Genome Medicine

REVIEW

Proteomics paves the way for Q fever diagnostics

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Abstract

Q fever is a worldwide zoonosis caused by *Coxiella burnetii*. The disease most frequently manifests clinically as a self-limited febrile illness, as pneumonia (acute Q fever) or as a chronic illness that presents mainly as infective endocarditis. The extreme infectivity of the bacterium results in large outbreaks, and the recent outbreak in the Netherlands underlines its impact on public health. Recent studies on the bacterium have included genome sequencing, the investigation of host-bacterium interactions, the development of cellular and animal models of infection, and the comprehensive analysis of different clinical isolates by whole genome and proteomic approaches. Current approaches for diagnosing Q fever are based on serological methods and PCR techniques, but the diagnosis of early stage disease lacks specificity and sensitivity. Consequently, different platforms have been created to explore Q fever biomarkers. Several studies using a combination of proteomics and recombinant protein screening approaches have been undertaken for the development of diagnostics and vaccines. In this review, we highlight advances in the field of *C. burnetii* proteomics, focusing mainly on the contribution of these technologies to the development and improvement of Q fever diagnostics.

Coxiella burnetii and the diagnosis of Q fever

Coxiella burnetii is the infectious agent responsible for Q fever, which occurs worldwide [1]. Many reservoirs have been reported, including mammals, birds and arthropods (mainly ticks), but infectious aerosols produced by farm animals and pets, including those from feces, milk, hides and wool, are the most frequent source of human infection [1]. Person-to-person transmission is rare [1,2], although sexual transmission has been documented [3]. Presentation of the disease is extremely variable. A non-immunized person develops a primary infection in 60% of cases (Table 1). This can lead to the acute disease (in 40% of cases), which mostly presents as a flu-like syndrome or as severe pneumonia; 2% of patients with acute disease are hospitalized [1]. In patients with pre-existing valvulopathy, infection can progress to the chronic form (in 2-5% of patients), which is characterized by blood-culture-negative endocarditis [1,4]. The fever and characteristic vegetations (a mixture of bacteria and blood clots on heart valves) are frequently absent, making diagnosis difficult [1]. Importantly, Q fever is associated with high morbidity and mortality in pregnant women [1,4], although only few such cases have been reported to date [2,4]. The incidence of Q fever was recently re-evaluated by analyzing Q fever data collected at the French National Reference Center (FNRC) between 1985 and 2009 [5]. During this 25-year period, the FNRC identified 32 outbreaks in Europe, indicating that the number of Q fever cases was increasing [5].

In the recent outbreak of Q fever in the Netherlands, a rapid increase in human Q fever cases (3,523 in total) was observed between 2007 (182) and 2009 (2,361) [2,6,7]. Q fever had already been endemic in the Netherlands, and the disease was previously diagnosed in dairy goats and dairy sheep in 2005 [2,7]. The sudden increase may have been linked to a more virulent subtype of *C. burnetii* [2,6,7]. Indeed, several genotypes of *C. burnetii* were involved in the Dutch outbreak. When tested by multiple-locus variable-number tandem repeat analysis (MLVA) typing, the strains were found to differ by only a single repeat difference and it was thought that they might represent microvariants of a hypervirulent strain [7]. The rising number of reported outbreaks over the past 10 years worldwide is, however, considered to be a consequence of more efficient detection [6]. In the Dutch outbreak, several factors were considered to have contributed to the increase in Q fever cases, including: (i) the high density of farms in the regions where the bacterium is endemic, (ii) asymptomatic infection in the majority of infected animals, and (iii) more efficient diagnostic tests [2,6,7]. Nevertheless, important factors still need to be assessed including the persistence of *C. burnetii* in the environment and in different hosts, and the potential to prevent and control the next outbreak. Q fever has

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Table 1. Main characteristics of the immune responses to *C. burnetii* infection occurring in the acute and chronic phases

Immune response	Acute phase	Chronic phase	Detection or exploration methods
Cells	T lymphocytes	Fewer T lymphocytes (CD4/CD8)	Flow cytometry
Total eradication of bacteria	No	No	qRT-PCR, qPCR
Granuloma formation	Yes	No, large vacuole	Immunohistochemistry
Detection of bacteria in granuloma/large vacuole	No, very weak	Yes	Immunohistochemistry and qRT-PCR, qPCR
Antibody production	IgG against phase I, IgM against phase II	IgG, IgM, IgA against both phase I and II	Serology (IFA)
Properties of monocytes from convalescent patients	Able to kill *C. burnetii* and migrate through the endothelium	Unable to kill *C. burnetii* or migrate through the endothelium	qRT-PCR, qPCR targeting *C. burnetii*, apoptosis detection (TUNEL assay)
Cytokines	IFN-γ and TNFα, mediated through TLR4 activation	IL-10	qRT-PCR targeting the specific cytokines
Immune response	Efficient	Deleterious	-

IFA, immunofluorescence assay; IFN-γ, Interferon-gamma; IgG, Immunoglobulin G; IgM, Immunoglobulin M; IgA, Immunoglobulin A; IL-10, Interleukin 10; qRT-PCR, quantitative real time polymerase chain reaction; qPCR, quantitative polymerase chain reaction; TLR4, Toll-like receptor 4; TNFα, tumor necrosis factor α; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

become a serious public health problem in many areas not previously known as endemic zones. The bacterium is highly infectious and, consequently, the Centers for Disease Control and Prevention (CDC) in the USA have classified it as a category B bioterrorism agent [8].

In the past decade, technological developments have contributed an improved understanding of some of the pathological aspects of the intracellular life-cycle of *C. burnetii* and the role of host immunity. The diagnosis of *C. burnetii* infection still lacks sensitivity and specificity, especially at the early stage of infection. Therefore, recent efforts have focused on identifying strain-specific or clinical-outcome-specific protein markers (Table 2). Here, we review recent studies on *C. burnetii*, focusing on the contribution of proteomic technologies to our understanding of *C. burnetii* infection and to the diagnosis of Q fever.

Coxiella burnetii

Bacteriology

C. burnetii is a small (0.3-1 μm) obligate intracellular Gram-negative coccobacillus. The cell wall structure of this bacterium displays characteristics similar to those of Gram-negative bacteria, but does not stain reliably with Gram stain; for this reason, Gimenez staining has been used historically [9]. *C. burnetii* has been classified as a member of the γ-proteobacteria [1,8].

Genetic variability of isolates

Currently, the genome sequences of six *C. burnetii* strains (CBuG Q212, CBuK Q154, Dugway 5J108-111, RSA331, RSA493 and MSU Goat Q177) are available; Nine Mile RSA493 was the first *C. burnetii* genome to be sequenced [10,11]. *C. burnetii* isolates also harbor different plasmid types (QpH1, QpRS, QpDG or QpDV), which define specific genovars [8]. It remains to be determined whether these plasmids are involved in virulence.

