Coxiella burnetii immunogenic proteins as a basis for new Q fever diagnostic and vaccine development

C. GERLACH[1], Ľ. ŠKULTÉTY[2,3], K. HENNING[1], H. NEUBAUER[1], K. MERTENS[1*]

[1]Friedrich-Loeffler-Institute, Institute of Bacterial Infections and Zoonoses, Naumburger Str. 96a, 07743 Jena, Germany; [2]Institute of Virology, Biomedical Research Center Slovak Academy of Sciences, Bratislava, Slovak Republic; [3]Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic

Summary. – *Coxiella burnetii* is the etiological agent of the zoonosis Q fever, which can cause an acute or a chronic, life-threatening disease in humans. It presents a highly stable cell form, which persists in the environment and is transmitted via contaminated aerosols. Ruminants are considered as the main reservoir for human infections but are usually asymptomatic. Subclinical infection in these animals and the occurrence of serologically negative shedders hamper the identification of infected animals with the currently used diagnostic techniques. This suboptimal sensitivity limits reliable identification of infected animals as well as the well-timed implementation of countermeasures. This review summarizes compounds, focusing on *C. burnetii* seroreactive proteins, which were discovered in recent immunoproteomic studies. We analyzed these proteins regarding their localization, function, frequency of citation, differences seen in various host species as well as sensitivity and specificity. Finally, proteins useful for the development of new diagnostic test systems as well as subunit vaccines were discussed.

Keywords: Coxiella burnetii; proteomic analysis; immunoreactive proteins; specificity; sensitivity; serological diagnostics

Introduction

Coxiella burnetii is the etiological agent of the zoonosis Q fever, which has been reported worldwide. This gram-negative bacterium forms a small cell variant that can be transmitted via contaminated aerosols. The organism is considered as one of the most infectious agents for humans with an $ID_{50} = 1$ (Vigil *et al.,* 2010). It replicates within eukaryotic cells in a progressing phagolysosome-like parasitophorous vacuole at acidic pH 5 (Voth and Heinzen, 2007). Because *C. burnetii* is highly stable and can remain infectious in the environment for an extended period of time, it was classified by the Centers for Disease Control, USA as a category B bioterrorism agent (Vigil *et al.,* 2010).

In humans, *C. burnetii* presents in 40% of cases as an acute and often self-limiting, febrile illness with severe headaches, fever or pneumonia. In 1–5% of primary infections, chronic Q fever may develop, which can be life-threatening and often presents as endocarditis (Maurin and Raoult, 1999; Landais *et al.*, 2007; Kampschreur *et al.*, 2014). In the EU countries, 648 and 833 human Q fever cases were reported in 2013 and 2015, respectively (ECDC/EFSA report, 2015 and 2016). However, during a massive epidemic that occurred in the Netherlands between 2007 and 2010, over 4000 acute and 284 chronic (mortality rate of 19%) human cases were recognized (Kampschreur *et al.*, 2010, 2014). These numbers illustrate that the infection has a major public health impact and it can re-emerge anytime from the endemic state into an outbreak of an unexpected dimension.

Ruminants are considered as the main reservoir for Q fever in men. In these animals, an infection is usually asymptomatic or may manifest as late term abortions or weak offspring. *C. burnetii* is massively shed in birth or abortion products, but also in milk, feces, and urine (Arricau-Bouvery *et al.*, 2003; Rodolakis *et al.*, 2007; Rousset *et al.*, 2009). Due

to the subclinical infection and unnoticed shedding that can be continuous or intermittent (Barlow *et al.*, 2008; Boarbi *et al.*, 2014), identification of the source of infection is usually problematic. Furthermore, the veterinary Q fever serodiagnostics have limited sensitivity. It was demonstrated to be 86% for milk samples and 84% for blood samples from cattle (Paul *et al.*, 2013), or as low as 58% for milk samples from goats (Hogerwerf *et al.*, 2014). Thus, it is not surprising that independent studies described the occurrence of serologically negative shedders. Ruminants with normal parturition can shed the bacterium via the milk or vaginal mucus even while specific antibodies are not detectable (Rousset *et al.*, 2009; Bottcher *et al.*, 2011; Niemczuk *et al.*, 2014; Bauer *et al.*, 2016). It was shown that at least 24% of these seronegative aborting and non-aborting goats shed the microorganism (Rousset *et al.*, 2009).

Like members of *Enterobacteriaceae, C. burnetii* exhibits a lipopolysaccharide (LPS) phase variation. Virulent phase I bacterium expresses a full-length, smooth LPS, whereas avirulent phase II exhibits a severely truncated, rough LPS, that develops after frequent passaging in immunoincompetent hosts (Raoult and Parola, 2007). For routine veterinary diagnosis IgG-based Q fever ELISA kits, containing mixed corpuscular antigens of *C. burnetii* phase I and II, are employed. These antigens are not well defined and might not be accessible to antibodies in the test systems. Antigenic variation among *C. burnetii* isolates may also contribute to suboptimal test sensitivity (Beare *et al.*, 2009). It was demonstrated by comparison of the commercial with experimental phase-specific ELISAs that 45% of sera are only phase II positive but phase I negative. Thus, these sera cannot be diagnosed as Q fever positive by commercial ELISA kit (Bottcher *et al.*, 2011).

The whole cell antigens may contain many conserved proteins, which can impair specificity through cross-reactions with other bacterial pathogens, such as *Bartonella* spp., *Legionella* spp. and *Chlamydia* spp. (La Scola and Raoult, 1996; Musso and Raoult, 1997; Lukacova *et al.*, 1999). This finding might relay to a comprehensive study of the large Dutch outbreak, which has shown only 52% specificity of a commercial ELISA (Hogerwerf *et al.*, 2014). A further drawback of currently used serodiagnostics is the inability to differentiate between recent and past infections or between infected and vaccinated animals (Horigan *et al.*, 2011). Therefore, efforts have to be made to improve uniformity, sensitivity, and specificity of the diagnostic kits by replacement of the currently used corpuscular whole cell antigens with well-defined antigens like immunogenic proteins. These compounds might be also beneficial for the development of a subunit vaccine (Arricau-Bouvery *et al.*, 2005; O'Neill *et al.*, 2014).

This review gives an overview of *C. burnetii* seroreactive proteins described in recent immunoproteomic studies. We analyzed the identified proteins regarding their localization,

function, the frequency of detection, differences seen in various host species as well as sensitivity and specificity. Finally, we discussed proteins which might be useful in diagnostic or subunit vaccine development.

Selection of immunoproteomic publications

The comprehensive literature search was performed using PubMed (https://www.ncbi.nlm.nih.gov/pubmed) as the main source of studies on *C. burnetii.* Nineteen articles focussing on the identification of immunoreactive proteins which were published since 2004 were analyzed. These publications are listed in Table 1 together with their basic characteristics, like the method of identification, species involved, and number of sera evaluated. The publications comprise eleven and four investigations, solely based on human and mice sera, respectively, and three studies that analyzed human and mice sera in parallel using the same techniques. Thus, fourteen articles investigated the reactivity of human, seven mice, and one guinea pig sera. Interestingly, one of the selected studies employed two different experimental procedures for evaluating the same human sera (Xiong *et al.*, 2012a). On the other hand, we excluded a publication describing a frequently published antigen which was recognized by sera from experimentally and naturally infected goats, since it did not report a screening method (Fernandes *et al.*, 2009).

Most of the studies used 2-dimensional gel electrophoresis followed by Western blotting for identification of reactive proteins. Other frequent methods are protein microarrays comprising up to 2000 *C. burnetii* open reading frames (ORFs) and ELISA. The latter is also used to validate results from experimental screening and to determine sensitivity and specificity of particular antigens. Western blot-like immunostrips have also been used to validate the obtained results (Vigil *et al.*, 2010). Interestingly, a unique procedure based on immunocapturing of antigens on biofunctionalized magnetic microspheres using polyclonal antibodies was presented by Flores-Ramirez *et al.* (2016). In most of these articles total protein extracts were analyzed, but few studied bacterial protein fractions enriched for outer membrane proteins (Papadioti *et al.*, 2011; Jiao *et al.*, 2014). These outer membrane proteins were found to be advantageous for the development of new vaccines due to their exposition to host immune cells (Hotta *et al.*, 2004; Papadioti *et al.*, 2011).

