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Data Article

Proteomics data of ovine mastitis associated with *Mannheimia haemolytica*



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ABSTRACT

Proteomics data have been obtained from experimental mastitis in ewes after intramammary challenge with *Mannheimia haemolytica*. Animals were sampled before and sequentially after challenge; blood plasma and milk whey samples were produced and were subjected to proteomics evaluation by means of two-dimensional gel electrophoresis and MALDI-TOF mass spectrometry. Full protein maps and differential proteomics in sequential samples from blood plasma and milk whey of experimental ewes were presented. Post-challenge, 33 and 89 proteins were identified with differential abundance in blood plasma and milk whey, respectively. Also, 74 proteins were identified with differential abundance between the inoculated and contralateral glands. The data provide further insight in the pathogenesis of mastitis in sheep and indicate potential biomarkers for the disease. The data are further discussed in the research article "Differential quantitative proteomics study of experimental *Mannheimia haemolytica* mastitis in sheep" [1].

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Specifications table

Subject area	Veterinary Science and Veterinary Medicine
More specific subject area	Sheep, Infectious Diseases
Type of data	Tables, figures
How data was acquired	Experimental infection of ewes Proteomics analysis by 2-DE and MALDI-TOF MS Matching of peptides and protein searches in MASCOT Server 2 software, under 'mammalia', excluding 'Homo sapiens' and 'rodents', in the Swiss-Prot database
Data format	Raw and analysed
Experimental factors	Blood plasma and milk whey samples from ewes with experimental mastitis, collected and analysed, in order to characterise the differential abundance of proteins during mastitis
Experimental features	Differential proteomics in sequential samples of blood plasma and milk whey collected from sheep with mastitis
Data source location	Karditsa, Greece, Veterinary Faculty, University of Thessaly
Data accessibility	All data are with article
Related research article	A.I. Katsafadou, G.T. Tsangaris, A.K. Anagnostopoulos C. Billinis, M.S. Barbagianni, N.G.C. Vasileiou, S.A. Spanos, V.S. Mavrogianni, G.C. Fthenakis, Differential quantitative proteomics study of experimental Mannheimia haemolytica mastitis in sheep. <i>J. Proteom.</i> 2019, in press

Value of the data

- Proteomics data from blood plasma and milk whey samples from ewes with experimental mastitis caused by *Mannheimia haemolytica* and the differential proteomics in sequential samples after challenge are presented for the first time.
- The data can be used for comparison to proteomics data from mastitis caused by other pathogens.
- Potential biomarkers can be selected among the proteins identified for improved diagnosis of the infection.

1. Data

After intramammary challenge of ewes with *Mannheimia haemolytica*, mastitis was induced, which was confirmed by clinical, microbiological, cytological and histopathological (Fig. 1) findings. Details of proteins obtained from blood plasma (Table 1, Fig. 2) and milk whey (Table 2, Fig. 3) samples collected before, as well as from milk whey samples obtained 12 h after (Table 3, Fig. 4) intramammary challenge with *Mannheimia haemolytica* were presented [1]. In the blood plasma and milk whey samples collected before inoculation, 19 and 40 proteins, respectively, were identified. In the milk whey sample collected post-inoculation, 65 proteins were identified.

Additionally, tabulated lists of observed proteins with changes in abundance, in post-challenge samples of blood plasma (Table 4), of milk whey of the inoculated side of the udder (Table 5) and of milk whey of the contralateral side of the udder (Table 6), collected sequentially, were also presented. In total, 33, 89 and 20 proteins with differential abundance were identified in the respective samples. Changes in protein abundance were separated into different classifications: (i) decrease (6, 18 and 1 proteins, respectively), (ii) new appearance (13, 53 and 8 proteins, respectively), (iii) increase (0, 3 and 0 proteins, respectively) and (iv) varying abundance (14, 15 and 11 proteins, respectively).