Voth *et al*. [12] suggested that *C. burnetii* plasmids play an important role in host-cell modifications [12]. Proteins encoded by plasmid QpH1 (such as CBUA0014) are translocated into the host cell by the Dot/Icm type IV secretion system (T4SS). Compared to those of other strictly intracellular bacteria, the *C. burnetii* genome harbors multiple copies of insertion sequence (IS) elements that are probably involved in genomic plasticity [13], but it possesses fewer pseudogenes, suggesting a recent genome reduction event [10]. The T4SS, together with genes encoding a large proportion of basic proteins, including ion exchangers that enable the bacterium to live in an acidic environment, are characteristic of the *C. burnetii* genome [10].

Phase variation

In addition to the reported genetic variability, antigenic variation due to different lipopolysaccharide (LPS) structures [3,14] and sugar compositions [15] is common among *C. burnetii* isolates. This is frequently referred to as phase variation. Smooth, full-length LPS is characteristic of isolates from naturally infected biological samples (phase I, virulent), whereas rough, truncated LPS is found in sub-cultured bacteria (phase II, avirulent). Unique *C. burnetii* carbohydrates have been identified and studied in detail [14,16]. Among these are 3-C(hydroxymethyl)-L-lyxofuranose, known as dihydroxystreptose (Strep), and 6-deoxy-3-methyl-D-gulopyranose, known as virenose (Vir), a unique marker of phase I virulent strains [14]. Recently, the virulent phase I and avirulent phase II variants of the Nine Mile RSA493 and RSA439 strains of *C. burnetii* were compared using tandem liquid chromatography mass spectrometry

Table 2. Main protein candidates for serodiagnosis that have been cross-validated by proteomic studies

Protein (locus-tag)	Identification	MW (kDa)	p*I*	Protein function	Nature of protein	Peptide signal sequence	Nature of analyzed samples	DT	Clinical significance	Ref(s)
CBU_0952	Acute disease antigen A (adaA)	25.9	8.67	Unknown	Membrane	MKKLTVTFLTFI SIFFAATAAFA	*Cb* isolates	BT, IP	Marker of acute Q fever	[31,63]
CBU_0612	Putative outer membrane chaperone protein (ompH, Skp)	18.8	9.71	Molecular chaperone, interacts with unfolded proteins	Membrane*	MIKRLLSAICLS VAMIWSVAA VAQTVGLVD	Patient sera, *Cb* NM II TPE	IP, RP	Marker of Q fever endocarditis, SP with Q fever patients (general)	[56,57]
CBU_0937	Hypothetical protein	51.4	8.99	Unknown	Membrane	MTSKLVISALGL CVSGALSTT LAST	mAbs, *Cb* NM II TPE, RP-based ELISA/HS	IP, BT	Marker of Q fever endocarditis. Marker of phase II	[17,56,58]
CBU_1910	Outer membrane protein (com1)	27.6	9.08	Protein disulfide oxidoreductase, unknown role in pathogenesis	Membrane	MKNRLTALF LAGTLTAGVAIA APSQF	mAbs, *Cb* NM II TPE	IP, BT, RP	SP with both acute Q fever and Q fever endocarditis. Marker of both phase I and phase II	[17,58, 65,67-69]
CBU_0236	Elongation factor Tu (tuf-2)	43.5	5.32	GTP-dependent binding of aminoacyl-tRNA in protein biosynthesis	Soluble*†		mAbs, TPE *Cb* NM II, HS, AS (infected/ vaccinated guinea pigs)	IP	SP, marker of acute Q fever	[31,58, 65,68]
CBU_0092	Tol-pal system protein (YbgF)	34.3	6.46	Critical for maintaining integrity of bacterial outer membrane Involved in protein-protein interactions	Membrane	MRLIKMKIKTLC VSSALAALM LSAPLTWADA	TPE *Cb* NM I and II HS Q-fever (general), AS (immunized guinea pigs) protein microarray	IP, RP	Phase II-specific marker (early diagnosis of acute Q fever), marker of Q fever (general)	[68,69]
CBU_0311	Outer membrane porin (*Coxiella* porin P1)	26.8	8.44	Able to form pore in lipid bilayers	Membrane*† OM location shown for *Cb* NM I	METTTKLAIGVS ALCCLASAA FAGGPD	ELISA and ELISPOT based on RP/AS IP: HS, and AS (infected/vaccinated guinea pigs)	RP, IP	Marker of Q fever (general), marker of acute Q fever; applications for drug and vaccine development	[31,67]
CBU_1718	Chaperonin (GroL)	58.284	5.14	Protein folding, ATP hydrolysis	Soluble†		HS/ TPE *Cb* NM II / RP; IP HS, and AS (infected/vaccinated guinea pigs)	IP, RP	Marker of Q fever (general), marker of acute Q fever	[31,65]
CBU_0229	50S ribosomal protein L7/ L12 (RplJ)	13.2	4.71	Binding site for several factors in protein synthesis	Membrane†	MAQLSKDDI LEAVANMSV MDVVDLVK AMEEKFGVS AQAAIAVAG PVAGGEA	IP: HS, and AS (infected/vaccinated guinea pigs)	BT, IP	Marker of both phase I and phase II, marker of acute Q fever	[17,31]
CBU_0263	DNA-directed RNA polymerase subunit alpha (rpoA)	35.5	5.61	DNA-dependent RNA polymerase transcription	Soluble		OMP fraction of *Cb* NM II and CbuG_ Q212 II; Phase 1 HS (chronic)	IP	Marker of chronic Q fever	[2,69]
CBU_1916	Universal stress protein family	15.78	6.58	Stress response	Soluble*		OMP fraction of *Cb* NM II and CbuG_ Q212 II; Phase 1 HS (chronic)	IP	Marker of chronic Q fever	[1,69]

I, phase I; II, phase II; AS, animal sera; BT, biotyping; *Cb*, *Coxiella burnetii*; DT, discovery technology; HS, human sera; IP, immunoproteomics; mAb, monoclonal antibody; MW, molecular weight; NM, Nine Mile; OM, outer membrane; OMP, outer membrane protein; p*I*, isoelectric point; RP, recombinant protein-based approaches; SP, seroreactive proteins; TPE, total protein extract.
Soluble (cytosolic). Membrane (having a signal peptide that directs protein to the cell membrane). *More than two-fold more abundant in the LCV stage than in the SCV stage. †Proteins common to SCV and LCV.

(LC-MS/MS) [17]. This study allowed the identification of strain-specific and clinical-outcome-specific protein markers [17] (Table 3). A total of 235 and 215 non-redundant proteins were identified from phase I and II variants, respectively. The most interesting outcomes of this work were the identification of 17 proteins that are involved in LPS biosynthesis, the first identification of DotD protein of the T4SS, and finally the identification of two ankyrins (CBU_0898 and CBU_1482). Biomarkers of LPS phase I were identified and might contribute to development of more sensitive diagnostic tests [17].

Culture conditions

C. burnetii is cultured in level 3 biosafety laboratory conditions. The bacterium can be propagated under laboratory conditions in cell lines [18] or in embryonated eggs [19, 20]. *C. burnetii* is able to infect various types of cells, including monocyte-macrophage systems and macrophage, fibroblast and epithelial cells [3,21]. The isolation of bacteria from clinical samples is carried out using the shell vial centrifugation technique [1]. *C. burnetii* was recently described as being cultivable in axenic medium (a medium that is free of contaminating organisms) under laboratory conditions [22]. The bacteria can be grown when incubated in a mesophilic atmosphere in an acidified citrate medium that is enriched with cysteine and casamino acids [22] and contains divalent metal cations. LimB (CBU_1224a) , a unique *C. burnetii* lipoprotein identified using matrix-assisted laser desorption ionization-time of flight/time of flight MS (MALDI-TOF/TOF MS), serves as surface receptor for such ions and may be involved in *C. burnetii* replication and pathogenesis [23]. Notably, the *C. burnetii* proteome includes a eukaryotic-like Δ24 sterol reductase homolog, CBU_1206, which might be involved in the intracellular growth of the bacterium [24].