Description of the identified immunoreactive proteins

In the 19 selected publications 169 immunoreactive proteins were described (Supplementary Table 1). At first, the cellular localization and function of these proteins were

Table 1. Selected *C. burnetii* immunoproteomic publications with the method of identification, host species and number of sera used

No. of study	Publications	Method of identification	Host species	Number of sera
(1)	**Chao *et al.*, 2005**	2D-GE and IB of *C. burnetii* Henzerling strain phase I and II	**Human**	n.s., IFA-positive for phase I and II
(2)	**Coleman *et al*, 2007**	2D-GE and IB of *C. burnetii* NM Crazy RSA 514 SCVs and LCVs	**Human**	2 convalescent-phase sera (recovered from acute Q fever)
(3)	**Beare *et al.*, 2008**	Microarrays with 1491 *C. burnetii* RSA 493 ORFs, ELISA for validation of Sp. and Se.	**Human**	55 acute, 5 chronic Q fever, 32 naïve samples
(4)	**Chen *et al.*, 2009**	ELISA with selected *C. burnetii* proteins	**Human**	55 acute, 5 chronic Q fever, 32 naïve samples
		T cell antigen analyses: ELISpot of the same proteins	**Mouse** (C57BL/6), normal and HLA DR4 transgenic, vaccinated with RSA 493	n.s.
(5)	**Sekeyova *et al.*, 2009**	2D-GE and IB of *C. burnetii* RSA 493 strain	**Human**	7 Q fever endocarditis, 5 acute Q fever, 3 naïve samples
(6)	**Sekeyova *et al.*, 2010**	2D-GE and IB of *C. burnetii* RSA 493, ELISA with identified recombinant proteins	**Human**	16 acute Q fever, 18 Q fever endocarditis, 14 naïve samples
(7)	**Vigil *et al.*, 2010**	Protein microarray with 1901 *C. burnetii* RSA 493 ORFs and Western-blot-like immunostrips	**Human**	40 acute Q fever, 20 naïve samples
(8)	**Vigil *et al.*, 2011**	Protein microarray with 2000 *C. burnetii* RSA 493 ORFs	**Human**	25 acute Q fever samples
(9)	**Papadioti *et al.*, 2011**	2D-GE and IB of sarcosyl-insoluble fraction (enriched in outer membrane proteins) of *C. burnetii* RSA 493 and CbuG_Q212 phase II	**Human**	1 chronic Q fever sample
(10)	**Kowalczewska *et al.*, 2012**	ELISA with 15 published (Sekeyova *et al.*, 2009) recombinant proteins	**Human**	16/26 acute Q fever, 18/27 Q fever endocarditis for prescreening/second assay, 14 naïve samples
(11)	**Flores-Ramirez *et al.*, 2016**	Immunocapturing of antigens by bio-functionalized magnetic microspheres (immobilized polyclonal antibodies)	**Human**	4 Q fever, 1 naïve sample
(12)	**Zhang *et al.*, 2004a**	1- and 2D-GE and IB with *C. burnetii* NMI (RSA 493) cell antigen or recombinant proteins	**Mouse** (BALB/c): Immunization with *C. burnetii* NMI: early or late sera = 2 or 5 weeks p.i.	4 per infection dose and incubation time
(13)	**Chen *et al.*, 2011**	ELISA with recombinant proteins; T cell epitope analyses: ELISpot, H-2 I-A[b] peptide binding assays, challenge experiments	**Mouse** (C57BL/6): Immunization with *C. burnetii* NMI whole cell vaccine or identified proteins	ELISA: n.s., T cell epitope analyses: 6 per protein and 5 per peptide
(14)	**Xiong *et al.*, 2014**	T cell epitope analyses: *in silico* prediction of H2 I-A[b] -affine peptides from known *C. burnetii* immunodominant proteins, ELISpot, challenge experiments	**Mouse** (C57BL/6): Immunization with peptides	15 for each peptide or peptide pool
(15)	**Xiong *et al.*, 2016**	T cell epitope analyses: in silico predictions of CD8+ T-cell epitopes from translocated T4SS substrates, ELISPOT, Immunization with recombinant *Listeria monocytogenes* vaccines and challenge experiments	**Mouse** (C57BL/6): Immunization with *C. burnetii* NMI or peptides (recombinant *L. monocytogenes* as vector), vaccination with whole cell vaccine	5 per group for immunization or vaccination, 6 per group for challenge experiment
(16)	**Xiong *et al.*, 2012**	2D-GE and IB of *C. burnetii* Xinquao strain	**Mouse** (BALB/c): Immunization with *C. burnetii* Xinquao strain	Pool of 8 per incubation time
			Human	2 late acute Q fever samples
		Microarray and IB with strongest immunoreactive, recombinant proteins from 2D-GE	**Human**	56 acute Q fever; 25 naïve samples; 10 for each rickettsial spotted fever, *Legionella pneumonia* and streptococcal pneumonia. Marked proteins were recognized by acute late Q fever sera

Table 1 (continued)

No. of study	Publications	Method of identification	Host species	Number of sera
(17)	**Wang *et al.*, 2013**	Micoarray with 101 *C. burnetii* RSA 493 proteins implicated in virulence-related functions	**Mouse** (BALB/c): Immunization with *C. burnetii* Xinquao strain	Pool of 10
			Human	10 early, 20 lage state, 7 convalescent, 9 chronic, 14 past Q fever samples. Marked proteins were recognized by late stage acute Q fever sera
(18)	**Jiao *et al.*, 2014**	2D-GE of surface exposed *C. burnetii* Xinqiao proteins, bioinformatic selection; Microarray with recombinant proteins	**Mouse:** Immunization with Xinquao strain	10 for *C. burnetii*, 10 for *Rickettsia rickettsii*, 10 for *R. heilongjiangensis*, 10 for *R. typhi*, 10 naïve samples
			Human	9 patients with IgG IFA titres > 1:800 and 1:400 antigen phase I and II, 10 from brucellosis and *Mycoplasma pneumonia*, 10 naïve sera
(19)	**Deringer *et al.*, 2011**	2D-GE and IB with whole-cell *C. burnetii* NMI and NMII protein extracts	**Guinea pig:** Immunization with killed *C. burnetii* NMI whole-cell vaccine	Pool of 3 sera, also for negative control

n.s. = not stated, NM = Nine Mile, Se. = sensitivity, Sp. = specificity.

analyzed. Based on gene annotations and *in silico* predictions presented in the respective publication we concluded, that only 43% of all identified proteins have a known or predicted localization (Fig. 1). Because some authors have suggested that surface exposition might increase the chance for recognition by immune cells (Hotta *et al.*, 2004; Papadioti *et al.*, 2011), we assumed that outer membrane proteins would dominate. Surprisingly, the majority of the identified proteins are located in the cytoplasm (27%), followed by proteins associated with the inner (8%) or outer membrane (7%). Only
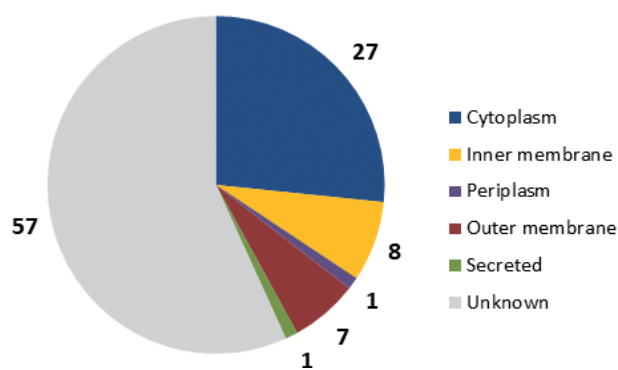

Fig. 1

Localization of identified *C. burnetii* antigens based on annotations in the respective publication

The functions of 43% of all identified proteins are stated in the publications as either known or predicted by defined algorithms, such as PSORTb 3.0.2 or SOSUI-GramN (Xiong *et al.*, 2014; Jiao *et al.*, 2014; Flores-Ramirez *et al.*, 2016). The remaining 57% are classified as proteins with unknown localization, or it was not stated.

one periplasmatic protein (1%) and one secreted protein (1%) were noted in the selected publications. Also, Jiao *et al.* (2014) who analyzed protein fractions enriched for surface-exposed antigens have revealed various cytoplasmic bacterial proteins. Subsequently, they concluded that besides technical reasons the identified cytoplasmic proteins might reside on the surface of *C. burnetii* and contribute to bacterium–host interactions. This observation was already described for other bacteria. For instance, the cytoplasmic aminopeptidase of *Rickettsiae* was detected in the outer membrane fraction of *Anaplasma marginale* (Santhanagopalan *et al.*, 2006), and the cytoplasmic disulfide oxidoreductase was present on the surface of *Ehrlichia chaffeensis* (McBride *et al.*, 2002). In *Staphylococcus aureus,* the cytoplasmic ATP-synthase F1 α and β subunits are located in the cell envelope (Gatlin *et al.*, 2006). Thus, we can legitimately speculate that the cytoplasmic seroreactive *C. burnetii* proteins may perform some additional moonlighting activities on the bacterial surface next to their cytoplasmic functions.