Finally, 79 proteins with differential abundance were identified between milk whey samples collected from the inoculated (74 proteins) and contralateral (5 proteins) glands of the experimental ewes (Table 7).

2. Experimental design, materials and methods

After intramammary inoculation of one mammary gland of ewes ($n = 5$) with *Mannheimia haemolytica* (1000–1250 cfu) performed on Day-0 (D0), mastitis was induced, as confirmed by clinical,

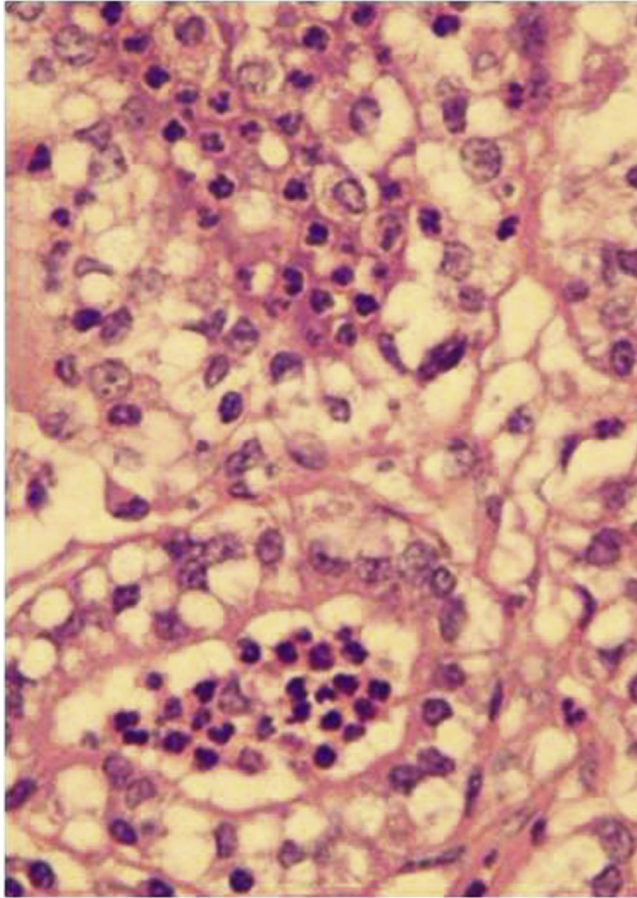


Fig. 1. Histological section of mammary parenchyma, from inoculated side of the udder on D2, with marked intra-alveolar neutrophilic infiltration and destruction of mammary alveoli.

Table 1

Details of all proteins identified in a blood plasma sample from one ewe, before deposition of *M. haemolytica* into one teat (identification by MALDI-TOF MS)^a.

Accession no.	Accession name	Description name	Theoretical MW	Theoretical pl	MASCOT score	Matched peptides	Coverage (%)
P12725	A1AT_SHEEP	Alpha-1-antiproteinase	46298	5.80	95	17/103	37
P15497	APOA1_BOVIN	Apolipoprotein A-I	30258	5.60	184	23/134	61
Q32PJ2	APOA4_BOVIN	Apolipoprotein A-IV	42991	5.20	100	18/129	49
P17690	APOH_BOVIN	Beta-2-glycoprotein 1	39538	9.70	66	11/139	46
Q9XT27	CERU_SHEEP	Ceruloplasmin	120020	5.44	54	14/104	24
P02676	FIBB_BOVIN	Fibrinogen beta chain	53933	9.20	76	27/160	37
P12799	FIBG_BOVIN	Fibrinogen gamma-B chain	50839	5.50	60	12/109	27
Q3SX14	GELS_BOVIN	Gelsolin	80966	5.50	112	20/98	36
P0CH25	HBA1_CAPHI	Haemoglobin subunit alpha-1	15212	9.40	50	5/61	52
P02075	HBB_SHEEP	Haemoglobin subunit beta	16120	6.90	132	12/71	86
P02077	HBBA_CAPHI		16068	6.91	61	7/61	46