Host-bacteria interactions

Physiopathology

Immune control of *C. burnetii* infection depends on T lymphocytes: chronic Q fever has been shown to develop preferentially in a nude mouse model that has a greatly reduced number of T cells [8]. In acute-phase disease, granuloma formation is a hallmark of an efficient immune response (Table 1), but *C. burnetii* is frequently missing from granulomas, resulting in the inability of PCR or immunocytochemistry tests to produce a positive diagnosis. In the chronic phase of infection, the immune response is inefficient or deleterious [8] (Table 1). The inoculum size, route of infection, host factors, and pathogenic potential of strains all play a role in the clinical presentation of acute Q fever [8]. Age, circadian rhythms and sex-related differences [25] may be involved in the development of the chronic form. Female sexual hormones (17-β-estradiol) are thought to have a protective role [8,26].

Intracellular survival

C. burnetii, an obligate intracellular bacterium, has evolved not only to survive but to thrive in the phagolysosome. The intracellular survival of *C. burnetii* is characterized by two distinct morphological forms: the large cell variant (LCV), which has evolved to persist within the acidified phagolysosome of monocytes or macrophages, and the 'spore-like' small cell variant (SCV), which can persist both in the phagolysosome and in extreme environmental conditions [27]. How *C. burnetii* mediates the establishment of the phagolysosomal-like compartment in which it resides and replicates is not well understood. We do know that bacterial protein synthesis is required for this process, suggesting that bacterial proteins directly influence the biogenesis of the *C. burnetii*-occupied vacuole [28,29]. Some of these mechanisms have been elucidated using proteomics and molecular biology [28-31]. Both developmental forms (SCV and LCV) were analyzed by a combination of two-dimensional electrophoresis (2-DE) and MS after differential fractionation [31]. Fifty proteins were identified *in vitro* from cytoplasm from Vero cells that were infected with *C. burnetii* (Table 3), but their roles have not been determined [31,32]. A Dot/Icm-dependent translocation in host cytoplasm was demonstrated for only a few of these, including *Coxiella* effector proteins such as CpeA (CBUA0006), CpeB (CBUA0013), CpeC (CBUA0014), CpeD (CBUA0015), CpeE (CBUA0016) and CpeF (CBUA0023) [29,32]. The T4SS candidate proteins identified by proteomic approaches remains to be functionally validated [31,32]. The majority of the identified proteins were found to be important for the intracellular survival of bacteria and were involved in RNA and DNA processing [33], confirming the results of Coleman *et al*. [31]. Notably, most of the identified proteins had basic physicochemical properties and contained eukaryotic motifs (such as ankyrin repeat-containing domains (Anks)) [33]. When *Legionella pneumophila* was used as a surrogate host, several different *C. burnetii* Anks could be delivered into the host cells by the *L. pneumophila* T4SS, suggesting that *C. burnetii* T4SS effector proteins affect host cell signal transduction pathways [28,34]. Moreover, when ectopically expressed, the *C. burnetii* Anks localized to a variety of subcellular regions in mammalian cells [32]. An understanding of the trafficking and role of Anks and of the secretion of T4SS effectors could help in selective drug design.

Current approaches for diagnosis of Q fever

The major issue for Q fever diagnosis is the non-specific clinical picture produced by the disease. Early stage

Table 3. Proteomic approaches for *C. burnetii* biomarker selection

Approach	Technique(s)	*Cb* isolates (culture method*)	Sera/mAbs	Applications	Identified proteins	Ref(s)
Biotyping						
Detection of specific markers for *Cb* isolates	MALDI-TOF MS	*Cb*: RSA493, BUD, Priscilla (CYSEE)	-	Optimization of method for typing *Cb* for specific strains or clinical isolates	RSA493-specific: CBU_1637, CBU_0401, CBU_0394, CBU_1592, CBU_1378, CBU_0403, CBU_0078, CBU_0961, CBU_1698, CBU_0644, CBU_1055 Priscilla-specific: CBU_0149, CBU_0438, CBU_0168, CBU_0745 BUD-specific: CBU_1989, CBU_2085	[20,54]
Identification of *Cb* strains and isolates	MALDI-TOF MS	*Cb*: NMI, Australian QD, M44, KAV, PAV, Henzerling, Ohio (CYSEE)	-	*Cb* isolate typing or diagnosis	Mass spectral peaks (1000-6000 Da), species-selective and strain-specific	[55]
Proteome of *Cb* NM I	2-DE and nanoLC-ESI MS/MS, LC-MALDI TOF/TOF MS, MALDI-TOF MS	TPE *Cb* RSA493, NM I (CC)	-	Markers of early stage infection, and therapeutic or vaccine development	197 distinct proteins. Vir and Strep: CBU_0691, CBU_0683 SP: CBU_0091, CBU_0109, CBU_0395, CBU_0867, CBU_1221, CBU_1268, CBU_1718, CBU_1910	[19]
In silico prediction of OMPs and identification of *Cb* (NM I) LP	Bioinformatics	*Cb* RSA493 genome sequence	-	Vaccine development or serodiagnosis	21 predicted OMPs and 9 LP; LP: CBU_1190 (LolA), CBU_1829 (LolB)	[59]
Proteome of *Cb* strain NM phase II	2-DE, 2D SDS-tricine PAGE, MALDI-TOF	TPE *Cb* NM II (CC, Vero)	-	Markers of active *Cb* infection, serology or therapeutic development	Proteins involved in *Cb* pathogenesis and survival mechanisms (NM II)	[94]
Proteome of *Cb* I and II	LC-MS/MS	*Cb* NM I and II (CYSEE)	-	Phase I and phase II distinct biomarkers, serology or biotyping (blood transfusion)	150 proteins reported (p/ >9. 5); virulence type I and T4SS: CBU_0884, CBU_0085, CBU_0318, CBU_0744, CBU_1099, CBU_1352, CBU_0338, DotD protein (CBU_1643); Anks: CBU_0898, CBU_1482; 7 enzymes involved in LPS phase I synthesis: CBU_0676, CBU_0678, CBU_0674, CBU_0681, CBU_0682, CBU_0683, CBU_0691, CBU_0846, CBU_1657	[17]
Cb secretome	Tricine-SDS PAGE, ESI-MS/MS, bioinformatics	*Cb* NM II (CC, Vero)	Cytoplasmic fraction from infected Vero cells	Characterization of T4SS, drug development	50 T4SS effector candidates: CBU_1440, CBU_0312, CBU_1091, CBU_1386, CBU_1518, Orf145, QpH1_p21, CBU_1297, CburD_01001397	[33]
Two *Cb* strains, subproteome (OMPs) and candidate proteins for serodiagnosis	Tris-Tricine SDS-PAGE, doubled SDS-PAGE, IP 2-DE, MALDI TOF/TOF	*Cb* strains NM RSA 493 II (acute) and CbuG_Q212 II (chronic), (CC, Vero)	OMP-enriched fraction, 1 HS (chronic)	Subproteome of *Cb*, chronic Q fever markers	86 identified OMPs. SP: htpB, CBU_0236, CBU_0263, CBU_1471, CBU_0572, CBU_0235, CBU_1916, CBU_0612, CBU_0937	[56]
Immunoproteomics or serology						
Candidate proteins for acute Q fever serodiagnosis	IP/serodiagnosis	17 *Cb* strains (CC) (BGM or L929)	RP *ada* (rada), AS immunized with rada	Marker of acute Q fever	adaA (CBU_0952)	[31,63]
Identification of *Cb* cell-variant-specific common SCV/LCV proteins	IP (2-DE, MALDI-TOF)	Purified SCVs and LCVs	HS (convalescent-phase, acute Q fever), AS from infected or vaccinated guinea pigs	Subunit vaccines or serodiagnostics for acute Q fever, unique SCV/LCV markers	Proteins that are differentially expressed in SCV and LCV forms. SP: CBU_1718, CBU_0236, CBU_0229, CBU_1943, CBU_1416, CBU_0952, CBU_0963, CBU_0737, CBU_0497, CBU_1200	[31]
SP for serodiagnosis of Q fever endocarditis	IP (2-DE/MALDI-TOF)	TPE *Cb* NM II (CC, Vero)	HS (acute Q fever or IE Q fever)	Markers of chronic Q fever (IE), serodiagnosis	CBU_0612, CBU_0480	[57]

