)
CBU0103		480	2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU0109		267	Protein microarray analysis	Human		Vigil et al. (2010)
CBU0115	mraZ	152	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU0140	ftsA	410	2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU0223	tuf-1	299	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU0229	rplL	126	2D/mass analysis	Human		Coleman et al. (2007)
			Protein microarray analysis	Human		Vigil et al. (2010)
CBU0235	fusA	699	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU0236	tuf-2	397	2D/mass analysis	Human		Coleman et al. (2007)
			2D/mass analysis	Human	Tested for Q fever diagnostic	Sekeyova et al. (2010)
			2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU0263	rpoA	327	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU0271	ssb	158	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU0299	rph	237	2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU0307		231	Protein microarray analysis	Human		Beare et al. (2008)
			2D/mass analysis	Human		Sekeyova et al. (2009)

(continued)

Table 16.1 (continued)

ID CBU No.	Gene	Size (AA)	Identification method	Sera reactive species	Annotation	References
CBU0309	htpG	633	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU0311	P1	223	Western blot and N-terminal seq	Mouse		Varghees et al. (2002)
					C57Bl/6 Mouse T cell antigen Tested for Q fever diagnostic	Chen et al. (2009)
CBU0366	phoR	252	Protein microarray analysis	Human		Beare et al. (2008)
CBU0383	tag	204	Protein microarray analysis	Human	Tested for Q diagnostic	Beare et al. (2008)
					C57Bl/6 Mouse T cell antigen Tested for Q fever diagnostic	Chen et al. (2009)
CBU0391	ribF	320	Protein microarray analysis	Human		Beare et al. (2008)
CBU0436		258	Protein microarray analysis	Human		Vigil et al. (2010)
CBU0479	kdsB	249	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU0480	argR	159	2D/mass analysis	Human	Recognized by chronic Q fever sera	Sekeyova et al. (2009)
CBU0497	fabF	414	2D/mass analysis	Human		Coleman et al. (2007)
CBU0528	rpsA	551	2D/mass analysis	Human		Sekeyova et al. (2009)
			2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU0545	lemA	192	Protein microarray analysis	Human		Beare et al. (2008)
			Protein microarray analysis	Human		Vigil et al. (2010)
CBU0572		458	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU0609		340	Protein microarray analysis	Human		Beare et al. (2008)

(continued)

Table 16.1 (continued)

ID CBU No.	Gene	Size (AA)	Identification method	Sera reactive species	Annotation	References
CBU0612	ompH	165	Protein microarray analysis	Human	Tested for Q fever diagnostic	Beare et al. (2008)
					Tested for Q fever diagnostic	Chen et al. (2009)
			2D/mass analysis	Human	Recognized by chronic Q fever sera	Sekeyova et al. (2009)
			Protein microarray analysis	Human		Vigil et al. (2010)
CBU0615	lpxA	259	Protein microarray analysis	Human		Vigil et al. (2010)
CBU0628	ppa	175	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU0630	mip	230	Protein microarray analysis	Human		Vigil et al. (2010)
CBU0632		99	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU0648	ribH	151	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU0653		198	Protein microarray analysis	Human		Vigil et al. (2010)
CBU0664		381	Protein microarray analysis	Human		Beare et al. (2008)
CBU0718		94	Protein microarray	Human		Beare et al. (2008)
					C57Bl/6 Mouse T cell antigen	Chen et al. (2009)
					Tested for Q fever diagnostic	
CBU0723		55	Protein microarray analysis	Human		Beare et al. (2008)
CBU0737	tig	442	2D/mass analysis	Human		Coleman et al. (2007)
CBU0750		324	2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU0754		348	Protein microarray analysis	Human		Beare et al. (2008)
CBU0760		124	Protein microarray analysis	Human		Beare et al. (2008)
CBU0774	pspC	82	Protein microarray analysis	Human		Beare et al. (2008)
CBU0776		283	Protein microarray analysis	Human		Beare et al. (2008)

(continued)

Table 16.1 (continued)

ID CBU No.	Gene	Size (AA)	Identification method	Sera reactive species	Annotation	References
CBU0781	ankG	338	Protein microarray analysis	Human	Tested for Q fever diagnostic	Beare et al. (2008)
					C57Bl/6 Mouse T cell antigen	Chen et al. (2009)
					Tested for Q fever diagnostic	
CBU0800		137	Protein microarray analysis	Human		Beare et al. (2008)
CBU0858	nadE	542	2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU0891		312	Protein microarray analysis	Human		Beare et al. (2008)
					C57Bl/6 Mouse T cell antigen	Chen et al. (2009)
					Tested for Q fever diagnostic	
			Protein microarray analysis	Human		Vigil et al. (2010)
CBU0895		203	Protein microarray analysis	Human		Vigil et al. (2010)
CBU0898		110	Protein microarray analysis	Human		Beare et al. (2008)
CBU0932	gpIK	501	2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU0935		129	Protein microarray analysis	Human		Beare et al. (2008)
CBU0937		465	2D/mass analysis	Human	Tested for Q fever diagnostic	Sekeyova et al. (2010)
			2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU0952	AdaA	227	Phage library	Mouse, Guinea pig	Tested for acute disease diagnostic	Zhang et al. (2005)
			2D/mass analysis	Human		Coleman et al. (2007)
					Tested for Q fever diagnostic	Chen et al. (2009)
			2D/mass analysis	Human		Sekeyova et al. (2009)