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Table 1 (continued)

Accession no.	Accession name	Description name	Theoretical MW	Theoretical pI	MASCOT score	Matched peptides	Coverage (%)
		Haemoglobin subunit beta-A					
P68056	HBBC_SHEEP	Haemoglobin subunit beta-C	15681	8.09	51	6/61	40
B6E141	HPT_CAPIB	Haptoglobin	45411	9.10	108	18/99	32
P81286	PLMN_SHEEP	Plasminogen (fragment)	38664	8.80	73	12/110	39
P18902	RET4_BOVIN	Retinol-binding protein 4	21397	5.30	54	9/102	51
Q29443	TRFE_BOVIN	Serotransferrin	79870	6.90	103	23/128	33
P14639	ALBU_SHEEP	Serum albumin	71139	5.80	193	34/154	56
P42819	SAA_SHEEP	Serum amyloid A protein	12680	6.10	65	8/95	53
P12303	TTHY_SHEEP	Transthyretin	15875	5.50	81	8/75	66

MS: mass spectrometre, MW: molecular weight, pI: isoelectric point.

^a Details of 'full protein map' performed in the sample are presented.

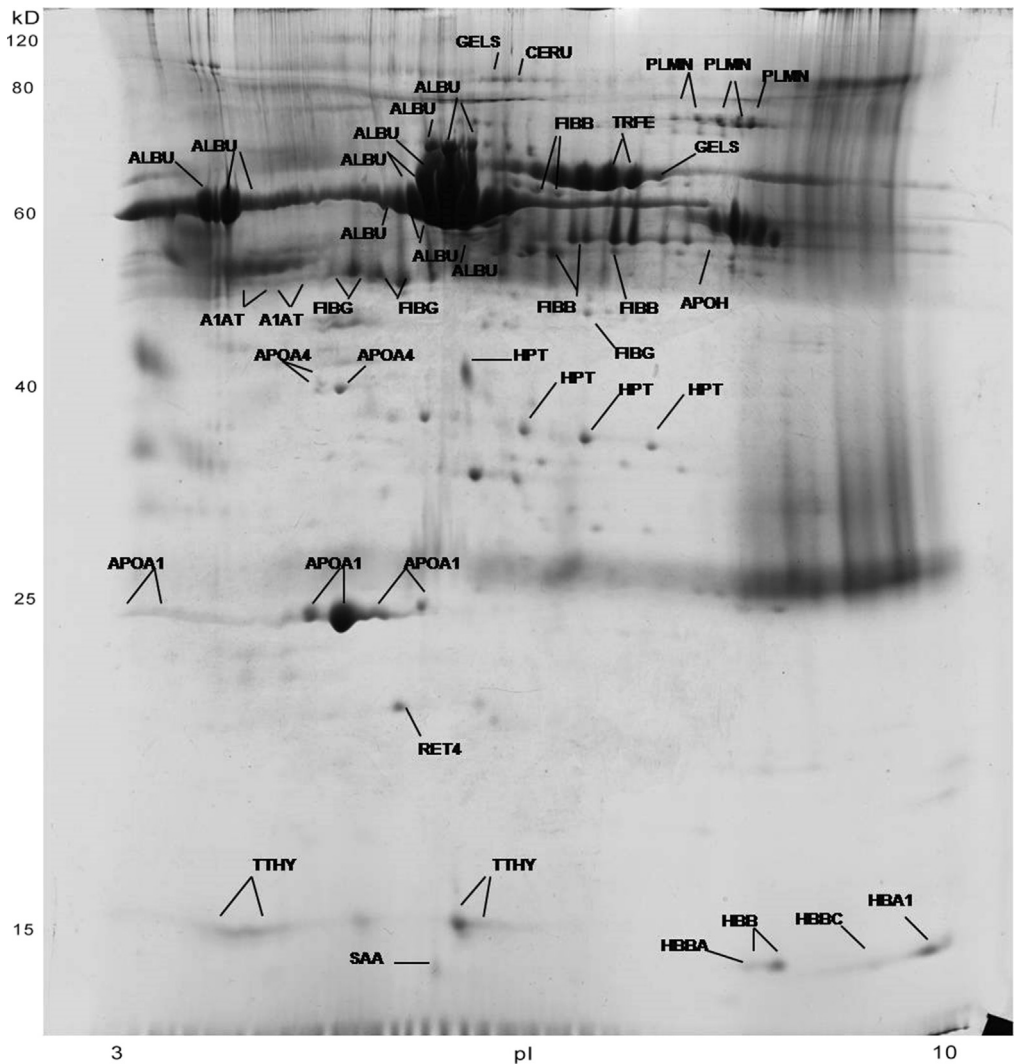


Table 2

Details of all proteins identified in two milk whey samples from two ewes, before deposition of *M. haemolytica* into one teat of each animal (identification by MALDI-TOF MS)^a.

Accession no.	Accession name	Description name	No. ewes ^b	Theoretical MW	Theoretical pI	MASCOT score	Matched peptides	Coverage (%)
P60713	ACTB_SHEEP	Actin, cytoplasmic 1	1	42052	5.18	74	11/79	45
P63258	ACTG_BOVIN	Actin, cytoplasmic 2	1	42108	5.20	75	11/72	37
P12725	A1AT_SHEEP	Alpha-1-antitrypsin	1	46298	5.80	127	15/60	34
P29701	FETUA_SHEEP	Alpha-2-HS-glycoprotein	1	39511	5.10	58	8/58	31
P09462	LALBA_SHEEP	Alpha-lactalbumin	2	16761	4.66	84	11/54	35
P04653	CASA1_SHEEP	Alpha-S1-casein	2	24347	5.20	63	8/75	46
P04654	CASA2_SHEEP	Alpha-S2-casein	2	26486	8.70	71	11/78	47
P15497	APOA1_BOVIN	Apolipoprotein A-I	1	30258	5.60	148	18/89	57
Q32PJ2	APOA4_BOVIN	Apolipoprotein A-IV	1	42991	5.20	66	11/84	37
P00829	ATPB_BOVIN	ATP synthase subunit beta, mitochondrial	1	56249	5.00	51	10/87	25