Continued overleaf

Table 3. Continued

Approach	Technique(s)	*Cb* isolates (culture method*)	Sera/mAbs	Applications	Identified proteins	Ref(s)
Identification of *Cb* proteins reacting with *Cb* mAbs	IP (2-DE/MALDI-TOF) RP-based ELISA	TPE *Cb* NM II (CC, Vero)	Specific mAbs; HS acute or chronic (IE)	Markers of chronic Q fever (IE), serodiagnosis	Q fever markers (general): CBU_1910, CBU_0236 Chronic Q fever marker (IE): CBU_0937	[58]
SP for early serodiagnosis of Q fever	IP (2-DE, LC-MS/MS) (pH 5-8, pH 3-10; phase I and phase II antigens)	TPE *Cb* NM I and II (CC, L929), *Cb* NM RSA493 I (CYSEE)	AS from immunized guinea pigs	Markers of early stage acute Q fever, serodiagnosis or vaccine development	Phase-I-specific SP: CBU_1706, CBU_12190, CBU_0495, CBU_0780, CBU_0955, Phase-II-specific SP: CBU_1290, CBU_0235, CBU_0215, CBU_0572, CBU_1398, CBU_0236, CBU_0092, CBU_1241, CBU_1396, CBU_1227, CBU_0481, CBU_0299, CBU_0481, CBU_0495, CBU_0482, CBU_0937, CBU_0236	[68]
RP-based approaches						
Candidate RPs for Q fever vaccine development and serodiagnosis	TAP products and expression of selected SP		HS (acute, chronic phase vaccine)	Serodiagnosis or vaccine development	SP: CBU_0008, CBU_0381, CBU_0612, CBU_0781, CBU_1115, CBU_1143, CBU_1157, CBU_1249, CBU_1853, CBU_1869 Top candidates: CBU_0781 (AnkG), CBU_1115 (lipoprotein), CBU_1143 (YajC)	[66]
Candidate RPs for Q fever vaccine development and serodiagnosis	Protein microarray		HS Q fever (general)	Markers of Q fever (general)	SP: CBU_1910, CBU_0891, CBU_0109, CBU_1143, CBU_0612, CBU_0092, CBU_0545, CBU_1398, CBU_0630, CBU_1513, CBU_1719, CBU_0229, CBU_0653	[69]
Candidate RPs for Q fever vaccine development and serodiagnosis	ELISA (HS), ELISPOT (AS)	11 RPs, *Cb* NM RSA493 I	HS Q-fever (IFA-positive, convalescent), HS chronic (transgenic mice, immunized with *Cb* NM RSA493 I)	Subunit vaccine development, serodiagnosis	SP: CBU_1910, CBU_1716, CBU_612, CBU_718, CBU_311	[67]
Identification of *Cb* SP	IP (2D, LC-MS), tripartite fusion RP	TPE *Cb* (I and II Henzerling strain) (CC, Vero)	HS Q fever (general)	Serodiagnosis or vaccine development	SP: CBU_0091, CBU_0109, CBU_0395, CBU_0867, CBU_1221, CBU_1268, CBU_1718, CBU_1910 Six RP(*Cb* II): GroEL, Com1, RecA, EF-Tu, OmpA-like and FtsZ	[65]

I, phase I; II, phase II; adaA, acute disease antigen A; AS, animal sera; *Cb*, *Coxiella burnetii*; IE, infective endocarditis; HS, human sera; IP, immunoproteomics; LP, lipoproteins; MAb, monoclonal antibody; MW, molecular weight; nanoLC-ESI MS/MS, nano liquid chromatography-electrospray ionization mass spectrometry; NM, Nine Mile; OM, outer membrane; OMP, outer membrane protein; RP, recombinant protein; SP, seroreactive proteins; TAP products, transcriptionally active PCR products, TPE, total protein extract; Vir and Strep: synthesis of virenose and streptose.
*CYSEE, cultured on yolk sacs in embryonated eggs; CC, cultured in cell lines.

detection of *C. burnetii* lacks specificity and is not sensitive enough for diagnosis of acute Q fever [35]. Moreover, the serological profiles of acute and chronic Q fever differ [5]. In acute cases, immunoglobulin M (IgM) is produced against phase I and II variants, and patients will have immunoglobulin G (IgG) antibodies against phase II antigens. In chronic Q fever, high levels of IgG against phase I and II antigens are produced [5] and persist for months or years after the initial infection. Increased IgG and immunoglobulin A (IgA) antibodies against phase I antigens are also often indicative of chronic Q fever [36]. In the early stage of infection (<10 days), the specific antibodies remain undetectable [37]. The prevalence of auto-antibodies, including antibodies similar to those seen in cases of rheumatoid arthritis and lupus, presents another problem in Q fever diagnosis [21].

Direct diagnosis

The laboratory diagnosis of Q fever depends on the stage of disease (acute or chronic), which in turn determines which sample should be used for analysis: blood, cerebrospinal fluid, bone marrow, cardiac valve biopsy, vascular aneurysm or graft, bone biopsy, liver biopsy, milk, placenta, fetal specimens in cases of abortion, or cell culture supernatants [38]. The choice of technique also depends on the available laboratory capabilities and on the clinical presentation of disease.