(continued)

Table 16.1 (continued)

ID CBU No.	Gene	Size (AA)	Identification method	Sera reactive species	Annotation	References
CBU0963	bcp	151	2D/mass analysis	Human		Coleman et al. (2007)
CBU0968		176	Protein microarray analysis	Human		Beare et al. (2008)
CBU1002	birA	323	Protein microarray analysis	Human		Beare et al. (2008)
CBU1065		184	Protein microarray analysis	Human		Beare et al. (2008)
CBU1094		380	Protein microarray analysis	Human		Vigil et al. (2010)
CBU1098		274	Protein microarray analysis	Human		Beare et al. (2008)
CBU1115		105	Protein microarray analysis	Human	Tested for Q fever diagnostic	Beare et al. (2008)
CBU1121		60	Protein microarray analysis	Human		Beare et al. (2008)
CBU1143	yajC	116	Protein microarray analysis	Human	Tested for Q fever diagnostic	Beare et al. (2008)
			Protein microarray analysis	Human		Vigil et al. (2010)
CBU1157		233	Protein microarray analysis	Human	Tested for Q fever diagnostic	Beare et al. (2008)
CBU1184		242	Protein microarray analysis	Human		Beare et al. (2008)
CBU1200	icd	427	2D/mass analysis	Human		Coleman et al. (2007)
CBU1241	mdh	328	2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU1249		203	Protein microarray analysis	Human	Tested for Q fever diagnostic	Beare et al. (2008)
CBU1260		248	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU1290	dnaK	656	2D/mass analysis	Human		Sekeyova et al. (2009)
			2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU1385	tsf	296	2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU1386	rpsB	313	Protein microarray analysis	Human		Vigil et al. (2010)
CBU1396	sucD	294	2D/mass analysis	Guinea pig		Deringer et al. (2011)

(continued)

Table 16.1 (continued)

ID CBU No.	Gene	Size (AA)	Identification method	Sera reactive species	Annotation	References
CBU1398	sucB	405	Protein microarray analysis	Human		Beare et al. (2008)
			Protein microarray analysis	Human		Vigil et al. (2010)
			2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU1416		216	2D/mass analysis	Human		Coleman et al. (2007)
CBU1433	nusA	503	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU1513		258	Protein microarray	Human		Vigil et al. (2010)
CBU1627	lcmE	1034	Protein microarray analysis	Human		Vigil et al. (2010)
CBU1697	nth	218	Protein microarray analysis	Human		Beare et al. (2008)
CBU1706		200	2D/mass analysis	Human		Sekeyova et al. (2009)
			2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU1718	groEL	552	2D/mass analysis	Human		Coleman et al. (2007)
			2D/mass analysis	Human		Sekeyova et al. (2009)
			2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU1719	groES	96	2D/mass analysis	Human		Sekeyova et al. (2009)
			Protein microarray analysis	Human		Vigil et al. (2010)
CBU1768		153	Protein microarray analysis	Human		Vigil et al. (2010)
CBU1770		432	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU1783	gap	334	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU1789		288	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU1835		459	Protein microarray analysis	Human		Beare et al. (2008)
CBU1853		149	Protein microarray analysis	Human	Tested for Q fever diagnostic	Beare et al. (2008)
CBU1863		603	Protein microarray analysis	Human		Vigil et al. (2010)

(continued)

Table 16.1 (continued)

