**SHORT COMMUNICATION**

**Protein candidates for Q fever serodiagnosis**

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**Abstract**

The discriminatory diagnosis of Q fever remains difficult because of the unspecific clinical presentations of the disease. Additionally, the diagnosis is often delayed because serodiagnosis is not sensitive enough in the early stages of the disease when the immune response is not yet efficient. Similarly, the diagnosis of Q fever endocarditis can only be performed in approximately 35%, mainly via serology, which was a criterion postulated by Duke. Owing to the discriminatory diagnosis of Q fever and the high number of tests requested, we focused on expressing several proteins for ELISA studies with *Coxiella burnetii*-infected sera. Previously, we selected a list of 31 candidates [Sekeyova et al. (2009) *Eur J Clin Microbiol Infect Dis* 28: 287–295], of which we have successfully cloned and expressed 21. Finally, 15 recombinant proteins were prescreened with the sera of patients with acute Q fever and Q fever endocarditis, respectively. Sera from a control group were also screened. The nine most immunoreactive proteins from the first assay were tested with the sera from a larger group of patients. Our study identified CBU_0092 as the best marker of acute Q fever but failed to isolate a highly specific and sensitive marker of Q fever endocarditis.

*Coxiella burnetii* is an obligate intracellular bacterial pathogen that causes Q fever disease in humans. Transmission to humans occurs through the inhalation of aerosols from infected animals. Two clinical forms of Q fever have been described as follows: (1) acute Q fever disease in which the majority of cases remain asymptomatic or the disease manifests as a self-limiting flu-like syndrome and (2) chronic Q fever, which is mainly associated with infective endocarditis in patients with pre-existing valvulopathy. Thus, the diagnosis remains difficult and is mainly based on serological methods and PCR (Frankel et al., 2011). However, these methods are time-consuming, fastidious and lack both sensitivity and specificity. Research over the last decade has focused on the development of serodiagnosis, vaccines, selection of candidate proteins through immune and proteomic studies and high-throughput screening technologies (Kowalczywska et al., 2011). A large list of recombinant proteins was generated, but only a few of them were seroreactive (Chao et al., 2005; Beare et al., 2008; Chen et al., 2009; Vigil et al., 2010, 2011). The aim of this study was to determine the seroreactivity of recombinant proteins (Sekeyova et al., 2009) by probing the sera from patients with acute Q fever and Q fever endocarditis.

We successfully cloned and expressed 21 C. burnetii proteins in *Escherichia coli* using Gateway Cloning Technology (Invitrogen) from a list of 31 targets selected by a previous immunoproteomic study (Sekeyova et al., 2009). The nucleic acid sequences of the ORFs were extracted from the genomic library (NCBI), and the predicted signal peptide sequences (http://bp.nuap.nagoya-u.ac.jp/sosui/sosuisignal/sosuisignal_submit.html) of six proteins (Supporting Information, Appendix S1) were removed. Expression and purification protocols were performed as previously described (Sekeyova et al., 2010; Vincentelli et al., 2011). DNA from the *C. burnetii* Nine Mile RSA 493 strain was extracted using commercially available kit (Qiagen, Chatsworth, CA) according to manufacturer’s instructions. The pDONR201 vector was used to generate an entry clone in *E. coli* DH5α cells, and the pETG-20A
Recombinant proteins as markers of Q fever

destination vector was used to generate expression clones containing an N-terminal 6His-tag plus a thioredoxin (TRX) fusion protein (Vincentelli et al., 2011) in E. coli Rosetta (DE3) pLysS (Novagen) cells. Proteins were purified by affinity chromatography using the N-terminal 6His-tag with nickel ion resin under either native (buffer A: 50 mM Tris–HCl, 300 mM NaCl, 250 mM imidazole, pH 8.0) or denaturing conditions (buffer A + 6 M urea, pH 8.0) depending on the purified protein yields (Sekeyova et al., 2010). We were unable to express or purify sufficient amounts of 10 of the proteins, including our best targets (CBU_612 and CBU_480). Quality controls for all experimental steps and modified ELISAs were conducted as previously described (Sekeyova et al., 2010).

Prescreening was performed with 15 recombinant proteins using the sera of 34 patients infected by C. burnetii (16 with acute Q fever and 18 with Q fever endocarditis) after obtaining their informed consent. Patients were diagnosed at the FNRC (Marseille, France) using serology and PCR (Frankel et al., 2011). The control group consisted of 14 healthy blood donors (HBD) who were IFA negative. The second assay was performed with the nine best protein targets using the sera of 53 patients infected by C. burnetii (26 with acute Q fever and 27 with Q fever endocarditis) and 26 HBD.

Currently, few studies have focused on the markers able to discriminate acute from chronic Q fever (Zhang et al., 2005; Sekeyova et al., 2009, 2010; Deringer et al., 2010; Vigil et al., 2010, 2011; Kowalczewska et al., 2011; Papadioti et al., 2011). To expand the library of available markers of Q fever disease, we cloned and expressed 21 proteins in E. coli (Sekeyova et al., 2009). First, we tested 15 recombinant proteins with the C. burnetii-infected sera of 34 infected patients and the sera of 14 healthy blood donors. Protein targets were prescreened for the general diagnosis of Q fever and for the discriminative diagnosis of Q fever (acute Q fever and Q fever endocarditis together). The nine most immunoreactive proteins were subjected to further screening with human sera from a larger group of patients (n = 79) (Appendix S1).