Immunodetection

In patients with chronic Q fever who are undergoing treatment, immunodetection of *C. burnetii* in fresh tissue samples or samples after formalin fixation and paraffin embedding may be very useful [1]. Several techniques can be employed: either an immunoperoxidase technique or immunofluorescence with polyclonal or monoclonal antibodies is frequently used [1]. New diagnostic tools, including autoimmunohistochemistry [39] and immunohistochemical peroxidase-based methods, have been reported for the diagnosis of blood culture-negative endocarditis [1]. The specificity of immunodetection is strongly correlated to the quality of the antibodies used.

Molecular tests

Several PCR-based assays have been developed in the past decade [1,37,40,41]. Although lacking sensitivity, PCR targeting the *htpAB*-associated repetitive element, which is present in 20 copies in the genome of *C. burnetii* [10], is routinely used to detect bacteria in cell cultures and clinical samples from both acute and chronic Q fever patients [1]. Light cycler nested-PCR (LCN-PCR) has been optimized for the early diagnosis of acute Q fever [40] when antibodies are absent [37]. This test, together with serology, is recommended in the first 2 weeks of acute Q fever [37]. Real-time quantitative PCR assay targeting the multicopy insertion sequences IS1111 and IS30a is also highly specific and sensitive [40,41]. Detection of the *adaA* gene (encoding acute disease antigen A) can be used to confirm acute Q fever [1]. Overall, PCR is useful for detecting *C. burnetii* in the early course of infection, following antigen shedding in livestock, or when applied to biopsies from patients with chronic Q fever. Molecular testing is generally recommended in addition to serology [2] but the possibility of reagent contamination leading to false-positive results is its major drawback [2,42,43].

Serology

The microbiological diagnosis of Q fever is usually based on serology and most commonly uses an indirect immunofluorescence assay (IFA) [2,5]. The cut-off for serological titres was first established in 1994 [44] but has been revised recently [5]. The diagnosis of Q fever is performed using different methods: a complement fixation test with commercially available antigen preparations combined with real-time PCR [45], enzyme-linked immunosorbent assay (ELISA) [46], IFA, and nested PCR [46]. ELISA helps in the diagnosis of Q fever after the fifth day of infection, whereas PCR is an efficient diagnostic tool during the first few days of infection [46].

According to guidelines for Q fever diagnosis, combined approaches, including PCR (≤7 days) and IFA (≥7 days), are strongly recommended in the early phase of infection [5,37,47] (Figure 1). In the case of chronic Q fever, especially with endocarditis, a positive result from systematic serological testing has been included as a major criterion in the modified Duke criteria [48]. When cross-reactivity with *Legionella micdadei* [49], *Bartonella* [50] and *Rickettsiae* [51] is observed, immunoblotting with adsorbed cross-reacting antigens is recommended.

The InoDiag automated fluorescence multiplexed antigen microarray method [52] has been compared with the IFA reference method for the detection of *C. burnetii* IgM. The advantages of the InoDiag technique are speed of analysis, the need for only a small quantity of sampled serum (5 μl) and multiplexing [53]. The sensitivity and specificity obtained by automated assay for diagnosis of the acute form were excellent, and the serological parameters obtained for serodiagnosis of Q fever endocarditis were also adequate [52]. This is a first step towards IFA standardization [52,53].

Proteomics

Recent technological developments in the field of molecular medicine have moved beyond genomics and transcriptomics to proteomics, with the goal of characterizing the impact of disease and therapy on cellular networks. Advances in proteomics-based research provide potential for the development of efficient diagnostic and therapeutic assays (Figure 1). Depending on the availability of clinical samples (storage, standardization and cohort), methods for proteomic analysis include mass spectrometry (MS), gel-based proteomics, 2-DE, differential gel electrophoresis (DIGE), immunoproteomics, recombinant protein-based arrays, and methods for the analysis of post-translational modifications (PTMs).

MS-based approaches

Several recent proteomics studies have been undertaken to identify clinical biomarkers that facilitate the accurate detection of the infectious agent, and offer new insights into inter- or intra-species relatedness. Several attempts have been made to characterize the whole proteome of *C. burnetii*, aiming to identify biomarkers that are useful in diagnosis or vaccine production for different strains or isolates (Table 3) [19,20,54]. These studies have helped to determine appropriate conditions for MS analysis, focusing on different matrices that can be used (such as α-cyano-4-hydroxycinnamic acid is a good matrix choice for samples with molecular weight (MW) <10,000 Da; sinapinic acid is an appropriate matrix choice for samples with MW >10,000 Da; or 2,5-dihydroxybenzoic acid is a good a matrix choice for hydrophobic compounds) and the nature of the sample (such as intact bacterial cells, cell-free extracts). Altogether, these studies have improved the MS-based laboratory pipeline.

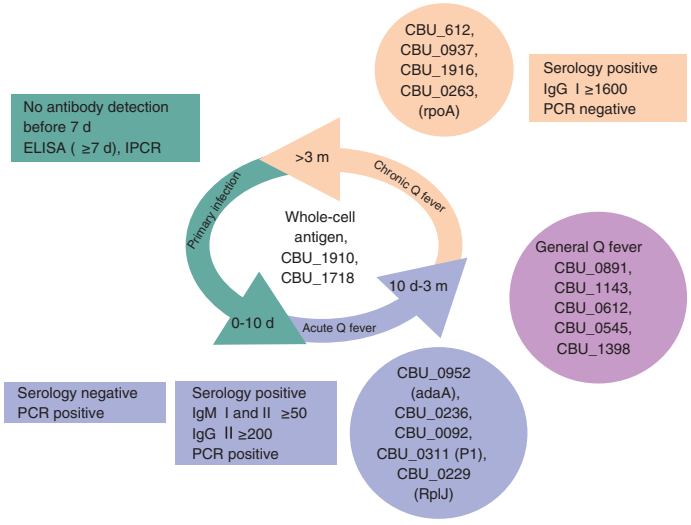

Figure 1. Flow diagram showing the evolution of Q fever disease in the absence of treatment. Q fever disease starts with asymptomatic primary infection (0-10 days), followed by acute Q fever (10 days to 3 months), and some subjects then develop the chronic form of disease (>3 months). The clinical sample used initially for the detection of *C. burnetii* at each stage is patient serum. The strategy for early-stage Q fever diagnosis consists of combined approaches, including PCR (≤7 days) and antigen detection by immunofluorescence assay (IFA) or enzyme-linked immunosorbent assay (ELISA) (≥7 days) performed on whole-cell antigen (formalin-inactivated bacteria). Immuno-PCR (IPCR) performed with whole-cell protein extracts may also be a promising detection tool. The diagnosis of chronic Q fever relies mainly on serology. The cut-off stands at (i) IgM phase II ≥25 and IgG phase II (and I) ≥200 for acute Q fever serodiagnosis; and (ii) IgG phase II and I ≥1,600 associated with the presence of IgA phase I ≥50 for chronic Q fever serodiagnosis. IgM may be still detectable in cases of chronic Q fever. The protein candidates for serodiagnosis selected by several proteomic studies are shown in the circles. In the centre, the most antigenic proteins, namely CBU_1910 (Com1) and CBU_1718 (GroEL), as well as whole-cell antigen, are versatile markers of Q fever.