ID CBU No.	Gene	Size (AA)	Identification method	Sera reactive species	Annotation	References
CBU1865		175	Protein microarray analysis	Human		Beare et al. (2008)
CBU1869		217	Protein microarray analysis	Human	Tested for Q fever diagnostic	Beare et al. (2008)
CBU1910	com1	252	Phage library screen, 2D analysis, protein array	Mouse, guinea pig, human		Hendrix et al. (1991)
					C57Bl/6 Mouse T cell antigen Tested for Q fever diagnostic	Chen et al. (2009)
			2D/mass analysis	Human	Tested for Q fever diagnostic	Sekeyova et al. (2010)
			Protein microarray analysis	Human		Vigil et al. (2010)
			2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU1940	atpE	100	Protein microarray analysis	Human		Beare et al. (2008)
CBU1943	atpA	515	2D/mass analysis	Human		Coleman et al. (2007)
			2D/mass analysis	Guinea pig		Deringer et al. (2011)
CBU1945	atpD	461	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU1958		32	Protein microarray analysis	Human		Beare et al. (2008)
CBU1966	hemA	413	Protein microarray analysis	Human		Beare et al. (2008)
CBU1967		409	Protein microarray analysis	Human		Beare et al. (2008)
CBU1969	dksA	147	Protein microarray analysis	Human		Beare et al. (2008)
CBU2020		476	Protein microarray analysis	Human		Beare et al. (2008)
CBU2029		210	2D/mass analysis	Human		Sekeyova et al. (2009)
CBU2065		212	Protein microarray analysis	Human		Beare et al. (2008)

References

Ackland JR, Worswick DA, Marmion BP (1994) Vaccine prophylaxis of Q fever. A follow-up study of the efficacy of Q-Vax (CSL) 1985–1990. Med J Aust 160:704–708

Andoh M, Zhang G, Russell-Lodrigue KE, Shive HR, Weeks BR, Samuel JE (2007) T cells are essential for bacterial clearance, and gamma interferon, tumor necrosis factor alpha, and B cells are crucial for disease development in *Coxiella burnetii* infection in mice. Infect Immun 75:3245–3255

Arricau-Bouvery N, Souriau A, Bodier C, Dufour P, Rousset E, Rodolakis A (2005) Effect of vaccination with phase I and phase II *Coxiella burnetii* vaccines in pregnant goats. Vaccine 23:4392–4402

Arricau-Bouvery N, Hauck Y, Bejaoui A, Frangoulidis D, Bodier CC, Souriau A, Meyer H, Neubauer H, Rodolakis A, Vergnaud G (2006) Molecular characterization of *Coxiella burnetii* isolates by infrequent restriction site-PCR and MLVA typing. BMC Microbiol 6:38–51

Baca OG, Paretsky D (1983) Q fever and *Coxiella burnetii*: a model for host-parasite interactions. Microbiol Rev 47:127–149

Beare PA, Samuel JE, Howe D, Virtaneva K, Porcella SF, Heinzen RA (2006) Genetic diversity of the Q fever agent. *Coxiella burnetii*, assessed by microarray-based whole-genome comparisons. J Bacteriol 188:2309–2324

Beare PA, Chen C, Bouman T, Pablo J, Unal B, Cockrell DC, Brown WC, Barbian KD, Porcella SF, Samuel JE, Felgner PL, Heinzen RA (2008) Candidate antigens for Q fever serodiagnosis revealed by immunoscreening of a *Coxiella burnetii* protein microarray. Clin Vaccine Immunol 15:1771–1779

Beare PA, Unsworth N, Andoh M, Voth DE, Omsland A, Gilk SD, Williams KP, Sobral BW, Kupko JJ III, Porcella SF, Samuel JE, Heinzen RA (2009) Comparative genomics reveal extensive transposon-mediated genomic plasticity and diversity among potential effector proteins within the genus *Coxiella*. Infect Immun 77:642–656

Benenson AS (1959) Q fever vaccine: efficacy and present status. In: Smadel JE (ed) Walter Reed Army Institute of Medical Science, Publ No 6th edn. US Government Printing Office, Washington, DC, p 47

Bengtson IA (1941) Complement fixation in "Q" fever. Proc Soc Exp Biol Med 46:665

Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res 27:573–580

Bilberstein EL, Riemann HP, Franti CE, Behymer DE, Ruppanner R, Bushnell R, Crenshaw G (1977) Vaccination of dairy cattle against Q fever (*Coxiella burnetii*): results of field trials. Am J Vet Res 38:189–193

Bildfell RJ, Thomson GW, Haines DM, McEwen BJ, Smart N (2000) *Coxiella burnetii* infection is associated with placentitis in cases of bovine abortion. J Vet Diagn Invest 12:419–425

Brennan RE, Russell K, Zhang G, Samuel JE (2004) Both inducible nitric oxide synthase and NADPH oxidase contribute to the control of virulent phase I *Coxiella burnetii* infections. Infect Immun 72:6666–6675

Brooks DL, Ermel RW, Franti CE, Ruppanner R, Behymer DE, Williams JC (1986) Q fever vaccination of sheep: challenge of immunity in ewes. Am J Vet Res 47:1235–1238

Brown GL (1973) Clinical aspects of "Q" fever. Postgrad Med J 49:539–541

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

Carrieri MP, Tissot-Dupont H, Rey D, Brousse P, Renard H, Obadia Y, Raoult D (2002) Investigation of a slaughterhouse-related outbreak of Q fever in the French Alps. Eur J Clin Microbiol Infect Dis 21:17–21

