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Protein composition of the phase I *Coxiella burnetii* soluble antigen prepared by extraction with trichloroacetic acid

G. FLORES-RAMÍREZ[1], M. KMEŤOVÁ[1], M. DANCHENKO[1], E. ŠPITALSKÁ[1], V. HAVLÍČEK[2], Ľ. ŠKULTÉTY[1,2*]

[1]Institute of Virology, Biomedical Research Center Slovak Academy of Sciences, Dúbravska cesta 9, 845 05 Bratislava, Slovak Republic; [2]Institute of Microbiology of the CAS, v.v.i., Prague, Czech Republic

Summary. – Q fever is a highly infectious, widespread airborne zoonosis caused by *Coxiella burnetii* bacterium. Humans usually acquire the disease by inhalation of contaminated aerosol produced by infected livestock. Vaccination is the most practical way for prevention and control of the disease in the exposed population. In this work, we reviewed the most important Q-fever outbreaks in Slovakia as well as the progress in vaccine development. One of them represents a soluble antigen complex produced by extraction with trichloroacetic acid from a highly purified *C. burnetii* phase I strain Nine Mile. It was developed at the Institute of Virology in Bratislava. The protein content of this vaccine was separated by gel electrophoresis and analyzed by mass spectrometry. The study has resulted in the identification of 39 bacterial proteins from which 12 were recognized as immunoreactive. Most of the proteins were involved in bacterium pathogenicity (41.6%) and cell wall maintenance (25%). Four of the immunoreactive proteins may possess the moonlighting activity. Definition of the vaccine components represents a prerequisite for vaccine standardization and approval by governmental authorities.

Keywords: Coxiella burnetii; Q fever; outbreaks in Slovakia; soluble antigen; chemovaccine; trichloroacetic acid; protein composition

Introduction

Coxiella burnetii is the causative agent of Q fever, a worldwide zoonotic disease with considerable economic impact in the livestock industry. Domestic animals as cattle, sheeps, and goats are the main reservoirs of *C. burnetii* and the source of Q fever in human.

The human infection is acquired by inhaling aerosolized microorganisms produced by infected livestock. The acute infection is typically asymptomatic or manifests as a febrile flu-like illness or pneumonia followed by a spontaneous recovery. In a small percent, patients may develop meningoencephalitis, hepatitis, endocarditis that require intensive care. Chronic infection is usually accompanied by severe symptoms such as pulmonary infections, endocarditis or hepatitis.

In Europe, Q fever is widely extended, several outbreaks have been reported in Bulgaria, Germany, France, Hungary, and Poland. The largest outbreak arose between 2007–2010 in the Netherlands which resulted in 4026 human cases (Van der Hoek *et al*., 2010; Georgiev *et al*., 2013; Gyuranecz *et al*., 2014).

The first outbreak of Q fever in Slovakia was recognized in 1954 among agricultural workers. Epidemiological investigations revealed that the source of infection was a sheep flock imported from Romania. Shortly after, an infection of textile plant workers occurred (Rehacek, 1987). During the next decades, many small epidemics were reported from factories processing imported wool or hides as well as from sheep and cattle farms with imported animals (Table 1). Within the period of 1972–1982 large-scale vaccination of the ruminants and their veterinary control were implemented, which resulted in significant reduction of Q fever cases in the country (Kováčová *et al*., 2002).

*Corresponding author. E-mail: viruludo@savba.sk; phone: +421-2-59302418.

Abbreviations: 2-DE = two-dimensional electrophoresis; SDS = sodium dodecyl sulfate; Abs = antibodies; ACN = acetonitrile; CBB = Coomassie Brilliant Blue; CMR = chloroform-methanol residue; ECL = enhanced chemiluminescence solution; IEF = isoelectric focusing; ESI = electrospray ionization; FA = formic acid; MS = mass spectrometry; NM = Nine Mile; PI = in phase I; TCA =trichloroacetic acid; WCV = whole inactivated bacterial cells