Characteristic and reproducible MS fingerprints containing unique biomarker profiles have also been obtained. This approach was applied for *C. burnetii* strain and phase identification by two independent laboratories for strains NMI, Australian QD, M44, KAV, PAV, Henzerling and Ohio [55] and for strains RSA493, BUD and Priscilla [20,54]. The method was validated by the prediction of samples in an independent test set with 100% sensitivity and specificity for five out of six strain classes [55]. Differences in the ion-signal profiles of three isolates, RSA493, BUD and Priscilla, were observed for peptides in the mass range 3-18 kDa [20,54]. In the recent work of Papadioti *et al.* [56], the outer-membrane protein (OMP) fractions of *C. burnetii* strains Nine Mile RSA 493 and CbuG_Q212 (phase II) were compared using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) combined with MS/MS analysis. Markers of chronic Q fever, such as CBU_0612 and CBU_0937, were identified [56-58] with agreement to predicted *in silico C. burnetii* OMPs [59].

When compared with conventional phenotypic and molecular identification methods, the implementation of MS in clinical laboratories could improve both the speed and sensitivity with which human pathogenic infections are diagnosed [60,61]. Nevertheless, proteomic approaches such as MALDI-TOF should not completely replace traditional diagnostic techniques in clinical microbiology, even though these traditional approaches have a number of shortcomings including the need for time-consuming biochemical and antibiotic sensitivity tests [60]. Recently, Hernychova *et al.* [62] demonstrated that *C. burnetii* can be identified rapidly at the species level by MALDI-TOF. To date, however, no routine method for the identification of *C. burnetii* clinical isolates has been shown to be fully reliable, probably because of the restrictions in culturing and handling *C. burnetii*. Further optimization of *C. burnetii* culture on solid media should facilitate its improved identification by routine biotyping.

Immunoproteomics

Despite the availability of sensitive and specific laboratory tests, the diagnosis of Q fever remains difficult. Moreover, a differential diagnosis to distinguish chronic (mainly endocarditis) from acute Q fever is greatly needed. Thus, several immunoproteomic studies, combining the use of combine 2-DE immunoblots and MS, have set out to find specific biomarkers of Q fever for the development of accurate diagnostic tools (Tables 2 and 3).

To date, only a few studies have investigated the possibility of differentiating between acute and chronic Q

fever [57,58,63]. Several markers have been proposed: (i) a marker of acute Q fever, adaA (CBU_0952) [63], and (ii) CBU_0612 (OmpH) and CBU_0480 (an arginine repressor), which were identified as promising markers for patients with Q fever endocarditis [57]. In another study, Q fever-specific proteins, namely the CBU_0937 protein, the OMP Com1 (CBU_1910) and elongation factor Tu (CBU_0236) were found to be discriminated by monoclonal antibodies [58]. Two of these proteins (CBU_0937 and CBU_1910) were cloned, expressed and tested by ELISA with sera from patients with acute and chronic Q fever [58]. Com1 (CBU_1910) has been widely studied [31,57,64-67] and is currently used for seroimmunological screening. Although tests using these immunoreactive proteins (CBU_0937 and CBU_1910) were neither sensitive nor specific enough for routine clinical application, the serological parameters for Com1 protein (CBU_1910) were cross-validated [58] and were in the same range as those reported by Beare *et al*. [66]. Moreover, Papadioti *et al*. [56] also demonstrated the seroreactivity of proteins CBU_0937 and CBU_0612 by two-dimensional immunoblot performed with serum from a patient with chronic Q fever [56].

Recent work by Deringer *et al*. [68] has raised the possibility of early- and late-stage serodiagnosis. These authors evaluated the IgG-specific response in a guinea pig model following vaccination with the Nine Mile strain of *C. burnetii*. Nine novel seroreactive *C. burnetii* proteins were identified (Table 3). Furthermore, several immunoreactive proteins from this study were identified in other studies as being immunoreactive with human Q fever sera [31,57,66,69]. This study did not, however, identify specific protein markers for each phase (I and II) separately. Notably, the identification of seroreactive *C. burnetii* proteins with low homology to other proteins seems to be promising for serodiagnosis because of the likelihood of low cross-reactivity. However, the low similarities of CBU_0937 with proteins in other bacteria were not sufficient for it to be considered useful as a specific marker. The serological operating parameters for Q fever serodiagnosis using CBU_0937 showed low sensitivity, even though the specificity was acceptable [58]. For patients with acute Q fever and endocarditis, the results were in the same range, indicating the low diagnostic potential of CBU_0937 [56,58].

Multiplexed biomarker protein patterns have a significantly higher positive predictive value (PPV) for disease discrimination. Immunoproteomic studies have been used to build a library of potential diagnostic or vaccine-related protein targets in several bacterial species: *Chlamydia trachomatis* [70-72], *Helicobacter pylori* [73-77], *Francisella tularensis* [78-82], *Shigella flexnerii* [83,84], *Tropheryma whipplei* [85,86] and *Bartonella henselae* [87,88]. Indeed, recent technological progress has enabled high-throughput, large-scale screening in miniaturized formats, such as protein microarrays. The laboratory pipeline could be enhanced by the validation of discovered diagnostic value (Figure 2). Some of the comprehensive studies performed on selected immunoproteomic targets were previously performed using molecular approaches. One such study involved *H. pylori* urease, which has diagnostic value (in the ^{13}C urea breath test (UBT) and in UBT-C13/UBT-C14 urease activity-based tests) and is a vaccine candidate [74]. In addition, these immunoproteomic studies were not applied for routine diagnostics, but contributed to the selection, and in some cases validation, of specific biomarkers. Immunoproteomics is time consuming, but has been an important first step in biomarker discovery. Further progress will probably depend on the miniaturization of clinical assays and the use of recombinant proteins.

Screening of recombinant proteins

Proteomics focuses on the large-scale study of an organism's proteins, particularly their structures, functions and expression. After the identification and subsequent verification of specific protein biomarkers, their utility as highly reliable, specific diagnostic markers can be investigated using complementary methods to previously used biological tests. The combination of immunoproteomic methods with protein expression and validation techniques provides an ideal basis for this highly demanding challenge (Figure 2). The study of Chao *et al*. [65] is an example of the integration of complementary technologies. Eleven protein candidates were selected using an immunoproteomic approach, and six (hsp60, Com-1, RecA, EF-Tu, OmpA-like protein and FtsZ) were successfully cloned [65], but these proteins were not tested for their diagnostic potential [65]. Other studies investigated diagnostic value using serodiagnostic screening with recombinant proteins [66,67,89]. High-throughput screening for the selection of serodiagnostic candidates has recently been performed [66,69]. Transcriptionally active PCR products (TAP products) corresponding to 1,988 *C. burnetii* open reading frames (ORFs) were tested using a protein microarray [66]. In total, 75% of the full-length proteins were produced using an *in vitro* transcription and translation system, and these were screened with sera from patients with Q fever and with sera from vaccinated individuals [66]. Fifty strongly immunoreactive protein candidates were proposed as serodiagnostic markers, including several previously identified proteins [31,57,64,65,67], Ank and multiple hypothetical proteins [66]. The top ten candidates, and the most reactive hypothetical membrane-associated protein CBU_0089, are listed in Table 3 [66]. In a study from the same group [67], all 11 of these recombinant