Chen C, Bouman TJ, Beare PA, Mertens K, Zhang GQ, Russell-Lodrique KE, Hogaboam JP, Peters B, Felgner PL, Brown WC, Heinzen RA, Hendrix LR, Samuel JE (2009) A systematic approach to evaluate humoral and cellular immune responses to *Coxiella burnetii* antigens. Clin Microbiol Infect 15:156–157

Chen C, Dow C, Wang P, Sidney J, Read A, Harmsen A, Samuel JE, Peters B (2011) Identification of CD4+ T cell epitopes in *C. burnetii* antigens targeted by antibody responses. PLoS One 6:e17712

Coleman SA, Fischer ER, Cockrell DC, Voth DE, Howe D, Mead DJ, Samuel JE, Heinzen RA (2007) Proteome and antigen profiling of *Coxiella burnetii* developmental forms. Infect Immun 75:290–298

Dellacasagrande J, Ghigo E, Raoult D, Capo C, Mege JL (2002) IFN-gamma-induced apoptosis and microbicidal activity in monocytes harboring the intracellular bacterium *Coxiella burnetii* require membrane TNF and homotypic cell adherence. J Immunol 169:6309–6315

Denison AM, Massung RF, Thompson HA (2007a) Analysis of the O-antigen biosynthesis regions of phase II isolates of *Coxiella burnetii*. FEMS Microbiol Lett 267:102–107

Denison AM, Thompson HA, Massung RF (2007b) IS1111 insertion sequences of *Coxiella burnetii*: characterization and use for repetitive element PCR-based differentiation of *Coxiella burnetii* isolates. BMC Microbiol 7:91–98

Deringer JR, Chen C, Samuel JE, Brown WC (2011) Immunoreactive *Coxiella burnetii* Nine Mile proteins separated by 2D electrophoresis and identified by tandem mass spectrometry. Microbiology 157:526–542

Derrick EH (1937) "Q" fever, a new entity: clinical features, diagnosis and laboratory investigation. Med J Aust 2:281–299

Dilbeck PM, McElwain TF (1994) Immunohistochemical detection of *Coxiella burnetii* in formalin-fixed placentas. J Vet Diagn Invest 6:125–127

Domingues P, Palkovic P, Toman R (2002) Analysis of phospholipids from *Coxiella burnetii* by fast atom bombardment mass spectrometry. A rapid method for differentiation of virulent phase I and low virulent phase II cells. Acta Virol 46:121–124

Dupont HT, Thirion X, Raoult D (1994) Q fever serology: cut off determination for microimmunofluorescence. Clin Diagn Lab Immunol 1:189–196

Engelhard VH (1994) Structure of peptides associated with class I and class II MHC molecules. Annu Rev Immunol 12:181–207

Enserink M (2010) Infectious diseases. Questions abound in Q-fever explosion in the Netherlands. Science 327:266–277

Fenollar F, Fournier PE, Raoult D (2004) Molecular detection of *Coxiella burnetii* in the sera of patients with Q fever endocarditis or vascular infections. J Clin Microbiol 42:4919–4924

Fernandes I, Rousset E, Dufour P, Sidi-Boumedine K, Cupo A, Thiery R, Duquesne V (2009) Evaluation of the recombinant heat shock protein B (HspB) of *Coxiella burnetii* as a potential antigen for immunodiagnostic of Q fever in goats. Vet Microbiol 134:300–304

Field PR, Santiago A, Chan SW, Patel DB, Dickeson D, Mitchell JL, Devine PL, Murphy AM (2002) Evaluation of a novel commercial enzyme-linked immunosorbent assay detecting *Coxiella burnetii*-specific immunoglobulin G for Q fever prevaccination screening and diagnosis. J Clin Mirobiol 40:3526–3529

Fishbein DB, Raoult D (1992) A cluster of *Coxiella burnetii* infections associated with exposure to vaccinated goats and their unpasteurized dairy products. Am J Tropic Med Hyg 47:35–40

Flores-Ramirez G, Toman R, Sekyova Z, Skultety L (2009) *In silico* prediction and identification of outer membrane proteins and lipoproteins from *Coxiella burnetii* by the mass spectrometry techniques. Clin Microbiol Infect 15:196–197

Fournier PE, Casalta JP, Habib G, Messana T, Raoult D (1996) Modification of the diagnostic criteria proposed by the Duke Endocarditis Service to permit improved diagnosis of Q fever endocoarditis. Am J Med 100:629–633

Fournier P-E, Raoult D (2003) Comparison of PCR and serology assays for early diagnosis of acute Q fever. J Clin Microbiol 41:5094–5098