Q6QAT4	B2MG_SHEEP	Beta-2-microglobulin	1	13570	6.10	56	5/37	44
P33048	CASB_CAPHI	Beta-casein	1	24906	5.10	54	8/67	34
Q1KYT0	ENOB_PIG	Beta-enolase	1	47443	8.93	69	44/177	52
P67976	LACB_SHEEP	Beta-lactoglobulin-1/B	2	20308	5.34	115	16/87	59
Q551S4	CAH3_PIG	Carbonic anhydrase 3	1	29678	8.76	62	44/177	55
A2VDP1	BRE1A_BOVIN	E3 ubiquitin-protein ligase	1	114272	5.60	60	15/86	21
P10790	FABPH_BOVIN	Fatty acid-binding protein, heart	1	14827	7.66	57	6/48	41
P02676	FIBB_BOVIN	Fibrinogen beta chain	1	53933	9.20	106	19/96	35
P00883	ALDOA_RABIT	Fructose-bisphosphate aldolase A	1	39774	9.20	50	12/139	36
O18751	PYGM_SHEEP	Glycogen phosphorylase, muscle form	1	97702	6.70	99	50/196	44
P01977	HBA1_TACAC	Haemoglobin subunit alpha-1	1	15509	9.50	51	5/58	46
P02102	HBE1_CAPHI	Haemoglobin subunit epsilon-1	1	16117	9.50	52	5/61	41
P19120	HSP7C_BOVIN	Heat shock cognate 71 kDa protein	1	71424	5.24	86	14/196	34
P13943	MMP1_RABIT	Interstitial collagenase	1	53877	6.30	50	9/62	20
P02669	CASK_SHEEP	Kappa-casein	1	21596	5.80	55	7/69	35
Q6EIZ0	K1C10_CANFA	Keratin, type I cytoskeletal 10	1	57847	4.90	69	16/110	25
A5JUY8	PERL_BUBBU	Lactoperoxidase	1	81559	9.70	53	16/101	24
Q29477	TRFL_CAPHI	Lactotransferrin	2	79361	9.50	194	32/127	49
P00339	LDHA_PIG	L-lactate dehydrogenase A chain	1	36880	9.10	65	18/127	43
Q8MJV0	MYH1_HORSE	Myosin-1	1	223772	5.49	51	17/127	13
Q9BE39	MYH7_BOVIN	Myosin-7	1	223889	5.50	59	23/78	14
P08049	NEP_RABIT	Nephrilysin	1	86212	5.20	50	9/58	15
P81265	PIGR_BOVIN	Polymeric immunoglobulin receptor	1	83695	7.70	51	11/68	14
P11979	KPYM_FELCA	Pyruvate kinase	1	58522	7.90	79	38/154	40
P28327	RK_BOVIN	Rhodopsin kinase	1	63464	5.90	50	9/64	23
Q29443	TRFE_BOVIN	Serotransferrin	1	79870	6.90	72	14/70	24
P14639	ALBU_SHEEP	Serum albumin	2	71139	5.80	292	38/96	58
P12303	TTHY_SHEEP	Transthyretin	1	15875	5.50	50	5/63	55