The annotated functions, which were available for approximately 50% of the identified proteins, were also evaluated (Fig. 2). The known or predicted roles of the proteins are evenly distributed among twelve categories with a slight preference for proteins involved in general metabolic pathways, such as energy production and conversion (13.6%). The second frequent class comprises enzymes involved in gene expression processes - transcription, translation and ribosomal structures (9.5%), followed by substrates (7.0%) of the type IV secretion system (T4SS). This observation underlines the hypothesis of Xiong *et al.* (2016) who noticed, that T4SS substrates are probable targets for the immune
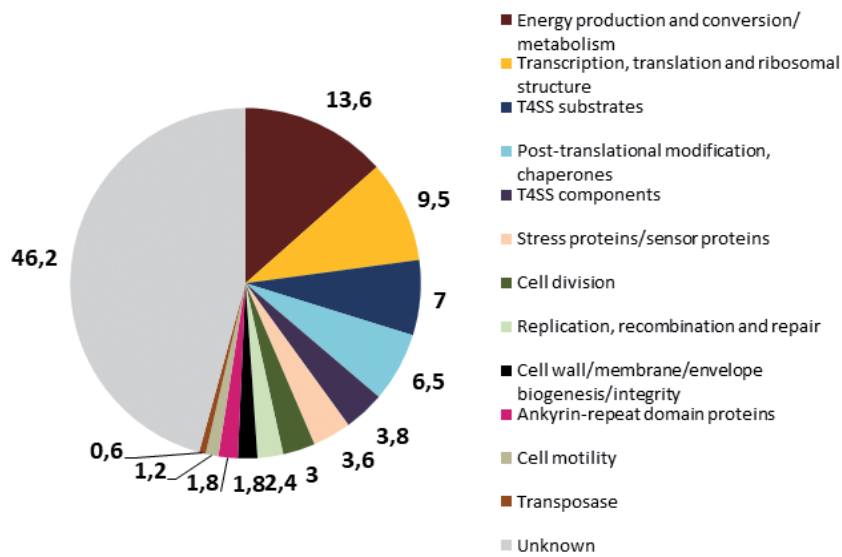
Fig. 2

Function of identified *C. burnetii* antigens based on annotations in the respective publication

The functions of more than half of all identified proteins are stated within the publications. Most were derived from COG (Jiao *et al.*, 2014) or UniProt databases (Flores-Ramirez *et al.*, 2016) as marked in Table 2. The remaining proteins are classified as proteins with unknown functions or functions were not stated.

system due to the cytosolic localization within the host and consequently have a high potential for major histocompatibility complex (MHC) l presentation to activate cytotoxic (CD8+) T cells. These T cells serve for an effective elimination of cells infected with intracellular bacteria even if they reside in phagosomes as it was described for *Salmonella typhimurium* and *Mycobacterium tuberculosis* (Harty and Bevan, 1999). Proteins that have chaperone activity or mediate post-translational modifications also tend to be immunogenic (6.5% of the reviewed proteins). Less represented groups of proteins can be related to the T4SS apparatus, sensor activity and stress response, cell division, DNA recombination and repair, cell wall biogenesis and integrity or ankyrin repeat domains, motility and DNA transposition.

The majority of immunogenic proteins seems to have housekeeping functions such as metabolism, gene expression, protein synthesis and DNA replication. Therefore, we speculate that these proteins might be the most presented antigens to immune cells due to high abundance during activated metabolism. This assumption is in good agreement with the result of Xiong *et al.* (2012a) who evaluated potential serodiagnostic markers for Q fever and identified 13 proteins with housekeeping function out of 20 antigenic proteins. They discovered that proportionally more of these proteins are recognized by the sera from patients with acute rather than with chronic or persistent infections. Apparently, the persistence of *C. burnetii* in patients with chronic Q fever is associated with unresponsiveness of lymphocytes due to

a lack of macrophage activation (Koster *et al.*, 1985; Stein *et al.*, 2000).

Comparison of antigenic profiles between the replicative large cell variants (LCVs) and metabolically dormant SCVs showed that the immunogenic proteins CBU1718, CBU0236, CBU0867, CBU1433, CBU0528, CBU0963, CBU1385, CBU0737 and CBU1416 are more abundant in LCVs (Ihnatko *et al.*, 2012; Papadioti *et al.*, 2012). Noticeably, eight of the nine antigens are involved in transcription, translation, chaperone or protein secretion activities. These findings confirm that the metabolically active LCVs are expressing housekeeping proteins that seem to be recognized by the immune cells.

Interestingly, among the identified immunoreactive proteins many virulence associated factors were described. The CBU0630 may play a role in survival and multiplication within the host cell, and it might be essential for cell entry (Ihnatko *et al.*, 2012). Similarly, CBU1260 (OmpA) was shown to be involved in invasion as its mutation strongly inhibits *C. burnetii* internalization and replication within host cells (Martinez *et al.*, 2014). Furthermore, CBU0612 (OmpH) is probably a major factor for adhesion to host cells (Sekeyova *et al.*, 2009) and the CBU0630 (MIP) is associated with macrophage infectivity (Flores-Ramirez *et al.*, 2016). In addition, CBU1967 and CBU1697 are classified (PATRIC, https://www.patricbrc.org) as multidrug resistance transporters of the Bcr/CflA family. Ankyrin-repeat domain proteins also play a role in the virulence mechanisms of

C. burnetii. They mimic eukaryotic proteins and modulate host cell processes including cell survival, signaling and vesicular trafficking (Brüggemann *et al.*, 2006). It was suggested that they are candidate T4SS substrates, which are secreted into the host cytosol (Voth and Heinzen, 2007; Voth *et al.*, 2009). Interestingly, five immunogenic proteins have also an antioxidant function (CBU0963, CBU1278, CBU1477, CBU1706, CBU1708), which may be crucial for survival within the acidic vacuole, the intracellular niche of *C. burnetii*.

Frequency of identified *C. burnetii* immunogens

Described seroreactive proteins vary drastically in their frequency of identification. Fig. 3a illustrates how many proteins have been identified in one, two or more immunoproteomic studies. Surprisingly, only a few proteins were found in more than two publications, indicating that there is no uniform pattern of antigens, even within individuals of the same host species. Most of the 169 *C. burnetii* immunodominant proteins (Supplementary Table 1) identified by screening with human, mouse, and guinea pig sera were published in only one or two studies (83%). However, 20 identified proteins were found frequently, in at least four or more publications. These proteins are listed in Table 2 with their characteristic features, including the strength of reactivity with selected sera, as well as sensitivity and specificity for detection of *C. burnetii* positive sera. According to the presented data, only five proteins (3%) were identified in at least half of the 19 publications (Table 1 and 2). The strongest immunodominant proteins are CBU0612 (OmpH), CBU0092 (YbgF), CBU0236 (Tuf-2), CBU1718 (GroEL), and

CBU1910 (Com1) which were mentioned in 9, 10, 11, 16, and 17 publications, respectively.

The differences in the immune system among the three analyzed host species are eventually responsible for the heterogeneity of antigen detection. Thus, we can assume, that the number of immunogenic proteins recognized more frequently will be higher if only one host species is analyzed. However, investigation of 14 publications that studied human sera (Table 1) has shown a similar frequency of antigen identification as mentioned above (Fig. 3b). From 132 proteins that react with human sera, 86% are found only in one or two studies. Similarly, approximately 2% are found in at least half of the 14 selected publications. CBU0236 (Tuf-2) was mentioned in eight, CBU1910 (Com1) in nine, and CBU1718 (GroEL) in ten articles. The seven publications using mice sera from two strains of genetically identical mice have also resulted in the identification of proteins that are rarely present in more than two studies (Fig. 3c). From the total of 64 immunogenic proteins recognized by mouse sera, 88% were identified only in one study, while 2% were present in more than a half of the selected publications. Repeatedly, CBU1910 (Com1) and CBU1718 (GroEL) were most frequently found. These antigens were also targeted in the guinea pig (Deringer *et al.*, 2011) and cattle studies (Vigil *et al.*, 2010).