Frangoulidis D, Schropfer E, Al Dahouk S, Tomaso H, Meyer H (2006) Comparison of four commercially available assays for the detection of IgM phase II antibodies to *Coxiella burnetii* in the diagnosis of acute Q fever. Ann N Y Acad Sci 1078:561–562

Frankel D, Richet H, Renvoise A, Raoult D (2011) Q fever in France, 1985–2009. Emerg Infect Dis 17:350–356

Fries LF, Waag DM, Williams JC (1993) Safety and immunogenicity in human volunteers of a choloform-methanol residue vaccine for Q fever. Infect Immun 61:1251–1258

Geddes AM (1983) Q fever. Br Med J 287:927–928

Genig VA (1968) A live vaccine1/M-44 against Q-fever for oral use. J Hyg Epidemiol Microbiol Immunol 12:265–273

Glazunova O, Roux V, Freylikman O, Sekeyova Z, Fournous G, Tyczka J, Tokarevich N, Kovacova E, Marrie TJ, Raoult D (2005) *Coxiella burnetii* genotyping. Emerg Infect Dis 11:1211–1217

Guatteo R, Beaudeau F, Joly A, Seegers H (2007) Performances of an ELISA applied to serum and milk for the detection of antibodies to *Coxiella burnetii* in dairy cattle. Revue Med Vet 158:250–252

Hackstadt T (1986) Antigenic variation in the phase I lipopolysaccharide of *Coxiella burnetii* isolates. Infect Immun 52:337–340

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

Hendrix LR, Samuel JE, Mallavia LP (1991) Differentiation of *Coxiella burnetii* isolates by analysis of restriction-endonuclease-digested DNA separated by SDS-PAGE. J Gen Microbiol 137:269–276

Hendrix LR, Mallavia LP, Samuel JE (1993) Cloning and sequencing of *Coxiella burnetii* outer membrane protein gene *com1*. Infect Immun 61:470–477

Hernychova L, Toman R, Ciampor F, Hubalek M, Vackova J, Macela A, Skultety L (2008) Detection and identification of *Coxiella burnetii* based on the mass spectrometric analyses of the extracted proteins. Anal Chem 80:7097–7104

Hoover TA, Culp DW, Vodkin MH, Williams JC, Thompson HA (2002) Chromosomal DNA deletions explain phenotypic characteristics of two antigenic variants, phase II and RSA 514 (Crazy), of the *Coxiella burnetii* Nine Mile strain. Infect Immun 70:6726–6733

Hoover TA, Vodkin MH, Williams JC (1992) A *Coxiella burnetii* repeated DNA element resembling a bacterial insertion sequence. J Bacteriol 174:5540–5548

Howe D, Barrows LF, Lindstrom NM, Heinzen RA (2002) Nitric oxide inhibits *Coxiella burnetii* replication and parasitophorous vacuole maturation. Infect Immun 70:5140–5147

Izzo AA, Marmion BP, Hackstadt T (1991) Analysis of the cells involved in the lymphoproliferative response to *Coxiella burnetii* antigens. Clin Exp Immunol 85:98–108

Jager C, Willems H, Thiele D, Baljer G (1998) Molecular characterization of *Coxiella burnetii* isolates. Epidemiol Infect 120:157–164

Jensen TK, Montgomery DL, Jaeger PT, Lindhardt T, Agerholm JS, Bille-Hansen V, Boye M (2007) Application of fluorescent *in situ* hybridization for demonstration of *Coxiella burnetii* in placentas from ruminant abortions. APMIS 115:347–353

Johnson JW, Eddy GA, Pederson CE Jr (1976) Biologic properties of the M-44 strain of *Coxiella burnetii*. J Infect Dis 133:334–338

Johnson JW, McLeod CG, Stookey JL, Higbee GA, Pedersen CE Jr (1977) Lesions in guinea pigs infected with *Coxiella burnetii* strain M-44. J Infect Dis 135:995–998

Kazar J, El-Najdawi E, Brezina R, Schramek S (1977) Search for correlates of resistance to virulent challenge in mice immunized with *Coxiella burnetii*. Acta Virol 21:422–430

Kazar J, Brezina R, Palanova A, Turda B, Schramek S (1982) Immunogenicity and reactogenicity of Q fever chemovaccine in persons professionally exposed to Q fever in Czechoslovakia. Bull World Health Organ 60:389–394

Kazar J, Votruba D, Propper P, Schramek S (1986) Onset and duration of immunity in guinea pigs and mice induced with different Q fever vaccines. Acta Virol 30:499–506

Klaassen CHW, Nabuurs-Franssen MH, Tilburg JJHC, Hamans MAWM, Horrevorts AM (2009) Multigenotype Q fever outbreak, the Netherlands. Emerg Infect Dis 15:613–614