Early stage detection of C. burnetii remains ambiguous and not sensitive enough to diagnose acute Q fever. The availability of early stage Q fever markers may improve diagnosis. ELISAs or arrays based on recombinant proteins are relatively easy to perform and may be additional diagnostic tools. Deringer et al. (2010) investigated early and late stage markers that could be useful in serodiagnosis. The seroreactive C. burnetii proteins identified were CBU_0932, CBU_1241, CBU_1396, CBU_1385, CBU_0299, CBU_0750, and CBU_0103, the weakly reacting both CBU_0140 and CBU_0858. None of these proteins were tested in the present study. Recombinant protein-based microarrays were explored using the sera from patients suffering from acute Q fever as well as healthy blood donors (Vigil et al., 2010). A total of 21 antigens were immunoreactive, and only 13 of them were specific to C. burnetii (Vigil et al., 2010). This study was recently revisited (Vigil et al., 2011) and focused on the discriminatory antigens of both acute and chronic Q fever. CBU_1910 was reported to be the most reactive antigen with high specificity (Vigil et al., 2010, 2011). Among the identified proteins, several had been tested in other studies (Coleman et al., 2007; Beare et al., 2008; Sekeyova et al., 2009, 2010; Deringer et al., 2010). The results from Vigil et al. (2010) and Beare et al. (2008) showed a similar range of reactivity for the best hits (CBU_1910, CBU_0891, CBU_1143, CBU_0612, CBU_0545, and CBU_1398). However, none of the markers discovered were selected for the present study except CBU_0612, which we were unable to express. In the revisited study, among the antigens that may be important for protective immunity, CBU_0092 was seroreactive with both acute and chronic Q fever sera (Vigil et al., 2011). The newly identified markers of acute Q fever are CBU_1920, CBU_1725, CBUK_1974, and CBU_1718 (Vigil et al., 2011). Unfortunately, we were unable to express CBU_1718 in our study.

Our best diagnostic marker of acute Q fever was CBU_0092 (Appendix S1), which was previously reported as a good marker (Beare et al., 2008; Deringer et al., 2010; Vigil et al., 2010, 2011; Papadioti et al., 2011). Its diagnostic power was strong enough for the general diagnosis of Q fever and might the better specificity for acute Q fever (L = 22). The second and third best targets for acute Q fever diagnosis were CBU_0271 (L = 5.33) and CBU_0632 (L = 3.7), respectively. With respect to the values of the standard operating parameters used in both our screenings, only CBU_0092 was both specific and sensitive enough for ‘routine’ applications in acute Q fever serodiagnosis.

The diagnosis of chronic Q fever remains difficult because of the varied and nonspecific clinical presentations of the disease. Despite advances in diagnostic techniques, the etiologic diagnoses of IE cannot be obtained in 30% of cases. Unlike bacterial cultures, serology has been included as a major criterion in the modified Duke criteria (Li et al., 2000; Frankel et al., 2011). It has raised interest in the discriminatory, noninvasive diagnosis of chronic Q fever. Only three (CBU_0443, CBU_1430, and CBUK_0188) of the 62 markers screened were highly specific for chronic Q fever (Vigil et al., 2011). Our best diagnostic markers of Q fever endocarditis were CBU_0937, CBU_2029, and CBU_0115. However, the L value was below 5 for all three markers, indicating that they do not significantly contribute to diagnosis. Because CBU_0937 was cross-validated in the recent work of Papadioti et al. (2011), we included it in our large-scale assay. CBU_0937 showed a very promising diagnostic
value for Q fever endocarditis ($L = 11.42$) in the first screening, which decreased with an increase in the number of patients ($L = 4.17$), demonstrating the impact of a larger trial group (Appendix S1). The previous results (Sekeyova et al., 2010) were confirmed in the present assay when the larger group was screened. Additionally, the three best targets from this study with specificity for Q fever endocarditis were not powerful enough for use as discriminating diagnostic markers in routine testing.

Our study contributes to the cross-validation of some seroreactive antigens of Q fever that were previously investigated, including CBU_0092 (Vigil et al., 2010, 2011; Papadioti et al., 2011). CBU_0092 was identified as the best marker for acute Q fever in our study. Unfortunately, we were unable to express our best identified markers for Q fever endocarditis (CBU_612 and CBU_480). Although CBU_0937 was recently described as demonstrating a promising Q fever endocarditis diagnostic value (Papadioti et al., 2011), we have shown that it is neither specific nor sensitive enough for ‘routine’ applications, which conformed our previous data (Sekeyova et al., 2010). We have illustrated the impact of trial group size on the results of such studies, thereby highlighting the importance of several trials to accurately validate markers.

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Test-operating parameters for all pre-screened ($n = 48$ human sera) and screened ($n = 79$ human sera) recombinant proteins.

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