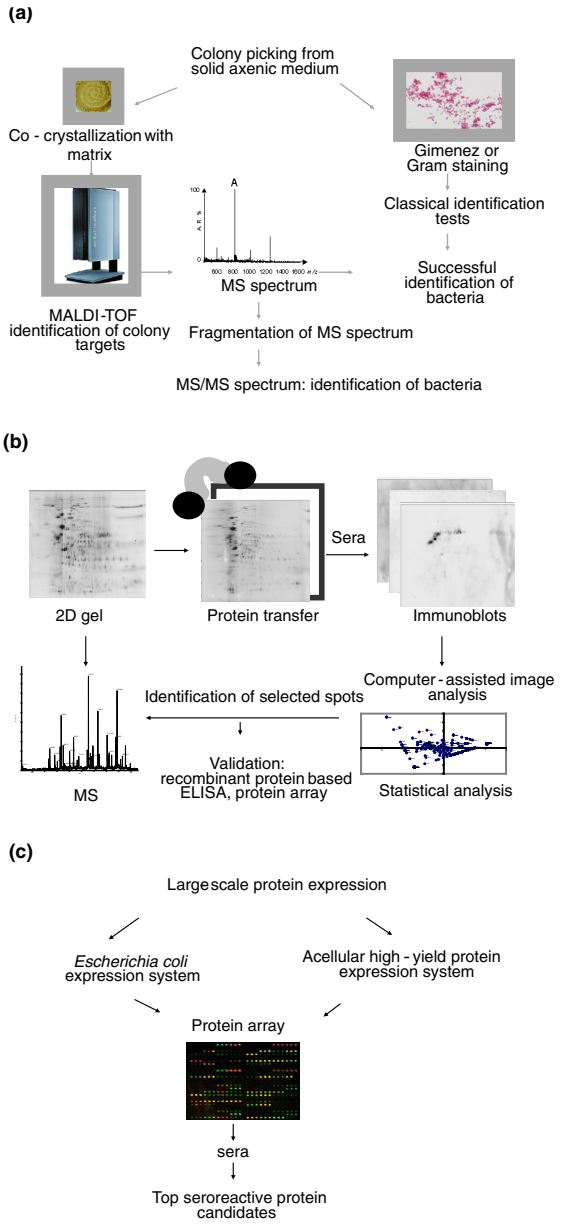

Figure 2. Proteomic technologies used in applied research on *C. burnetii* pathogenesis. Several proteomic approaches have been used to identify biomarkers of Q fever or to characterize the proteome of *C. burnetii*. **(a)** Laboratory pipeline for biotyping. Biotyping of *C. burnetii* is not yet applied routinely because of restrictions in manipulating the organism, which is a potential bioterrorism agent. With the recent development of axenic solid culture for *C. burnetii*, however, the laboratory isolation of strains from blood- culture-negative samples associated with endocarditis (chronic Q fever) or from a variety of other samples such as blood or rhinopharyngeal swabs (acute Q fever) might be possible. The sample or bacterial products are subjected to analysis by matrix-assisted laser desorption ionization-time of flight/time of flight mass spectrometry (MALDI-TOF/TOF MS). Colonies of *C. burnetii* are picked from solid medium, co-crystallized with matrix, and processed for MALDI TOF MS analysis. The obtained MS spectra are analyzed against an available database, which allows identification of the bacteria. In parallel, classical phenotypic identification methods, including Gimenez- or Gram-staining of bacteria and biochemical tests, can be applied to confirm the identity of bacteria. **(b)** Laboratory pipeline for immunoproteomics. The whole-cell protein extract or fraction (such as sarcosyl-insoluble fraction, containing mainly membrane proteins) is resolved on two-dimensional (2D) acrylamide gels. The resolved proteins are stained (using silver nitrate or Coomassie blue) or transferred onto nitrocellulose or polyvinylidene fluoride (PVDF) membranes and then processed for immunoblotting with sera. The sera are from patients or animals with Q fever (general, acute or chronic Q fever) and from naïve subjects (control group). The immunoblots are analyzed and compared to silver-stained gels (using commercially available software). This analysis can be improved by statistical methods (such as principal component analysis (PCA)), which allows more discriminating spot selection. All the selected spots are subjected to MS identification. In some studies, the best targets are validated by using different approaches, such as recombinant-based enzyme-linked immunosorbent assay (ELISA) or protein array. **(c)** Laboratory pipeline for recombinant protein-based approaches. The large-scale recombinant protein systems allow a high level of genome coverage (>75% of predicted open reading frames (ORFs)). *C. burnetii* proteins are expressed using *Escherichia coli* or acellular translation systems (such as the rapid translation system (RTS)). Expressed and/or purified recombinant proteins are transferred to arrays and screened with serum samples from patients and control subjects. Seroreactivity is detected using a fluorescently labeled anti-human IgG antibody. The arrays are read using a laser confocal scanner and the signal intensity of each protein is quantified. The results are analyzed and normalized using statistical tools. The normalized intensity is shown according to a color scale. The top seroreactive proteins are selected by using a determined cut-off.

proteins were able to differentiate a majority of IFA-positive sera from IFA-negative sera, but the reaction was stronger when sera from patients with endocarditis was used rather than sera from patients with acute Q fever. In the study by Vigil *et al.* [69], 84% of the entire proteome was expressed using a rapid translation system and screened with serum samples from 40 acute Q fever patients and 20 healthy individuals [69]. Only 21 antigens reacted strongly with IgG antibodies from infected *C. burnetii* patients [69]. Of these, 13 were specific to *C. burnetii* and eight cross-reacted with sera from healthy blood donors. As expected, CBU_1910 was the most reactive antigen with high specificity [69]. Among the identified proteins, several had already been identified in other studies and tested in a proof-of-principle diagnostic assay [31,57,58,66,68]. The results from Vigil *et al.* [69] and Beare *et al.* [66] showed similar ranges of reactivity for the best candidate protein biomarkers (CBU_1910, CBU_0891, CBU_1143, CBU_0612, CBU_0545, and CBU_1398).

In addition, several biomarkers were selected using immunoproteomic studies [31,57,68] and were reported to be promising proteins for Q fever serodiagnosis. In a large-scale comprehensive study, only about 1% of the

Table 4. Advantages and limitations of proteomic technologies in clinical microbiology