Table 1. Cases of Q fever in Slovakia

Year, place	Number of reported cases	Source of infection	References
1953, Western Slovakia	25 textile plant workers	Sheeps imported from Romania*	Bardos *et al.* (1956)
1954, Western Slovakia	51 farmworkers	Sheeps from the herd as above*	Bardos *et al.* (1956)
1954, Gbely, Holíč	unknown	Cattle	Sádecký and Ábel (1978)
1954, not mentioned	40 workers	Processing of imported wool	Literák, Řeháček (1996)
1957, Zemné, Nové Zámky District	28 humans	Dairy cows from Topoľčany district	Literák, Řeháček (1996)
1959, not mentioned	14 workers	Processing of imported wool and hides from Mongolia and China	Literák and Řeháček (1996)
1962, Sokoľany, Košice District	13 farmworkers	Cattle	Mittermayer *et al.* (1964),
1962, Bardejov District	10 farmworkers on exchange visits + 2 clinically latent + 1 infected lab. personnel	Visiting recent and past foci of Coxielosis	Janok *et al.* (1964)
1962, Trenčianska Teplá, Trenčín District	farmworkers	Imported rams from England	Literák and Řeháček (1996)
1963, Pinciná, Lučenec District	5 humans	Dairy cows	Literák and Řeháček (1996)
1963, Dobročská Lehota, Lučenec District	37 humans	Sheeps	Literák and Řeháček (1996)
1965–1966, Sokoľany, Kosice District, Kečkovce, Svidnik District	139 farmworkers	Cattle	Fricova *et al.* (1967), Literák and Řeháček (1996)
1967, Slavošovice, Rochovice, Rožňava District	26 humans	Sheeps	Literák and Řeháček (1996)
1969–1970, Budulov, Košice District	54 humans	Dairy cows	Literák and Řeháček (1996)
1970, Balvany, Čalovo (Veľký Meder), Komárno District	28 humans	Dairy cows	Literák and Řeháček (1996)
1975, Košice District	Farmworkers	Processing of sheep wool	Sádecký and Ábel (1978)
1993 (April), Jedľové Kostol'any, Nitra District	113 humans (84%) aerosol transmission (16%) farm workers	Goats (abortion)	Varga *et al.* (1997)
1993, Rimavská Sobota District	11 farmworkers	Goats (abortion)	Varga *et al.* (1997)
2007, Košice, Košice District	Veterinary students (≥1:800 IgG in 20 students = 8.2 %)	Professionally exposed	Dorko *et al.* (2008)

The largest outbreak of human Q fever in Slovakia began as a result of imports of 1181 goats from Bulgaria in September 1992. They were temporarily housed at Rimavská Sobota District, and 500 of them were transferred in January 1993 to the farm in the village Jedľové Kostoľany, Nitra District (Dorko *et al.*, 2012; Varga, 1997). Within the next two months, several abortions occured which resulted in two outbreaks. During the first one, 11 humans who were in direct contact with animals were infected (Dorko *et al.*, 2012). The second epidemic, however, involved as many as 113 human cases (Table 1). Epidemiological investigations revealed that the source of infection was a contaminated aerosol in a local pub that arose from the clothing of farm workers who witnessed the abortion of one of the goat shortly before (Kováčová *et al.*, 1998; Varga, 1997; Rehacek *et al.*, 1996).

Q fever outbreaks usually occur from occupational exposure involving veterinarians, meat processing plant workers, dairy workers, livestock farmers, and laboratory personnel working with *C. burnetii*. Thus, prevention and control efforts should be directed primarily toward these groups and environments. The most efficient way of avoidance is a vaccination of the exposed populations. Although clinical data are lacking, vaccination should also be considered for persons who are at higher risk for development of chronic Q fever, including those with cardiac valve defects or prostheses, those with vascular aneurysms, and immunocompromised patients. Therefore, development of an effective vaccine against Q fever with minimal adverse reactions become a subject of interest to many researchers.

One of the first vaccines was prepared in 1948, only 12 years after the discovery of *C. burnetii.* This early whole cell vaccine (Smadel vaccine) was developed from formalin-killed and ether-extracted *C. burnetii,* containing 10% yolk sac debris (Waag *et al.*, 2002). Later, it was observed, that purification with the aim to remove chicken proteins and lipids, and isolation of the whole inactivated bacterial cells