La Scola B (2002) Current laboratory diagnosis of Q fever. Semin Pediatr Infect Dis 13:257–262

Li Q, Niu D, Wen B, Chen M, Qiu L, Zhang J (2005) Protective immunity against Q fever induced with a recombinant PI antigen fused with HspB of *Coxiella burnetii*. Ann N Y Acad Sci 1063:130–142

Lukacova M, Gajdosova E, Skultety L, Kovacova E, Kazar J (1994) Characterization and protective effect of a 29 kDa protein isolated from *Coxiella burnetii* by detergent Empigen BB. Eur J Epidemiol 10:227–230

Lukacova M, Barak I, Kazar J (2008) Role of structural variations of polysaccharide antigens in the pathogenicity of Gram-negative bacteria. Clin Microbiol Infect 14:200–206

Luoto L, Casey ML, Pickens EG (1965) Q fever studies in Montana: detection of asymptomatic infection among residents of infected dairy premises. Am J Epidemiol 81:356–369

Marmion B (2007) Q fever: the long journey to control by vaccination. Med J Aust 186:164–166

Marmion BP, Storm PA, Ayres JG, Semendric L, Mathews L, Winslow W, Turra M, Harris RJ (2005) Long-term persistence of *Coxiella burnetii* after acute primary Q fever. Q J Med 98:7–20

Meiklejohn G, Reimer LG, Graves PS, Helmick C (1981) Cryptic epidemic of Q fever in a medical school. J Infect Dis 144:107–113

Million M, Thuny F, Richet H, Raoult D (2010) Long-term outcome of Q fever endocarditis: a 26-year personal survey. Lancet Infect Dis 10:527–535

Moos A, Hackstadt T (1987) Comparative virulence of intra- and interstrain lipopolysaccharide variants of *Coxiella burnetii* in the guinea pig model. Infect Immun 55:1144–1150

Morisawa Y, Wakiguchi H, Takechi T, Kurashige T, Nagaoka H (2001) Intractable Q fever treated with recombinant gamma interferon. Pediatr Infect Dis J 20:546–547

Muller HP, Schmeer N, Rantamaki L, Semler B, Krauss H (1987) Isolation of a protein antigen from *Coxiella burnetii*. Zentralbl Bakteriol Mikrobiol Hyg A 265:277–289

Narasaki CT, Mertens K, Samuel JE (2011) Characterization of the GDP-D-mannose biosynthesis pathway in *Coxiella burnetii*: the initial steps for GDP-β-D-virenose biosynthesis. PLoS One 6(10):e25514

Neth OW, Falcon D, Peromingo E, Soledad Camacho M, Rodriguez-Gallego C, Obando I (2011) Successful management of chronic multifocal Q fever osteomyelitis with adjuvant interferon-gamma therapy. Pediatr Infect Dis J 30(9):810–812

Omsland A, Cockrell DC, Fischer ER, Heinzen RA (2008) Sustained axenic metabolic activity by the obligate intracellular bacterium *Coxiella burnetii*. J Bacteriol 190:3203–3212

Omsland A, Cockrell DC, Howe D, Fischer ER, Virtaneva K, Sturdevant DE, Porcella SF, Heinzen RA (2009) Host cell-free growth of the Q fever bacterium *Coxiella burnetii*. Proc Natl Acad Sci U S A 106:4430–4434

Ormsbee RA, Marmion BP (1990) The disease. In: Marrie TJ (ed) Q fever, vol 1. CRC Press, Boca Raton, p 226

Ormsbee RA, Bell EJ, Lackman DB, Tallent G (1964) The influence of phase on the protective potency of Q fever vaccine. J Immunol 92:404–412

Palkovicova K, Ihnatko R, Vadovic P, Betinova E, Skultety L, Frangoulidis D, Toman R (2009) A monoclonal antibody specific for a unique biomarker, virenose, in a lipopolysaccharide of *Coxiella burnetii*. Clin Microbiol Infect 15:183–184

Panning M, Kilwinski J, Greiner-Fischer S, Peters M, Kramme S, Frangoulidis D, Meyer H, Henning K, Drosten C (2008) High throughput detection of *Coxiella burnetii* by real-time PCR with internal control system and automated DNA preparation. BMC Microbiol 8:77–84

Peter O, Dupuis G, Peacock MG, Burgdorfer W (1987) Comparison of enzyme-linked immunosorbent assay and complement fixation and indirect fluorescent-antibody tests for detection of *Coxiella burnetii* antibody. J Clin Microbiol 25:1063–1067

Pierce CY, Barr JR, Woolfitt AR, Moura H, Shaw EI, Thompson HA, Massung RF, Fernandez FM (2007) Strain and phase identification of the U.S. category B agent *Coxiella burnetii* by matrix assisted laser desorption/ionization time-of-flight mass spectrometry and multivariate pattern recognition. Anal Chem Acta 583:23–31