Kowalczewska *et al.* (2011) described these immunodominant proteins as the most versatile markers of Q fever. Wang *et al.* (2013) proposed that CBU1718 is an excellent molecular marker for serodiagnosis of both, acute and chronic Q fever. Furthermore, CBU1910 was suggested to be a key antigen, which may induce protective immunity (Xiong *et al.*, 2012b). The next most frequently described *C. burnetii* antigen, CBU0236 (Tuf-2) is a candidate marker of acute
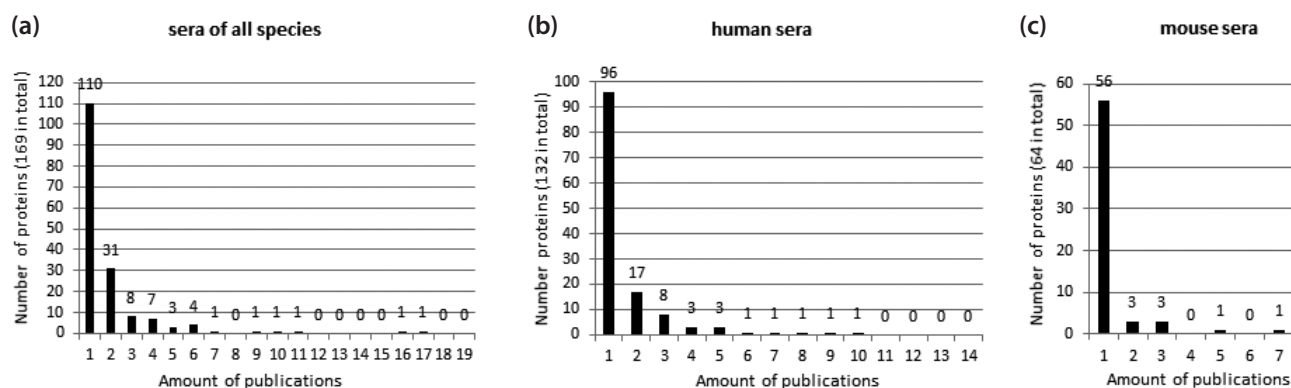

Fig. 3

Citation frequency of identified *C. burnetii* immunogenic proteins
For each protein the identifications listed in Table 1 and Supplementary Table 1 were counted either for all 169 proteins (**a**), 132 proteins reactive with human sera (**b**) or 64 proteins reactive with mouse sera (**c**).

Q fever (Kowalczewska *et al.*, 2011). Similarly, CBU0092 (YbgF) was proposed to be a phase II specific marker that can be employed for early diagnosis of acute infection (Kowalczewska *et al.*, 2012). It was also supposed as essential for protective immunity (Vigil *et al.*, 2011). CBU0612 (OmpH) is also a promising candidate marker for acute and chronic Q fever (Kowalczewska *et al.*, 2011). It may be important for adhesion to host cells (Sekeyova *et al.*, 2009) and for induction of a protective immunity (Vigil *et al.*, 2011).

Despite extensive genomic plasticity and diversity among potential effector proteins described by Beare *et al.* (2009), sequence analyses of all 169 identified immunogenic proteins showed identical protein sequences between all published strains (data not shown). Thus, the presence of strain-specific antigens of *C. burnetii* can probably be excluded, despite the possibility of posttranslational modifications. It rather seems that the bacterium lacks active antigenic proteins which may induce a conserved immune response or produce molecules that can inhibit it. Indeed, Shannon *et al.* (2005) have identified significant differences in human dendritic cell (DC) activation between *C. burnetii* phase I and II. Infection with the virulent phase I cells did not induce activation of DC in contrast to infection with the avirulent phase II. The latter has resulted in 10-fold higher IL-12 and tumor necrosis factor (TNF) production. Thus, the authors proposed that full-length LPS of phase I may mask the toll-like receptor (TLR) ligands from innate immune cells allowing *C. burnetii* replication without inflammatory response in immune competent hosts (Shannon *et al.*, 2005). The bacterium directly limits inflammasome activation (Cunha *et al.*, 2015) and proinflammatory response of primary bovine macrophages by inhibition of translation and release of IL-1β. *C. burnetii* also restricts stimulation of the increased expression of the activation markers CD40, CD80 and CD86, and MHC molecules in these cells (Sobotta *et al.*, 2016). Besides inhibition of the inflammatory response, *C. burnetii* prevents host cell apoptosis (Voth *et al.*, 2007; Lührmann and Roy, 2007; Klingenbeck *et al.*, 2013; Eckart *et al.*, 2014). This mechanism ensures survival within host cells and leads to a restriction in antigen uptake or presentation of bacterial proteins to surrounding innate immune cells. Thus, *C. burnetii* is a highly specialized organism which can subvert host cell functions by prevention of TLR recognition, inhibition of apoptosis and inflammation as well as modulation of diverse vesicle traffic pathways (Cunha *et al.*, 2015). We can conclude that these evasion strategies probably account for the inconsistent seroreactivity of most identified *C. burnetii* antigens as it is evident from the huge fraction of proteins found once.
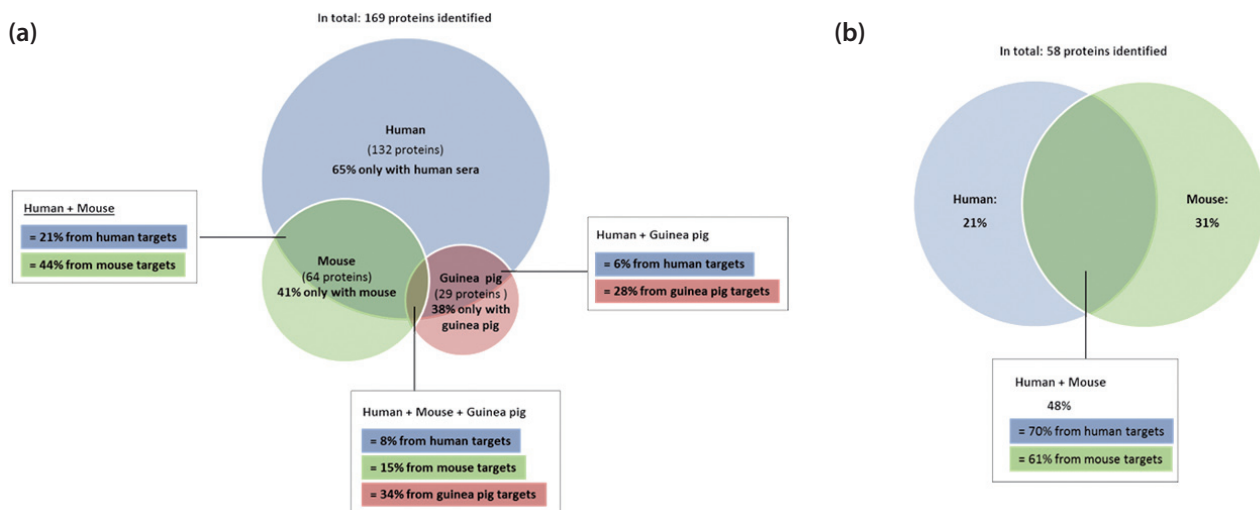

Fig. 4

Distribution of *C. burnetii* immunogenic proteins between species-specific and common antigens
(a) The Venn diagram comprises all immunogenic proteins identified in studies 1 to 19 listed in Table 1. For each species, varying numbers of proteins were identified, illustrated by the different circle sizes: 132 proteins are seroreactive in humans versus 64 and 29 seroreactive proteins in mice and guinea pigs. The ratios are related to the total number of found proteins in the respective host species. The ratios for proteins that are recognized by two or all three host species (illustrated by shared areas) are shown for each host species. The numbers in brackets indicate absolute protein numbers. **(b)** The Venn diagram comprises immunogenic proteins identified in publications 16, 17 and 18 as listed in Table 1, that analyzed human and mouse sera in parallel with the same techniques (Xiong *et al.*, 2012a; Wang *et al.*, 2013; Jiao *et al.*, 2016). In total 58 proteins were identified and the ratios for antigens found exclusively in humans, mice or both were averaged. Bold ratios are related to all proteins from the three studies and the others refer to the total number in the respective host species.

Host species-specific and common *C. burnetii* antigens

The distribution of all identified immunogenic proteins among the host species is demonstrated in Fig. 4a. The number of determined reactive proteins varies drastically from 132 that were recognized by human sera versus 62 and 29 with mice and guinea pig sera, respectively. Due to these discrepancies, the ratios of antigens found either solely in one, two or all three species are related to the total number of identified proteins in the respective species. The majority (65%) of immunogenic proteins identified with human sera is found only in human sera, while 21% and 6% are also recognized by mouse or guinea pig sera, respectively. Only 8% are shared by all three host species. Thus, most of *C. burnetii* antigens recognized by humans are apparently not common with antigens causing a response in mice or guinea pigs. The species-specific antigenic response may arise from differences in immune systems as these vary significantly in humans and mice (Mestas and Hughes, 2004). Different infection stages, as well as, individual variabilities may also contribute to differences in antibody generation.