(WCV), contributed to less reactogenic and highly immunogenic preparations (Waag, 2007). Hendrix and Chen (2012) also showed that the vaccines prepared from virulent phase I microorganisms (WCV-PI) are more effective than the attenuated phase II vaccines (Arricau-Bouvery and Rodolakis, 2005). Nevertheless, this WCV was banned in the most countries around the world due to the prevalence of adverse effects (local and systemic reactions) that may occur in people with a history of Q fever or those that were previously vaccinated (Waag *et al*., 2007). Q-Vax® is the only commercially available human Q fever WCV-PI vaccine which is licensed in Australia. It is a monovalent vaccine developed in 1989 by the Commonwealth Serum Laboratories from formalin-inactivated purified whole cells of phase I *C. burnetii* Henzerling strain (Porter *et al*., 2011). It has a reported efficacy over 98 % (Trubiano *et al*., 2012; Sellens *et al*., 2016; Waag *et al*., 2002) with protection up to 5 years (Ruiz and Wolfe, 2014). However, according to the rules of Australian National Vaccination Programme, strict pre-vaccination protocols such as serology and intradermal skin testing with diluted vaccine have to be implemented prior administration to avoid adverse effects (Sellens *et al*., 2016).

The acellular vaccines, or chemovaccines, are a new generation of vaccines with reduced adverse reactions that were shown to be as effective against Q fever as the whole cell vaccine (Parker *et al*., 2006). The chloroform-methanol residue vaccine (CMR) was prepared thanks to the cooperation of Rocky Mountain Laboratories with the US Army Research Institute for Infectious Disease in the late 70s. The phase I Henzerling strain inactivated with formalin was lyophilized and refluxed with a chloroform-methanol (4:1) mixture (Kersh *et al*., 2013). The prepared vaccine was able to induce protection in animals and human volunteers (Oyston and Davies, 2011; Fries *et al*., 1993). The chemovaccine, developed in former Czechoslovakia, is a soluble antigen complex produced by extraction of highly purified phase I *C. burnetii* Nine Mile strain EP3 using trichloroacetic acid (TCA). This extract contains proteins and LPS and it is highly reactogenic in humans (Kazar *et al*., 1982). In laboratory animals (mice and rabbits) the TCA extract induces the formation of antibodies against antigens 1 and 2, which was demonstrated by serodiagnosis as well as with opsonization phagocytosis and serum protective tests (Kazar *et al*., 1978). It also induces the cell-mediated component of immunity (Kazar *et al*., 1983). According to the record of the Department of Rickettsiology, Institute of Virology, BMC SAS, there were two major periods of immunization using this vaccine. The first was from 1972 to 1982 and the second from 1986 to 2004 during which 1421 and almost 300 individuals were vaccinated, respectively. They were mainly veterinarians, laboratory, farm and abattoir workers, and soldiers (Kazar *et al*., 1982, 1983). While significant protective efficacy and antibodies response were observed in the vaccinated individuals, no significant side effects were noted. Very mild systemic and local adverse reactions were described only in previously exposed individuals. Nevertheless, the vaccine production was stopped in 2002 due to insufficient knowledge of the composition of the vaccine. To fill the knowledge gap, we performed comprehensive characterization of the protein composition of the TCA extract.

Material and Methods

Preparation of the TCA extract of C. burnetii RSA 493. The optimal condition of extraction by TCA was applied (Lukacova *et al*., 1989). The purified inactivated *C. burnetii* RSA 493 phase I cells (100 mg) were resuspended in 20 ml of Mill-Q H_2O and 30 ml of 16.7 % (w/v) TCA solution was added to obtain the final concentration of 10 % (w/v) of TCA. The extraction was performed at 0°C for 45 min in an ice bath containing NaCl under constant stirring. After neutralization with sodium hydroxide, the mixture was spun down at 16,000 x g and 4°C for 40 min. Three drops of 10% (v/v) phenol was added to avoid microbial contamination. The supernatant was then dialyzed using 3,000 MW cut off membrane for 3 days against Mill-Q H_2O water (2 l changed once a day) at 4°C. This step was necessary to remove the salt. Then the sample was frozen at -80°C followed by lyophilization for 3 days using Power dry PL3000 instrument (Thermo Scientific).