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Fig. 2. 2-DE gel with annotation of representative protein spots, obtained from a blood plasma sample collected from one ewe before intramammary inoculation with *M. haemolytica* (protein identification by MALDI-TOF MS). A1AT: Alpha-1-antitrypsin, ALBU: Serum albumin, APOA1: Apolipoprotein A-I, APOA4: Apolipoprotein A-IV, APOH: Beta-2-glycoprotein 1, CERU: Ceruloplasmin, FIBB: Fibrinogen beta chain, FIBG: Fibrinogen gamma-B chain, GELS: Gelsolin, HBA1: Haemoglobin subunit alpha-1, HBB: Haemoglobin subunit beta, HBBA: Haemoglobin subunit beta-A, HBBC: Haemoglobin subunit beta-C, HPT: Haptoglobin, PLMN: Plasminogen, RET4: Retinol-binding protein 4, SAA: Serum amyloid A protein, TRFE: Serotransferrin, TTHY: Transthyretin.

Table 2 (continued)

Accession no.	Accession name	Description name	No. ewes ^b	Theoretical MW	Theoretical pI	MASCOT score	Matched peptides	Coverage (%)
A4UMC5	TFP11_RABIT	Tuftelin-interacting protein 11	1	96645	5.56	52	6/63	17
Q3MHN5	VTDB_BOVIN	Vitamin D-binding protein	1	54904	5.20	60	10/69	27

MS: mass spectrometre, MW: molecular weight, pI: isoelectric point.

^a Combined details of 'full protein map' performed in the sample are presented.

^b No. of ewes in samples of which the respective protein was identified.