A more precise conclusion about the portion of host species-specific anti-*C. burnetii* antigen responses can be drawn from those three publications, which analyzed human and mouse sera in parallel using the same techniques (Xiong *et al.*, 2012a; Wang *et al.*, 2013; Jiao *et al.*, 2014; Ref. 16–18, Table 1). In this case, the ratios can be related to the total number of proteins (Fig. 4b). The 58 identified immunoreactive proteins were used for calculating the ratios of proteins exclusively found in human, mouse or the both sera. Approximately half (48%) of the total proteins found are recognized by human and mouse sera, particularly 66% in the publication of Xiong *et al.* (2012), 44% in Wang *et al.* (2013) and 41% in Jiao *et al.* (2014). These common antigens represent very promising immune targets for sensitive Q fever detection in various hosts, including humans and ruminants.

Sensitivity and specificity of identified immunogenic proteins

In some of the selected immunoproteomic studies, sensitivity and specificity of ELISAs based on seroreactive proteins were analyzed (Beare *et al.*, 2008; Chen *et al.*, 2009; Sekeyova *et al.*, 2010) or protein microarrays (Xiong *et al.*, 2012a). Sensitivity ranged from 40% to 60%. However, there are some proteins which are connected with very low sensitivity (8.3% for CBU1628 and 11.6% for CBU1627) (Beare *et al.*, 2008). This is in agreement with the rare presence in the 19 selected studies (Supplementary Table 1). On the other hand, there are also some proteins which are connected to very

high sensitivity such as CBU1718 (GroEL) with 88% (Xiong *et al.*, 2012a) and CBU0092 (YbgF) with 72% (Sekeyova *et al.*, 2010). These exceptionally high values correlate with the frequency of citations as they belong to the most often identified proteins (Table 2).

Regarding the specificity of tests, some of the immunodominant proteins including the most frequently published antigens, like CBU1718 (GroEL), CBU0236 (Tuf-2), CBU1398 (SucB), CBU1290 (DnaK) and CBU0235 (FusA) are highly conserved among bacteria species (Ihnatko *et al.*, 2012). This correlates with their housekeeping functions (Table 2). Also, CBU0612 (OmpH), which belongs to the most often identified antigens is described as widely distributed in various bacteria (Sekeyova *et al.*, 2009). Majority of the most immunogenic *C. burnetii* antigens tend to be conserved proteins and are surely also immune targets in other bacterial species. This makes their application for detection of *C. burnetii*-specific epitopes difficult. For example, rabbit *C. burnetii* phase II polyclonal antisera reacted with recombinant *Bartonella* CBU1398 (SucB), a highly conserved enzyme of the TCA cycle (Gilmore *et al.*, 2003).

Immunoproteomic studies that compared reactivity of single proteins with sera from Q fever patients and other infections revealed significant differences. Xiong *et al.* (2012a) analyzed the major reactive proteins in humans, CBU1718 (GroEL), CBU0092 (YbgF), CBU0229 (RipL), CBU0630 (Mip), CBU0612 (OmpH), CBU1910 (Com1) and CBU1290 (DnaK) with sera of patients with rickettsial spotted fever, streptococcal pneumonia or *Legionella pneumonia*. The proteins showed moderate cross-reactivity with Q fever patient sera. Thus, a combination of antigens was suggested to enhance sensitivity and specificity of detection (Xiong *et al.*, 2012a). In another study eight from 16 surface-exposed *C. burnetii* antigens (CBU0067, CBU0227, CBU0630, CBU1078, CBU1290, CBU1385, CBU1594 and CBU1706) reacted significantly higher with Q fever sera from mice than with sera of mice infected *with Rickettsia rickettsia, R. heilongjiangensis* or *R. typhi* (Jiao *et al.*, 2014). These results make particular seroreactive proteins promising molecules for Q fever detection in humans without significant cross-reactions from related bacteria and pathogens causing similar symptoms.

Regarding the cross-reactivity with naïve sera, one study shows that most identified proteins (13 of 21 proteins) were Q fever-specific: CBU1910, CBU0891, CBU0109, CBU1143, CBU0612, CBU0092, CBU0545, CBU1398, CBU0630, CBU1513, CBU1719, CBU0229 and CBU0653 (Vigil *et al.*, 2010). Nearly half of them belong to the most frequently reactive antigens confirming their diagnostic potential.

Thus, the mentioned publications demonstrate the suitability of many identified immunogenic proteins for specific detection of Q fever.

Table 2. Most frequently identified *C. burnetii* immunogenic proteins sorted by the total frequency of citations

CBU gene kDa, pI	Localization	Protein function (PATRIC, unless otherwise specified)	Identified in (reference No.):						Total No.
			Human: 14 publications		Mouse: 7 publications		Guinea pig: 1 publication		
			Reacted with	No.	Reacted with	No.	Reacted with	No.	
1910 *com1* 27.6, 9.1	U./OM (14)	Outer membrane protein Com1, post-translational modification, protein turnover, chaperones* (18)	(1), (3): Sp. 90%, Se. 50%, (5), (6): total sera: Sp. 71%, Se. 47%, endocarditis: Sp. 71%, Se. 55%, acute: Sp. 71%, Se. 37.5%, (7): most reactive, (8), (11), (16): Se. 52%, (18): reacted with 6 of 9 sera	9	(12): early and late sera, (13): most potent, 2 T cell epitopes, (14 and 15): each 1 T cell epitope, (16), (17), (18): cross-reacted with *Rickettsia spp.*	7	(19): good reactivity for both phases	1	17
1718 *groEL* 58.3, 5.1	C (14)	Heat shock protein 60 family chaperone GroEL, HspB, protein folding, adhesion# (11), post-translational modification, protein turnover, chaperones* (18)	(1), (2): reacted with 2 of 2 sera, (5), (8), (9), (11), (16): reacted with 2 of 2 sera, (16): Se. 88%, (17): 85% of late stage acute Q fever sera and 67% of chronic sera (18): highest (reacted with 7 of 9 sera)	10	(14) and (15): each 1 T cell epitope, (16), (17), (18): cross-reacted with *Rickettsia spp.*	5	(19): weak reactivity for both phases	1	16
0236 *tuf-2* 43.6, 5.3	C (11)	Translation elongation factor Tu, protein synthesis# (11), translation, ribosomal structure and biogenesis* (18)	(1), (2): reacted with 1 of 2 sera, (5), (6), (9), (11), (16): reacted with 2 of 2 sera, (18): reacted with 5 of 9 sera	8	(16), (18): cross-reacted with *Rickettsia spp.*	2	(19): good reactivity for both phases	1	11
0092 *ybgF* 34.3, 6.5	U./OM (14)	Cell division coordinator CpoB, tol-pal system protein YbgF (15), function U.* (18)	(5), (7), (8), (10): best marker for acute sera, (16): reacted with 1 of 2 sera, (16): Se. 72%	6	(14): 1 T cell epitope, (15): 2 T cell epitopes, (16)	3	(19): good reactivity for phase I	1	10
0612 *ompH* 18.8, 9.5	PP/OM (14)	Outer membrane chaperone Skp (OmpH) precursor, may be important for adhesion to host cells (5), cell wall/ membrane/ envelope biogenesis* (18)	(3): strong, Sp. 81.2%, Se. 51.6%, (4): Sp. 81.2%, Se. 51.6%, T cells recognize human, not murine MHC, (5): only for endocarditis sera, (7), (8), (9), (16): Se. 48%	7	(14): 1 T cell epitope, (16)	2			9
1398 *sucB* 46.0, 5.4	C (11)	Dihydrolipoamide succinyltransferase component (E2) of 2-oxoglutarate dehydrogenase complex, TCA cycle, lysin degradation# (11), energy production and conversion* (18)	(3): strong, (7), (11), (16): reacted with 1 of 2 sera	4	(12): late sera, (16)	2	(19): good reactivity for both phases	1	7
0952 *adaA* 25.9, 9.3	OM (18)	Hypothetical protein, acute disease antigen (2), function U.* (18)	(2): reacted with 1 of 2 sera, (4): Sp. 100%, Se. 25%, no human or murine T cell recognition, (5), (16): reacted with 1 of 2 sera, (18): reacted with 7 of 9 sera	5	(18): cross-reacted with *Rickettsia* spp.	1			6
0937 51.4, 9.0	OM (11)	Hypothetical protein, type IV secretion system# (11)	(5), (6): total sera: Sp. 93%, Se. 38%, endocarditis: Sp. 93%, Se. 39%, acute: Sp. 93%,Se. 37.5%, (9), (10), (11)	5			(19): good reactivity for phase II	1	6