Two dimensional electrophoresis (2-DE). Aliquots (500 µg) of the lyophilized extract were dissolved in a sample buffer (8 mol/l Urea, 2 mol/l thiourea, 1% (v/v) ASB14, 1% (w/v) Triton X-100) containing 1% (v/v) of carrier ampholytes pH range 3–10 or 4–7 (GE Healthcare), and 1.7% of DeStreak reagent (GE Healthcare). Immobilized pH gradient strips (pH 3–10 or pH 4–7, 18 cm, GE Healthcare, Sweden) were passively rehydrated overnight (approx. 16 h) in the dark at RT, then placed into Multiphor II apparatus (GE Healthcare, Sweden) and isoelectric focusing was performed using the following protocol; 100V in gradient for 1 h, 500V in gradient for 1 h, 1000V in gradient for 1 h, 6,000V in gradient for 3 h, and 6,000V for 18 h. The strips were then rinsed in deionized water and incubated in equilibration buffer (50 mmol/l Tris-HCl, pH 8.8, 6 mol/l urea, 30% (v/v) glycerol, 2% (w/v) SDS containing 1% (w/v) dithiothreitol (DTT, Sigma-Aldrich)) for 15 min, followed by 4% (w/v) iodoacetamide (IAA, Sigma-Aldrich) with 0.08% (w/v) bromphenol blue. The second dimension separation was carried out on 16% polyacrylamide gels (20 cm x 20 cm x 1 mm) in Tris-glycine running buffer, using a Protean XL (Bio-Rad, USA) device. It was performed at 5 mA/gel for 60 min, followed by 45 mA/gel until tracking dye migrated to the bottom of the gels. Protein spots were visualized with colloidal Coomassie Brilliant Blue (CBB) or used for Western blot.

Western blot. After the electrophoresis, the gel was washed for 5 min in Milli-Q water. A PVDF membrane 0.22 µm (Merck, Germany) was activated with methanol, and the proteins were transferred using a semi-dry blotting apparatus (Multiphore II, Amersham). The voltage was set according to gels size (surface area (cm^2) * 0.8). The membrane was blocked with 5% non-fat dry milk (Biorad) in PBS-Tween 0.1%

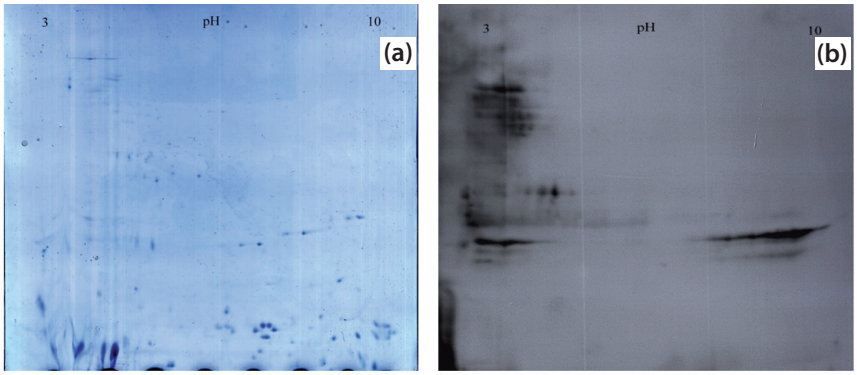

Fig. 1

(a) The representative 2-DE gel (3–10pI) and (b) Western blot analysis of phase I *C. burnetii* RSA soluble antigen

(PBS-T) overnight at 4°C. Then, it was five times washed with PBS-T and incubated for 2.5 h in 1:200 diluted rabbit serum containing polyclonal antibody (to the Phase I & II *C.burnetii* antigens) in 2.5% non-fat dry milk at room temperature (RT). The membrane was washed 5 times for 5 min with 200 ml of PBS-T and incubated with secondary antibody (IgG HRP-linked 1:1,500 Polyclonal Swine, Anti-Rabbit Immunoglobulin, Dako) for 1 h in 2.5% non-fat dry milk in PBS-T at RT. Finally, the membrane was washed five times with PBS-T and developed by ECL system (GE Healthcare).