Technical approach	Advantages	Limitations
MS-based approaches		
Biotyping	• MALDI-TOF MS has several applications in diagnosis and clinical microbiology, including the identification of bacteria and characterization of bacterial proteomes • Selection of protein targets for diagnostic, vaccine or therapeutic development • Applicable to a variety of samples: including bacterial colonies, clinical samples such as blood cultures or urine, and environmental samples • Availability of commercial database (includes 1,660 bacteria isolates and 66 cell types) • Low cost and easy handling compared with classical phenotype-based bacterial identification	• When several bacterial species are present (such as *Streptococcus* spp. or anaerobic Gram-negative bacteria in addition to *C. burnetii*) poor mass-peak signals may result, which may not be distinguished from the signals produced by the culture medium • Limitations in *C. burnetii* handling (such as level 3 biosafety laboratory needed, difficulties of culturing an intracellular bacteria) • Blood culture is slow (with slow-growing bacteria) or negative (for example in cases of blood-culture-negative endocarditis caused by *C. burnetii*) and insufficiently sensitive (for example when the patient has previously received antibiotics)
SDS-PAGE coupled to nano-LC proteome identification	• In general, better coverage and sensitivity when compared with MALDI-TOF MS • Identification of low molecular weight proteins • Suitable for investigations of PTMs	• Requires culture of the pathogen, for *C. burnetii* this involves labor-intensive purification from eukaryotic cells and processing of samples • Labor-intensive, time-consuming • Requires skilled operators • Costly
Immunoproteomics (2-DE coupled to MALDI-TOF)	• Low cost • Resolved proteins contain PTMs that can be visualized on gels (isoforms) • Robust and suitable method for biomarker selection • Can provide an individual profile of reactivity for each patient sample • Can be used with monoclonal antibodies	• Requires the culture and purification of *C. burnetii* in a level 3 biosafety laboratory, and is labor-intensive • Requires large samples • Variable findings can result, depending on culture conditions, strains, technology and operator skills • Differences between 2D stained gels and immunoblots • Limitations with 2-DE in resolving basic membrane proteins, and low and high MW proteins • In general, low genome coverage (5-30% of total predicted ORFs)
Recombinant protein-based approaches		
Protein array	• Does not require the culturing or handling of *C. burnetii* • Miniaturized systems require small amounts of clinical samples (such as 1-2 μl sera) and allows high-throughput screening (>75% of total predicted ORFs) • Low cost, does not require specific operator skills	• *Escherichia coli* system produces proteins without their PTMs (phosphorylations, glycolysations), which are known to be antigenic • Misfolded or multimeric proteins may not be recognized • Requires costly laboratory equipment (fluorescent scanner and spot robot)
ELISA	• Easy to perform, does not require sophisticated technology	• Lower sensitivity than protein array or IPCR

IPCR, immuno-PCR; LC, liquid chromatography; MALDI-TOF MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry; ORF, open-reading frame; PTM, post-translational modification.

whole proteome of *C. burnetii* expressed *in vitro* showed seroreactivity [69]. This proportion of reactive antigens is comparable to that reported by Beare *et al.* [66]. Altogether, the data suggest that a limited number of proteins are involved in the humoral response to *C. burnetii* [66,69].

Recently, protein microarrays were used to evaluate the humoral response to *C. trachomatis* [90,91]. Sera from mice immunized with live and non-viable elementary bodies were screened with 99% of the genomic and plasmid proteins expressed *in vitro* [90]. The results revealed that 185 proteins elicited a strong early and sustained antibody response in mice. Indeed, most of these proteins have already been reported as seroreactive [90,91]. In similar work, 933 genomic- and plasmid-derived recombinant glutathione S-transferase (GST) fusion proteins were tested with sera from 99 women with urogenital infections. Among 27 seroreactive serum samples, 12 proteins had already been reported as having

diagnostic value and a further 15 proteins were newly identified [91]. Both studies narrowed down the number of seroreactive targets, showing that the number of proteins involved in the humoral host response is limited. A miniaturized protein microarray model has been used to investigate both the humoral response against *Burkholderia pseudomallei*, the causative agent of melioidosis (classified among the group B bioterrorism weapons by the CDC [92]), and *B. henselae*, the causative agent of cat scratch disease and infective endocarditis [93]. A few specific and sensitive antigens with diagnostic value are now available for a number of infectious diseases. The diagnostic potential of recombinant proteins might be useful in complementing the usual tests, but is insufficient to replace whole antigen- based serology of Q fever. The major drawback of recombinant proteins, generally expressed using *Escherichia coli*-based systems, is the lack of PTMs, which are of increasing interest for translational and clinical applications.

Conclusions and future directions

The future of diagnostic testing relies upon the development of new technologies, and proteomics is rapidly contributing to this area (Table 4). More sensitive and specific tests for early-stage Q fever detection as well as reliable methods for clinical follow-up of patients are needed.

Proteomics is paving the way for serodiagnosis development by first selecting seroreactive protein candidates and then validating them in recombinant-protein-based screening systems, such as classic ELISAs and large-scale comprehensive protein arrays. To date, however, none of the proteomics-based techniques has been applied for routine diagnosis of Q fever, mainly because the majority of the resulting discoveries are awaiting large-scale validation. Moreover, the equipment and resources available in diagnostic laboratories outside of large hospitals are generally insufficient for proteomic investigations, and the technology remains expensive and time-consuming.

One of the most important challenges in Q fever diagnosis is the detection of *C. burnetii* during the early stage of disease, because asymptomatic seroconversion is observed in only 60% of patients. Optimization of the conditions for obtaining specific MALDI-TOF signatures of *C. burnetii*-infected serum (acute Q fever) will be the first step towards the routine application of this technology. Moreover, in the event of a *C. burnetii* outbreak, MS-based approaches could be useful in strain subtyping, which in turn allows preventive measures and treatments to be used. Strain-specific proteins have been already characterized [19,20,94]. Considering that the immune response to recombinant proteins is limited, their routine use in acute Q fever diagnosis is doubtful. Nevertheless, diagnoses made using recombinant-protein-based microarrays might enhance the discriminatory power of whole antigen-based serology and PCR. This can be particularly useful when searching for serum markers of chronic infection in 'at risk' patients.

The specificity of Q fever serology might also be enhanced by employing monoclonal antibodies raised against *C. burnetii*, and these are available in several laboratories [58,95-101]. Such approaches can be useful for the detection of *C. burnetii* infection using serum samples and immuno-PCR (IPCR) [102,103] when no such infection has been detected by classic whole antigen-based ELISA. Routine clinical applications are still needed for the detection of intracellular pathogens. An immune-MALDI-TOF MS [104] could be another alternative for investigating chronic Q fever samples (such as biopsies of infected organs). Even in this post-genomic era, however, new technologies are not yet able to replace the isolation and culturing of pathogens. The development of *C. burnetii* axenic medium was an enormous breakthrough [22] that has allowed the genetic manipulation of these bacteria. Indeed, a *C. burnetii* genetic mutant lacking the FtsZ protein has been generated [105]. Genetic manipulation of these bacteria will allow several new areas of investigation, including further proteomic studies of the immune response to *C. burnetti*, studies of the effectors of the T4SS, and investigation of the intracellular survival mechanisms of *C. burnetii*. The information garnered in such studies will, in turn, facilitate the development of more specific and sensitive diagnostic assays for Q fever.

Abbreviations

adaA, acute disease antigen A gene; Ank, ankyrin repeat-containing domain; CDC, Centers for Disease Control and Prevention; 2-DE, two-dimensional electrophoresis; ELISA, enzyme-linked immunosorbent assay; FNRC, French National Reference Center; IFA, immunofluorescence assay; IgG, Immunoglobulin G; IgM, Immunoglobulin M; IgA, Immunoglobulin A; IPCR, immuno-PCR; IS, insertion sequence; LCV, large cell variant; LPS, lipopolysaccharide; MS, mass spectrometry; MW, molecular weight; OMP, outer-membrane protein; ORF, open-reading frame; PTM, post-translational modification; SCV, small cell variant; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; T4SS, type IV secretion system; UBT, urea breath test.

Competing interests

The authors declare that they have no competing interests.

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