Table 2 (continued)

CBU gene kDa, pI	Localization	Protein function (PATRIC, unless otherwise specified)	Identified in (reference No.):						Total No.
			Human: 14 publications		Mouse: 7 publications		Guinea pig: 1 publication		
			Reacted with	No.	Reacted with	No.	Reacted with	No.	
1290 *dnak* 70.8, 5.1	C (11)	Chaperone protein DnaK, protein folding, plasminogen activity[#] (11), post-translational modification, protein turnover, chaperones* (18)	**(5)**, **(11)**, **(16)**: reacted with 2 of 2 sera, **(16)**: Se. 48%	4	**(18)**: significantly higher than with *Rickettsia* spp.	1	**(19)**: good reactivity for both phases	1	6
0630 *mip* 26.0, 10.2	OM/C (11)	Hypothetical protein, Peptidyl-prolyl cis-trans isomerase Mip, macrophage infectivity, adhesin (11), post-translational modification, protein turnover, chaperones* (18)	**(7)**, **(16)**: Se. 60%, **(18)**: reacted with 5 of 9 sera	3	**(14)**: 1 T cell epitope, **(16)**, **(18)**: strongest, significantly higher than with *Rickettsia* spp.	3			6
0307 24.9, 9.9	OM (14)	Hypothetical protein, OmpA-like protein, cell envelope integrity[#] (11)	**(3)**: strong, **(5)**, **(11)**	3	**(14)**: 1 T cell epitope	1	**(19)**: co-identified with a protein in both phases	1	5
0311 26.8, 8.4	OM (11)	Hypothetical protein, outer membrane protein P1, porin (11), function U.* (18)	**(4)**: Sp. 78.1%, Se. 43.3%, T cells recognize murine and human MHC, **(11)**	2	**(13)**, **(14)**: 1 T cell epitope, **(15)**: 2 T cell epitopes	3			5
0229 *rpIL* 13.2, 4.4	IM/ PP/C (18)	LSU ribosomal protein L7p/L12p (P1/P2), translation, ribosomal structure and biogenesis* (18)	**(2)**: reacted with 2 of 2 sera, **(7)**, **(16)**: reacted with 2 of 2 sera, Se. 68%, **(18)**: reacted with 8 of 9 sera	4					4
0891 34.4, n.s.	U. (3)	Hypothetical exported protein (3)	**(3)**: strong, **(4)**: Sp. 80.6%, Se. 41.6%, T cells recognize murine MHC, **(7)**: 2nd most reactive protein, **(8)**	4					4
1719 *groES* 10.5, 5.2	C (18)	HSP60 family co-chaperone GroES, post-translational modification, protein turnover, chaperones[#] (18)	**(5)**, **(7)**, **(18)**: reacted with 8 of 9 sera	3	**(18)**: not significantly higher than with *Rickettsia* spp.	1			4
1385 *tsf* 32.0, 5.8	C (18)	Translation elongation factor Ts, translation[#] (11), ribosomal structure and biogenesis* (18)	**(11)**, **(18)**: reacted with 7 of 9 sera	2	**(18)**: significantly higher than with *Rickettsia* spp.	1	**(19)**: good reactivity for both phases	1	4
1706 22.0, 5.1	C (11)	Alkyl hydroperoxide reductase subunit C-like protein, stress protein[#] (11), antioxidant defense (Ihnatko *et al.*, 2012), post-translational modification, protein turnover, chaperones* (18)	**(5)**, **(11)**	2	**(18)**: significantly higher than with *Rickettsia* spp.	1	**(19)**: good reactivity for phase I	1	4
0235 *fusA* 77.9, 5.1	C (11)	Translation elongation factor G, translation[#] (11)	**(5)**, **(9)**, **(11)**	3			**(19)**: co-identified with a protein in both phases	1	4
1241 *mdh* 35.0, 4.9	PP (11)	Malate dehydrogenase, TCA cycle, cystein, methionine metabolism[#] (11)	**(11)**, **(16)**: reacted with 1 of 2 sera	2	**(16)**	1	**(19)**: good reactivity for both phases	1	4

Table 2 (continued)

CBU gene kDa, pI	Localization	Protein function (PATRIC, unless otherwise specified)	Identified in (reference No.):							Total No.
			Human: 14 publications		Mouse: 7 publications		Guinea pig: 1 publication			
			Reacted with	No.	Reacted with	No.	Reacted with	No.		
0737 *tig* 50.2, 5.1	C (11)	Cell division trigger factor, protein export, chaperone[#] (11)	(2): reacted with 1 of 2 sera, (11), (16): reacted with 2 of 2 sera	3	(16)	1				4

Publications are specified with numbers in brackets from Table 1. The complete list of all 169 immunogenic proteins with the total number of identified proteins per host species is in Supplementary Table 1. 2D-GE = 2D-gel elektrophoresis, C = cytoplasm, EC = extracellular, H-2 I-A[b] = MHC class II molecule, HLA = human leucocyte antigen, IB = immunoblotting, IFA = immunefluorescence assay, IM = inner membrane, LCV = large cell variant, No. = number of publications, n.s. = not stated, NM = Nine Mile, OM = outer membrane, PP = periplasm, p.i. = post infection, SCV = small cell variant, Se. = sensitivity, Sp. = specificity, U. = unknown. *COG annotation (18), [#]UNIPROT annotation (11).

Candidate proteins for new vaccines

Identification of immunodominant proteins is not only required for the improvement of diagnostics, but also for the development of subunit vaccines. For an effective immunity against *C. burnetii,* both the humoral and cellular immune response, and especially their interplay with CD4+ T cells are needed (Zhang *et al.*, 2004b and 2007; Andoh *et al.*, 2007; Chen *et al.*, 2011). Naïve mice that received serum from vaccinated mice were protected against *C. burnetii* challenge as stated by Vigil *et al.* (2011). Also, a cellular immune response contributes to protective immunity. Adoptive transfer of CD8+ and CD4+ T cells conferred measurable protection against *C. burnetii* challenge (Xiong *et al.*, 2014, 2016; Zhang *et al.*, 2007). *C. burnetii* causes death in the SCID and T cell deficient mice, but not in B cell deficient mice. Therefore, T cells seem to be essential for Q fever immunity (Andoh *et al.*, 2007).

The following immunogenic proteins were described to have a potential as candidates for subunit vaccines: CBU0311 (P1) (Ihnatko *et al.*, 2012), CBU1910 (Com1), CBU0092 (YbgF), CBU0612 (OmpH), CBU0891, CBU1143 (YajC) and CBU0545 (LemA) (Vigil *et al.*, 2011). To confirm their potential as a vaccine, mouse bone marrow-derived dendritic cells were stimulated with recombinant proteins and transferred into naïve mice before *C. burnetii* challenge. Com1 (CBU1910) and Mip1 (CBU0630) in contrast to GroEL (CBU1718) were identified as key antigens to induce a protective immune response and to stimulate IFN-γ producing CD4+ (Th1) and CD8+ (Tc1) T cells (Xiong *et al.*, 2012b).

In another study, CD4+ T cell epitope peptides derived from major immunodominant proteins were investigated in mice resulting in a pool of seven peptides that conferred significant resistance to *C. burnetii* challenge (Xiong *et al.*, 2014). This confirms the importance of protein or peptide combinations not only for sensitive Q fever diagnostic but also for the development of an effective vaccine. Moreover, 29 *C. burnetii* CD8+ T cell peptide epitopes were delivered via expression in a *Listeria monocytogenes* strain for cytosol targeting and induced strong CD8+ T-cell IFN-γ recall responses after infection as well as measurable protection *in vivo* (Xiong *et al.*, 2016). Thus, these *in vivo* experiments emphasize, that stimulation of B and T (CD4+ and CD8+) cells confer protection against *C. burnetii* challenge.

Conclusion

Altogether 169 *C. burnetii* antigenic proteins were identified in the 19 immunoproteomic studies published during the last two decades. Twenty of these proteins are shown as the most frequently recognized antigens by human, mouse or guinea pig sera. CBU1910 (Com1), CBU1718 (GroEL), CBU0236 (Tuf-2), CBU0092 (YbgF) and CBU0612 (OmpH) were highlighted as immunodominant markers that might serve as promising candidates for better diagnostic tools and vaccines. Since sensitivity and specificity of tests are essential characteristics, we stress the importance of combining specific proteins and peptides for a robust Q fever detection.

Supplementary information is available in the online version of the paper.