Trypsin digestion and mass spectrometry analyses. The excised spots were washed with agitation in 50% (v/v) acetonitrile (ACN) (Merck, Germany) in 50 mmol/l NH_4HCO_3 (Ambic; Fluka,) at RT. After complete destaining, gel pieces were dehydrated with 100% ACN for 10 min at RT, reduced in 10 mmol/l DTT, and alkylated with 50 mmol/l iodoacetamide. The gel plugs were washed and dehydrated again with 100% ACN, and then incubated for 14–16 h at 37°C in digestion solution (10 ng/µl of lyophilized sequencing grade modified trypsin (Promega, USA). The resulting peptides were acidified in extraction solution (1% (v/v) formic acid (FA, Sigma Aldrich) in 5% (v/v) ACN) followed by dehydration of the gel pieces in 70% ACN. The tryptic peptides were analyzed by automated nanoflow reverse-phase chromatography using the nanoAcquity UPLC system coupled to a Q-TOF Premier (Waters, UK) as described earlier (Skultety *et al.*, 2011). The data were processed using the ProteinLynx Global Server (PLGS) v. 3.0 (Waters, UK). All data were lock spray calibrated against [Glu1]-Fibrinopeptide B (Sigma Aldrich, USA). The results were searched against the full proteome sequences derived from *C. burnetii* Nine Mile phase I which was downloaded from UniProt (in October 2016). Tentative peptide and protein identifications were ranked, and scored by their relative correlation to a number of well-established models of known and empirically derived physicochemical attributes of proteins and peptides. During database searches, one missed cleavage site was allowed. The precursor peptide mass tolerance was set to ± 20 ppm, and fragment mass tolerance to ± 40 ppm. The search was performed with Cys carbamidomethylation and Met oxidation as fixed and variable modifications, respectively. A minimum of two matched peptides and three or more consecutive fragment ions from the same series were required for protein identification. Protein identifications were accepted after manual inspection of probabilistic based PLGS assignment at 95% confidence level. Only those proteins are listed in the tables which were found at least twice out of three replicates.

Results and Discussion

In order to reveal protein composition of the soluble antigen used as a chemovaccine, the purified *C. burnetii* cells in phase I was subjected to extraction with TCA. The proteins were then resolved within the pI range 3–10 and mass range 15–150 kDa using 2-DE. The analyses were performed on samples prepared in two biological replicates. A representative gel is shown in Fig. 1a. Image analysis revealed 82 protein spots which were reproducibly detected on CBB-stained gels.

The protein spots were excised from the gels, digested with trypsin, and analyzed by LC-MS/MS. Subsequently, data were processed by PLGS and the experimentally recorded MS spectra matched against the fully sequenced *C. burnetii* Nine Mile phase I database. Specifically, 39 proteins were identified (Table 2). The 10 kDa chaperonin was found in six (4, 5, 70, 71, 72, 73) spots, the hypothetical exported protein CBU_1095 was identified in five (14, 16, 17, 58, 59) spots, and the Tol system periplasmic component ybgF (52, 53, 54, 62), uncharacterized protein CBU_0089a (12, 19, 71, 72), and 50S ribosomal protein L7/L12 (6, 7, 74, 75) in four spots. The others were recognized in less than 3 spots. Most of the identified proteins (42%) were associated with cytoplasm, 17% with the periplasm, 8% with the inner membrane, and 33% with the outer membrane of the bacterium (Fig. 2a). Interestingly, as many as 41% of the identified immunoreactive proteins may have moonlighting activity.

Table 2. Identified proteins resolved from the TCA extract of *C. burnetii* cells