Raoult D, Houpikian P, Tissot Dupont H, Riss JM, Arditi-Djiane J, Brouqui P (1999) Treatment of Q fever endocarditis: comparison of 2 regimens containing doxycycline and ofloxacin or hydroxychloroquine. Arch Intern Med 159:167–173

Redd T, Thompson HA (1995) Secretion of proteins by *Coxiella burnetii*. Microbiology 141:363–369

Richardus JH, Donkers A, Dumas AM, Schaap GJP, Akkermans JPWM, Huisman J, Valkenburg HA (1987) Q fever in the Netherlands: a sero-epidemiological survey among human population groups from 1968-1983. Epidemiol Infect 98:211–219

Russel-Lodrigue KE, Andoh M, Poels MW, Shive HR, Weeks BR, Zhang GQ, Tersteeg T, Masegi T, Hotta A, Yamaguchi T, Fukushi H, Hirai K, McMurray DN, Samuel JE (2009) *Coxiella burnetii* isolates cause genogroup specific virulence in mouse and guinea pig models of acute Q fever. Infect Immun 77:5640–5650

Samoilis G, Aivaliotis M, Vranakis I, Papadioti A, Tselentis Y, Tsiotis G, Psaroulaki A (2010) Proteomic screening for possible effector molecules secreted by the obligate intracellular pathogen *Coxiella burnetii*. J Proteome Res 9:1619–1626

Sawyer LA, Fishbein DB, McDade JE (1987) Q fever: current concepts. Rev Infect Dis 9:935–946

Schmeer N, Muller P, Langel J, Krauss H, Frost JW, Wieda J (1987) Q fever vaccines for animals. Zentralbl Bakteriol Microbiol Hyg A 267:79–88

Schmeer N, Muller H-P, Baumgartner W, Wieda J, Krauss H (1988) Enzyme-linked immunosorbent fluorescence assay and high-pressure liquid chromatography for analysis of humoral immune responses to *Coxiella burnetii* proteins. J Clin Microbiol 26:2520–2525

Schneeberger PM, Hermans MHA, van Hannen EJ, Schellekens JJA, Leenders ACAP, Wever PC (2010) Real-time PCR with serum samples is indispensible for early diagnosis of acute Q fever. Clin Vaccine Immunol 17:286–290

Schramek S, Mayer H (1982) Different sugar compositions of lipopolysaccharides isolated from phase I and pure phase II cells of *Coxiella burnetii.* Infect Immun 38:53–57

Sekeyova Z, Kowalczewska M, Decloquement P, Pelletier N, Spitalska E, Raoult D (2009) Identification of protein candidates for the serodiagnosis of Q fever endocarditis by an immunoproteomic approach. Eur J Clin Microbiol Infect Dis 28:287–295

Sekeyova Z, Kowalczewska M, Vincentelli R, Decloquement P, Flores-Ramirez G, Skultety L, Raoult D (2010) Characterization of antigens for Q fever serodiagnostics. Acta Virol 54:173–180

Shaw EI, Moura H, Woolfitt AR, Ospina M, Thompson HA, Barr JR (2004) Identification of biomarkers of whole *Coxiella burnetii* phase I by MALDI-TOF mass spectrometry. Anal Chem 76:4017–4022

Smadel JE, Snyder MJ, Robbins FC (1948) Vaccination against Q fever. Am J Hyg 47:71

Stein A, Kruszewska D, Gouvernet J, Raoult D (1997) Study of the 16S-23S ribosomal DNA internal spacer of *Coxiella burnetii*. Eur J Epidemiol 13:471–475

Stoker MG, Fiset P (1956) Phase variation of the Nine Mile and other strains of *Rickettsia burnetii*. Can J Microbiol 2:310–321

Svraka S, Toman R, Skultety L, Slaba K, Homan WL (2006) Establishment of a genotyping scheme for *Coxiella burnetii*. FEMS Microbiol Lett 254:268–274

Thiele D, Willems H, Krauss H (1994) The 16S/23S ribosomal spacer region of *Coxiella burnetii*. Eur J Epidemiol 10:421–426

Tilburg JJHC, Melchers WJG, Pettersson AM, Rossen JWA, Hermans MHA, van Hannen EJ, Nabuurs-Franssen MH, de Vries MC, Horrevorts AM, Klaassen CHW (2010) Interlaboratory evaluation of different extraction and real-time PCR methods for detection of *Coxiella burnetii* DNA in serum. J Clin Microbiol 48:3923–3927