References

Raoult D, Parola P (2007): Rickettsial diseases. Informa Healthcare, USA. https://doi.org/10.3109/9781420019971

Andoh M, Zhang G, Russell-Lodrigue KE, Shive HR, Weeks BR, and 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. https://doi.org/10.1128/IAI.01767-06

Arricau Bouvery N, Souriau A, Lechopier P, and Rodolakis A (2003): Experimental Coxiella burnetii infection in pregnant goats: excretion routes. Vet. Res. 34, 423–433. https://doi.org/10.1051/vetres:2003017

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. https://doi.org/10.1016/j.vaccine.2005.04.010

Barlow J, Rauch B, Welcome F, Kim SG, Dubovi E, and Schukken Y (2008): Association between Coxiella burnetii shedding in milk and subclinical mastitis in dairy cattle. Vet. Res. 39, 23. https://doi.org/10.1051/vetres:2007060

Bauer AE, Hubbard KR, Johnson AJ, Messick JB, Weng HY, Pogranichniy RM (2016): A cross sectional study evaluating the prevalence of Coxiella burnetii, potential risk factors for infection, and agreement between diagnostic methods in goats in Indiana. Prevent. Vet. Med. 126, 131–137. https://doi.org/10.1016/j.prevetmed.2016.01.026

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. https://doi.org/10.1128/CVI.00300-08

Beare PA, Unsworth N, Andoh M, Voth DE, Omsland A, Gilk SD, Williams KP, Sobral BW, Kupko JJ, 3rd, 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. https://doi.org/10.1128/IAI.01141-08

Boarbi S, Mori M, Rousset E, Sidi-Boumedine K, Van Esbroeck M, Fretin D (2014): Prevalence and molecular typing of Coxiella burnetii in bulk tank milk in Belgian dairy goats, 2009-2013. Vet. Microbiol. 170, 117–124.

Bottcher J, Vossen A, Janowetz B, Alex M, Gangl A, Randt A, and Meier N (2011): Insights into the dynamics of endemic Coxiella burnetii infection in cattle by application of phase-specific ELISAs in an infected dairy herd. Vet. Microbiol. 151, 291–300. https://doi.org/10.1016/j.vetmic.2011.03.007

Bruggemann H, Cazalet C, Buchrieser C (2006): Adaptation of Legionella pneumophila to the host environment: role of protein secretion, effectors and eukaryotic-like proteins. Curr. Opin. Microbiol. 9, 86–94. https://doi.org/10.1016/j.mib.2005.12.009

Chao CC, Chen HW, Li X, Xu WB, Hanson B, Ching WM (2005): Identification, cloning, and expression of potential diagnostic markers for Q fever. Ann. NY Acad. Sci. 1063, 76–78. https://doi.org/10.1196/annals.1355.010

Chen C, Bouman TJ, Beare PA, Mertens K, Zhang GQ, Russell-Lodrigue 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 immunoreactive antigens. Clin. Microbiol. Infect. 15 (Suppl. 2), 156–157. https://doi.org/10.1111/j.1469-0691.2008.02206.x

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. https://doi.org/10.1371/journal.pone.0017712

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. https://doi.org/10.1128/IAI.00883-06

Cunha LD, Ribeiro JM, Fernandes TD, Massis LM, Khoo CA, Moffatt JH, Newton HJ, Roy CR, Zamboni DS (2015): Inhibition of inflammasome activation by Coxiella burnetii type IV secretion system effector IcaA. Nat. Commun. 6, 10205. https://doi.org/10.1038/ncomms10205

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. https://doi.org/10.1099/mic.0.043513-0

Eckart RA, Bisle S, Schulze-Luehrmann J, Wittmann I, Jantsch J, Schmid B, Berens C, Luhrmann A (2014): Antiapoptotic activity of Coxiella burnetii effector protein AnkG is controlled by p32-dependent trafficking. Infect. Immun. 82, 2763–2771. https://doi.org/10.1128/IAI.01204-13

European Food Safety Authority and European Centre for Disease Prevention and Control (2016): The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. EFSA J. 14, 4634. https://doi.org/10.2903/j.efsa.2016.4634

European Food Safety Authority and European Centre for Disease Prevention and Control (2015): Scientific Report of EFSA and ECDC. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA J. 13, 3991.

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. https://doi.org/10.1016/j.vetmic.2008.08.024

Flores-Ramirez G, Danchenko M, Quevedo-Diaz M, Skultety L (2016): Reliable tool for detection of novel Coxiella burnetii antigens, using immobilized human polyclonal antibodies. J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci. 1047, 84–91.

Gilmore RD, Jr., Carpio AM, Kosoy MY, Gage KL (2003): Molecular characterization of the sucB gene encoding the immunogenic dihydrolipoamide succinyltransferase protein of Bartonella vinsonii subsp. berkhoffii and Bartonella

quintana. Infect. Immun. 71, 4818–4822. https://doi.org/10.1128/IAI.71.8.4818-4822.2003

Harty JT, Bevan MJ (1999): Responses of CD8(+) T cells to intracellular bacteria. Curr. Opin. Immunol. 11, 89–93.

Hogerwerf L, Koop G, Klinkenberg D, Roest HI, Vellema P, Nielen M (2014): Test and cull of high risk Coxiella burnetii infected pregnant dairy goats is not feasible due to poor test performance. Vet. J. 200, 343–345. https://doi.org/10.1016/j.tvjl.2014.02.015

Horigan MW, Bell MM, Pollard TR, Sayers AR, Pritchard GC (2011): Q fever diagnosis in domestic ruminants: comparison between complement fixation and commercial enzyme-linked immunosorbent assays. J. Vet. Diagn. Invest. AAVLD 23, 924–931. https://doi.org/10.1177/1040638711416971

Hotta A, Zhang GQ, Andoh M, Yamaguchi T, Fukushi H, Hirai K (2004): Use of monoclonal antibodies for analyses of Coxiella burnetii major antigens. J. Vet. Med. Sci. 66, 1289–1291.

Ihnatko R, Shaw E, Toman R (2012): Proteome of Coxiella burnetii. Adv. Exp. Med. Biol. 984, 105–130. https://doi.org/10.1007/978-94-007-4315-1_6

Janeway CA Jr, Travers P, Walport M, Shlomchik MJ (2001: Immunobiology: The Immune System in Health and Disease. 5th edition. New York : Garland Science; T cell-mediated cytotoxicity. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27101/

Jiao J, Xiong X, Qi Y, Gong W, Duan C, Yang X, Wen B (2014): Serological characterization of surface-exposed proteins of Coxiella burnetii. Microbiology 160, 2718–2731. https://doi.org/10.1099/mic.0.082131-0

Kampschreur LM, Delsing CE, Groenwold RH, Wegdam-Blans MC, Bleeker-Rovers CP, de Jager-Leclercq MG, Hoepelman AI, van Kasteren ME, Buijs J, Renders NH, Nabuurs-Franssen MH, Oosterheert JJ, Wever PC (2014): Chronic Q fever in the Netherlands 5 years after the start of the Q fever epidemic: results from the Dutch chronic Q fever database. J. Clin. Microbiol. 52, 1637–1643. https://doi.org/10.1128/JCM.03221-13

Kampschreur LM, Wegdam-Blans MC, Thijsen SF, Groot CA, Schneeberger PM, Hollander AA, Schijen JH, Arents NL, Oosterheert JJ, Wever PC (2010): Acute Q fever related in-hospital mortality in the Netherlands. Neth. J. Med. 68, 408–413.

Klingenbeck L, Eckart RA, Berens C, Luhrmann A (2013): The Coxiella burnetii type IV secretion system substrate CaeB inhibits intrinsic apoptosis at the mitochondrial level. Cell. Microbiol. 15, 675–687. https://doi.org/10.1111/cmi.12066

Koster FT, Williams JC, Goodwin JS (1985): Cellular immunity in Q fever: specific lymphocyte unresponsiveness in Q fever endocarditis. J. Infect. Dis. 152, 1283–1289. https://doi.org/10.1093/infdis/152.6.1283

Kowalczewska M, Nappez C, Vincentelli R, La Scola B, Raoult D (2012): Protein candidates for Q fever serodiagnosis. FEMS Immunol. Med. Microbiol. 64, 140–142. https://doi.org/10.1111/j.1574-695X.2011.00912.x

Kowalczewska M, Sekeyova Z, Raoult D (2011): Proteomics paves the way for Q fever diagnostics. Genome Med. 3, 50. https://doi.org/10.1186/gm266

La Scola B, Raoult D (1996): Serological cross-reactions between Bartonella quintana, Bartonella henselae, and Coxiella burnetii. J. Clin. Microbiol. 34, 2270–2274.