Protein function	Accession	Gene locus	Description	MW (kDa)	pI (pH)	Spot number	PLGS Score	Peptides	Coverage (%)	Localisation	Immunreactive
M	B5QS73	CBU_0089a	Uncharacterized protein CBU_0089a	12	10,3	12, 19, 71, 72	3806	10	53	U	
T	Q83F57	CBU_0092	Tol system periplasmic component ybgF	34	6,5	52, 53, 54, 62	3015	25	30	P	X
U	Q83F41	CBU_0110	Hypothetical exported protein CBU_0110	18	9,3	36, 39, 40	9472	25	38	U	
U	Q83F37	CBU_0114	UPF0234 protein CBU_0114	18	9,0	24	1157	14	34	C	
A	Q83F36	CBU_0115	Transcriptional regulator MraZ	17	5,1	47	4241	16	41	C	
D	Q83AQ8	CBU_0182	Superoxide dismutase [Cu-Zn] sodC	21	9,6	44, 45	2611	8	38	P	
T	Q83ET5	CBU_0225	Transcription termination/antitermination protein NusG	22	8,9	25, 56	2581	10	29	C	
R	P0C8S3	CBU_0229	50S ribosomal protein L7/L12	13	4,5	6, 7, 74, 75	16932	19	37	C	
R	Q83EL5	CBU_0304	Translation initiation inhibitor	16	9,0	11	2978	12	14	C	
E	Q83EL2	CBU_0307	Outer membrane protein CBU_0307	25	10,1	49, 51	4491	21	44	O	X
U	B5QS99	CBU_0562a	Uncharacterized protein CBU_0562a	15	6,9	9, 71, 72	13213	23	35	U	
J	Q83DT1	CBU_0612	Outer membrane protein ompH	19	10,2	41, 42, 43	28438	53	68	P, O	
J	P51752	CBU_0630	Peptidyl-prolyl cis-trans isomerase Mip	26	10,2	50, 66	1367	18	31	O	X
R	Q83D76	CBU_0864	30S ribosomal protein S6	15	7,9	20	2435	15	36	C	
R	Q83D73	CBU_0867	50S ribosomal protein L9	17	6,2	10	1539	8	30	C	
T	Q83D29	CBU_0915	Enhanced entry protein enhB.1	19	8,7	22, 23	5305	16	23	U(MA)	
D	Q83D04	CBU_0943	Rhodanese-related sulfurtransferase	17	8,8	35	2223	13	38	U	
J	Q83CZ8	CBU_0952	adA protein	26	9,3	28, 29, 33	5256	37	41	O	X
D	Q83CY8	CBU_0963	Putative peroxiredoxin bcp	17	8,0	21	9546	22	45	C (MA)	
U	Q83CL9	CBU_1095	Hypothetical exported protein CBU_1095	20	7,1	14, 16, 17, 58, 59	15173	18	36	U	
U	Q83CI0	CBU_1135	Hypothetical exported protein CBU_1135	16	9,3	41	6194	9	57	S	
M	Q83C87	CBU_1241	Malate dehydrogenase mdh	35	4,9	54	2022	13	20	C	X
J	O87712	CBU_1290	Chaperone protein DnaK	71	5,0	1	1067	21	25	C (MA)	X
T	Q83C41	CBU_1293	Protein GrpE	23	5,2	48	3338	18	27	C	
A	Q9X5U9	CBU_1385	Elongation factor Ts	32	5,8	61, 63	2466	17	35	C	
A	Q83BN9	CBU_1464	DNA-binding protein HU	10	10,0	39, 40	16895	16	40	C	
T	Q83BI9	CBU_1519	Protein-export protein SecB	18	4,3	46	1303	6	19	I	X
U	Q83BB8	CBU_1594	GatB/YqeY domain protein	17	5,9	13	3823	8	26	C	
T	Q83B63	CBU_1652	IcmX protein	41	6,0	65	1092	11	17	U	
U	Q83B41	CBU_1677	Hypothetical cytosolic CBU_1677	17	6,2	15, 18, 57	4250	23	75	C	
J	P19685	CBU_1708	Superoxide dismutase [Fe] sodB	22	6,2	26, 27	3427	12	26	P (MA)	X
M	Q83B07	CBU_1715	Glycine cleavage system H protein gcvH	15	3,8	55, 67	5919	8	44	C	X
J	P19421	CBU_1718	60 kDa chaperonin GroEL	58	5,0	68	3910	46	48	C (MA)	X
T	P19422	CBU_1719	10 kDa chaperonin	10	5,0	4, 5, 70, 71, 72, 73	18554	16	99	C	
M	Q83AV0	CBU_1778	Fructose-bisphosphate aldolase fabA	40	5,3	76, 77	687	8	25	C (MA)	X
U	B5QSG2	CBU_1847b	Uncharacterized protein CBU_1847b	13	8,5	37, 38	18406	33	53	U	
M	Q83AI4	CBU_1902	Peptidase, M16 family	52	6,3	64	1120	10	20	P	
J	H7C7D7	CBU_1910	Outer membrane protein com1	28	9,4	30, 31, 32	7924	20	31	O	X
U	Q83A32	CBU_2079	Uncharacterized protein CBU_2079	14	8,6	34	3902	16	19	U	

The highest PLGS score, coverage, and numbers of peptides identified are shown. Localization: C = cytoplasmic, MA = protein with potential moonlighting activities, O = outer membrane protein, I = inner membrane, P = periplasmic protein, U = unknown. Immunoreactive proteins: X = present. Function: M = metabolic pathway, E = cell envelope integrity, T = protein & peptide secretion & trafficking, J = pathogenicity & pathogenesis, A = transcription, D = detoxification, R = translation.