Tissot-Dupont H, Vaillant V, Rey S, Raoult D (2007) Role of sex, age, previous valve lesion, and pregnancy in the clinical expression and outcome of Q fever after a large outbreak. Clin Infect Dis 44:232–237

To H, Hotta A, Zhang GQ, Nguyen SV, Ogawa M, Yamaguchi T, Fukushi H, Amano K, Hirai K (1998) Antigenic characteristics of polypeptides of *Coxiella burnetii* isolates. Microbiol Immunol 42:81–85

Varghees S, Kiss K, Frans G, Braha O, Samuel J (2002) Cloning and porin activity of the outer membrane protein P1 from *Coxiella burnetii*. Infect Immun 70:6741–6750

Vigil A, Ortega R, Nakajima-Sasaki R, Pablo J, Molina DM, Chao C-C, Chen H-W, Ching W-M, Felgner PL (2010) Genome-wide profiling of humoral immune response to *Coxiella burnetii* infection by protein microarray. Proteomics 10:2259–2269

Vigil A, Chen C, Jain A, Nakajima-Sasaki R, Jasinskas A, Pablo J, Hendrix LR, Samuel JE, Felgner PL (2011) Profiling the humoral immune response of acute and chronic Q fever by protein microarray. Mol Cell Proteomics 10(10):M110.006304

Vodkin MH, Williams JC (1986) Overlapping deletion in two spontaneous phase variants of *Coxiella burnetii.* J Gen Microbiol 132:2587–2594

Vodkin MH, Williams JC, Stephenson EH (1986) Genetic heterogeneity among isolates of *Coxiella burnetii*. J Gen Microbiol 132:455–463

Waag DM, Bolt CR, Marrie TJ, Williams JC (1991) The biology of *Coxiella burnetii*. In: Williams JC, Thompson HA (eds) Q fever. CRC Press, Boca Raton, pp 164–167

Waag DM, England MJ, Pitt ML (1997) Comparative efficacy of a *Coxiella burnetii* chloroform:methanol residue (CMR) vaccine and a licensed cellular vaccine (Q-Vax) in rodents challenged by aerosol. Vaccine 15:1779–1783

Waag DM, England MJ, Tammariello RF, Byrne WR, Gibbs P, Banfield CM (2002) Comparative efficacy and immunogenicity of Q fever chloroform:methanol residue (CMR) and phase I cellular (Q-Vax) vaccine in cynomolgus monkeys challenged by aerosol. Vaccine 20:2623–2634

Waag DM, England MJ, Bolt CR, Williams JC (2008) Low-dose priming before vaccination with the phase I chloroform-methanol residue vaccine against Q fever enhances humoral and cellular immune responses to *Coxiella burnetii*. Clin Vaccine Immunol 15:1505–1512

Williams JC, Cantrell JL (1982) Biological and immunological properties of *Coxiella burnetii* vaccines in C57BL/10ScN endotoxin-nonresponder mice. Infect Immun 35:1091–1102

Williams JC, Damrow TA, Waag DM, Amano K (1986) Characterization of a phase I *Coxiella burnetii* chloroform-methanol residue vaccine that induces active immunity against Q fever in C57BL/10ScN mice. Infect Immun 51:851–858

Williams JC, Peacock MG, Waag DM, Kent G, England MJ, Nelson G, Stephenson EH (1990) Vaccines against coxiellosis and Q fever. Ann N Y Acad Sci 590:88–111

Wisniewski HJ, Kleiman MW, Lackman DB, Krumbiegel ER (1969) Demonstration of inapparent infection with disease agents common to animals and man. Health Lab Sci 6:173–177

Zhang GQ, Samuel JE (2003) Identification and cloning potentially protective antigens of *Coxiella burnetii* using sera from mice experimentally infected with Nine Mile phase I. Ann N Y Acad Sci 990:510–520

Zhang YX, Zhi N, Yu SR, Li QR, Yu GC, Zhang X (1994) Protective immunity induced by 67K outer membrane protein of phase I *Coxiella burnetii* in mice and guinea pigs. Acta Virol 38:327–332

Zhang GQ, Kiss K, Seshadri R, Hendrix LR, Samuel J (2004) Identification and cloning of immunodominant antigens of *Coxiella burnetii*. Infect Immun 72:844–852

Zhang G, To H, Russell KE, Hendrix LR, Yamaguchi T, Fukushi H, Hirai K, Samuel JE (2005) Identification and characterization of an immunodominant 28-kilodalton *Coxiella burnetii* outer membrane protein specific to isolates associated with acute disease. Infect Immun 73:1561–1567

Zhang G, Russell-Lodrigue KE, Andoh M, Zhang Y, Hendrix LR, Samuel JE (2007) Mechanisms of vaccine-induced protective immunity against *Coxiella burnetii* infection in BALB/c mice. J Immunol 179:8372–8380