Landais C, Fenollar F, Thuny F, Raoult D (2007): From acute Q fever to endocarditis: serological follow-up strategy. Clinical infectious diseases: an official publication of the Infect. Dis. Soc. Am. 44, 1337–1340.

Luhrmann A, Roy CR (2007): Coxiella burnetii inhibits activation of host cell apoptosis through a mechanism that involves preventing cytochrome c release from mitochondria. Infect. Immun. 75, 5282–5289. https://doi.org/10.1128/IAI.00863-07

Lukacova M, Melnicakova J, Kazar J (1999): Cross-reactivity between Coxiella burnetii and chlamydiae. Folia Microbiol. 44, 579–584. https://doi.org/10.1007/BF02816263

Martinez E, Cantet F, Fava L, Norville I, Bonazzi M (2014): Identification of OmpA, a Coxiella burnetii protein involved in host cell invasion, by multi-phenotypic high-content screening. PLoS Pathogens 10, e1004013. https://doi.org/10.1371/journal.ppat.1004013

Maurin M, Raoult D (1999): Q fever. Clin. Microbiol. Rev. 12, 518–553.

Mestas J, Hughes CCW (2004): Of Mice and Not Men: Differences between Mouse and Human Immunology. J. Immunol. 172, 2731–2738. https://doi.org/10.4049/jimmunol.172.5.2731

Musso D, Raoult D (1997): Serological cross-reactions between Coxiella burnetii and Legionella micdadei. Clin. Diagn. Lab. Immunol. 4, 208–212.

Niemczuk K, Szymanska-Czerwinska M, Smietanka K, Bocian L (2014): Comparison of diagnostic potential of serological, molecular and cell culture methods for detection of Q fever in ruminants. Vet. Microbiol. 171, 147–152. https://doi.org/10.1016/j.vetmic.2014.03.015

O'Neill TJ, Sargeant JM, Poljak Z (2014): A systematic review and meta-analysis of phase I inactivated vaccines to reduce shedding of Coxiella burnetii from sheep and goats from routes of public health importance. Zoonoses Public Health 61, 519–533.

Papadioti A, De Bock PJ, Vranakis I, Tselentis Y, Gevaert K, Psaroulaki A, Tsiotis G (2012): Study of the whole cell lysate of two Coxiella burnetii strains using N-terminomics. J. Proteome Res. 11, 3150–3159. https://doi.org/10.1021/pr201175m

Papadioti A, Markoutsa S, Vranakis I, Tselentis Y, Karas M, Psaroulaki A, Tsiotis G (2011): A proteomic approach to investigate the differential antigenic profile of two Coxiella burnetii strains. J. Proteomics 74, 1150–1159. https://doi.org/10.1016/j.jprot.2011.04.016

Paul S, Toft N, Agerholm JS, Christoffersen AB, Agger JF (2013): Bayesian estimation of sensitivity and specificity of Coxiella burnetii antibody ELISA tests in bovine blood and milk. Prevent. Vet. Med. 109, 258–263. https://doi.org/10.1016/j.prevetmed.2012.10.007

Rahman KS, Chowdhury EU, Poudel A, Ruettger A, Sachse K, Kaltenboeck B (2015): Defining species-specific immunodominant B cell epitopes for molecular serology of

Chlamydia species. Clin. Vaccine Immunol. 22, 539–552. https://doi.org/10.1128/CVI.00102-15

Rodolakis A, Berri M, Hechard C, Caudron C, Souriau A, Bodier CC, Blanchard B, Camuset P, Devillechaise P, Natorp JC, Vadet JP, Arricau-Bouvery N (2007): Comparison of Coxiella burnetii shedding in milk of dairy bovine, caprine, and ovine herds. J. Dairy Sci. 90, 5352–5360. https://doi.org/10.3168/jds.2006-815

Roest HI, Bossers A, Rebel JM (2013): Q fever diagnosis and control in domestic ruminants. Develop. Biol. 135, 183–189. https://doi.org/10.1159/000188081

Rousset E, Berri M, Durand B, Dufour P, Prigent M, Delcroix T, Touratier A, Rodolakis A (2009): Coxiella burnetii shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds. Appl. Environ. Microbiol. 75, 428–433. https://doi.org/10.1128/AEM.00690-08

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. https://doi.org/10.1007/s10096-008-0621-4

Sekeyova Z, Kowalczewska M, Vincentelli R, Decloquement P, Flores-Ramirez G, Skultety L, and Raoult D (2010): Characterization of antigens for Q fever serodiagnostics. Acta Virol. 54, 173–180. https://doi.org/10.4149/av_2010_03_173

Shannon JG, Howe D, Heinzen RA (2005): Virulent Coxiella burnetii does not activate human dendritic cells: role of lipopolysaccharide as a shielding molecule. Proceedings of the National Academy of Sciences of the United States of America 102, 8722–8727. https://doi.org/10.1073/pnas.0501863102

Sobotta K, Hillarius K, Mager M, Kerner K, Heydel C, Menge C (2016): Coxiella burnetii Infects Primary Bovine Macrophages and Limits Their Host Cell Response. Infect. Immun. 84, 1722–1734. https://doi.org/10.1128/IAI.01208-15

Stein A, Lepidi H, Mege JL, Marrie TJ, Raoult D (2000): Repeated pregnancies in BALB/c mice infected with Coxiella burnetii cause disseminated infection, resulting in stillbirth and endocarditis. J. Infect. Dis. 181, 188–194. https://doi.org/10.1086/315166

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: MCP 10, M110.006304. https://doi.org/10.1074/mcp.M110.006304

Vigil A, Ortega R, Nakajima-Sasaki R, Pablo J, Molina DM, Chao CC, Chen HW, Ching WM, Felgner PL (2010): Genome-wide profiling of humoral immune response to Coxiella burnetii infection by protein microarray. Proteomics 10, 2259–2269. https://doi.org/10.1002/pmic.201000064

Voth DE, Heinzen RA (2007): Lounging in a lysosome: the intracellular lifestyle of Coxiella burnetii. Cell. Microbiol. 9, 829–840. https://doi.org/10.1111/j.1462-5822.2007.00901.x

Voth DE, Howe D, Heinzen RA (2007): Coxiella burnetii inhibits apoptosis in human THP-1 cells and monkey primary alveolar macrophages. Infect. Immun. 75, 4263–4271. https://doi.org/10.1128/IAI.00594-07

Voth DE, Howe D, Beare PA, Vogel JP, Unsworth N, Samuel JE, Heinzen RA (2009): The Coxiella burnetii ankyrin repeat domain-containing protein family is heterogeneous, with C-terminal truncations that influence Dot/Icm-mediated secretion. J. Bacteriol. 191, 4232–4242. https://doi.org/10.1128/JB.01656-08

Wang X, Xiong X, Graves S, Stenos J, Wen B (2013): Protein array of Coxiella burnetii probed with Q fever sera. Sci. China Life Sci. 56, 453–459. https://doi.org/10.1007/s11427-013-4472-6

Xiong X, Wang X, Wen B, Graves S, Stenos J (2012a): Potential serodiagnostic markers for Q fever identified in Coxiella burnetii by immunoproteomic and protein microarray approaches. BMC Microbiol. 12, 35. https://doi.org/10.1186/1471-2180-12-35

Xiong X, Meng Y, Wang X, Qi Y, Li J, Duan C, Wen B (2012b): Mice immunized with bone marrow-derived dendritic cells stimulated with recombinant Coxiella burnetii Com1 and Mip demonstrate enhanced bacterial clearance in association with a Th1 immune response. Vaccine 30, 6809–6815. https://doi.org/10.1016/j.vaccine.2012.09.017

Xiong X, Qi Y, Jiao J, Gong W, Duan C, Wen B (2014): Exploratory study on Th1 epitope-induced protective immunity against Coxiella burnetii infection. PloS One 9, e87206. https://doi.org/10.1371/journal.pone.0087206

Xiong X, Jiao J, Gregory AE, Wang P, Bi Y, Wang X, Jiang Y, Wen B, Portnoy DA, Samuel JE, Chen C (2016): Identification of Coxiella burnetii CD8+ epitopes and delivery by attenuated Listeria monocytogenes as a vaccine vector in a C57BL/6 mouse model. The J. Infect. Dis. volume, pages??? Zhang G, Samuel JE (2004b): Vaccines against Coxiella infection. Exp. Rev. Vaccines 3, 577–584.

Zhang G, Kiss K, Seshadri R, Hendrix LR, Samuel JE (2004a): Identification and cloning of immunodominant antigens of Coxiella burnetii. Infect. Immun. 72, 844–852. https://doi.org/10.1128/IAI.72.2.844-852.2004

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. https://doi.org/10.4049/jimmunol.179.12.8372