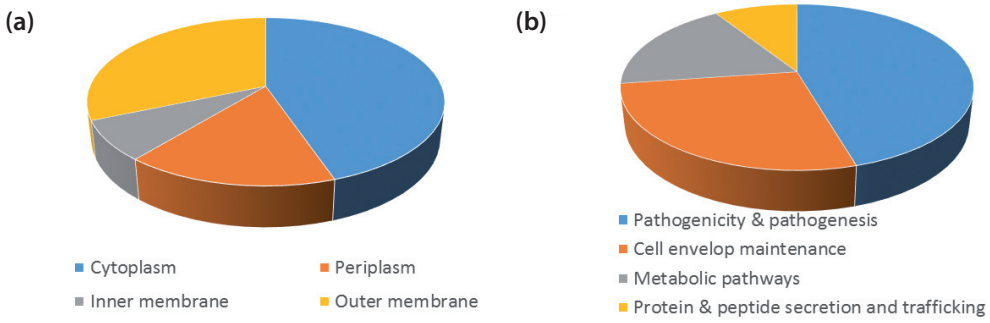

Fig. 2

Pie diagram of identified immunoreactive proteins extracted by TCA from *C. burnetii*
(a) Cell compartment localization; (b) Functional ontology.

The function was assigned to all identified proteins using UniProt database and then sorted into four groups (Fig. 2b). Proteins involved in pathogenicity & pathogenesis were the most frequent (41.66%), the second group corresponds to the cell envelope maintenance (25%), followed by metabolic pathways (16.66%). The last group is formed by proteins involved in protein & peptide secretion and trafficking (8.3%) (Fig. 2b).

Immunoblot analysis detected 12 immunoreactive proteins (Fig. 1b, Table 2). Interestingly, four of them may have moonlighting activity. All these proteins have been previously reported in other studies (Flores-Ramirez *et al.*, 2017; Chen *et al.*, 2011; Papadioti *et al.*, 2011; Sekeyova *et al.*, 2009; Beare *et al.*, 2008; Jian *et al.*, 2014, Toman *et al.*, 2013). These include the general markers for Q fever chaperones 60 kDa (Groel) and the chaperonin dnaK (Xiong *et al* 2012) as well as the virulent factors (ompH and Mip), markers for acute Q fever (the omp's AdA and com1), and another stress protein with moonlighting activity (superoxide dismutase [Fe] sodB) that are involved in pathogenesis and pathogenicity (Flores-Ramirez *et al.*, 2014; Skultety *et al.*, 2011). The component of the tol-pal system ybgF which mediates the peptidoglycan synthesis and outer membrane constriction during cell division (Deringer *et al.*, 2011) and the marker of acute Q fever (omp-A like protein CBU_0307) participate in the stabilization and maintenance of the cell envelope. The secB is engaged in protein trafficking. The glycine cleavage system protein H (gcvH), malate dehydrogenase (mdh), and fructose-bisphosphate aldolase (fabA) are metabolic enzymes. The last one is involved in the glycolysis and gluconeogenesis with virulence function in several bacteria (Shams *et al.*, 2014).

Conclusion

In this work proteomic approach based on 2-D electrophoresis, mass spectrometry, and bioinformatics was employed to identify and characterize the protein composition of the soluble antigen possessing phase I antigen reactivity that was used as a chemovaccine against Q fever in men. It was successfully administered in the past to more than 1700 volunteers. The vaccine was prepared from *C. burnetii* cells of strain Nine Mile EP3 in phase I using TCA extraction under the optimal conditions.

The proteomic analysis resulted in the identification of 39 unique proteins of *C. burnetii* from which 12 were recognized as immunoreactive. The localization and function of these proteins were assigned by bioinformatics. It was suggested that the identified proteins are primarily extracted from the surface of the *C. burnetii* cells. These proteins may form a part of the active components of the vaccine, which challenges the immune system of the host to generate antibodies that can fight the disease. Nevertheless, further studies have to be performed to analyze the proteins and the saccharide moiety of the extract which is involved in inducing protection. Because, some proteins, if included in the vaccine, may be immunosuppressive, whereas in other cases immune responses to some proteins may actually enhance disease. Thus, it is critical to identify those proteins that are essential for inducing protection and eliminate the others.

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