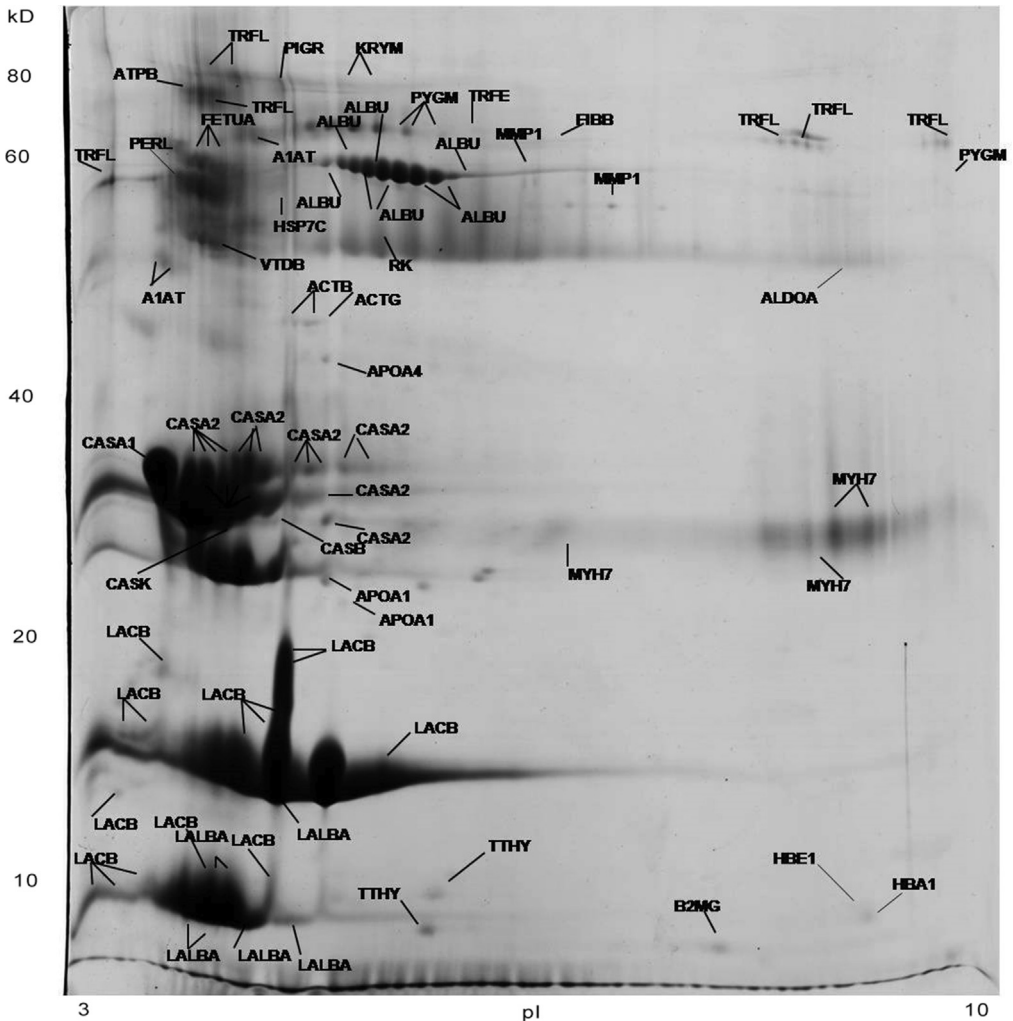


Fig. 3. 2-DE gel with annotation of representative protein spots, obtained from a milk whey sample from one ewe before intramammary inoculation with *M. haemolytica* (protein identification by MALDI-TOF MS). A1AT: Alpha-1-antiproteinase, ACTB: Actin, cytoplasmic 1, ACTG: Actin, cytoplasmic 2, ALBU: Serum albumin, ALDOA: Fructose-bisphosphate aldolase A, APOA1: Apolipoprotein A-I, APOA4: Apolipoprotein A-IV, B2MG: Beta-2-microglobulin, CASA1: Alpha-S1-casein, CASA2: Alpha-S2-casein, CASK: Kappa-casein, FIBB: Fibrinogen beta chain, HBA1: Haemoglobin subunit alpha-1, HBE1: Haemoglobin subunit epsilon-1, HSP7C: Heat shock cognate 71 kDa protein, KPYM: Pyruvate kinase, LACB: Beta-lactoglobulin-1/B, LALBA: Alpha-lactalbumin, MMP1: Interstitial collagenase, MYH7: Myosin-7, PERL: Lactoperoxidase, PIGR: Polymeric immunoglobulin receptor, PYGM: Glycogen phosphorylase, muscle form, RK: Rhodopsin kinase, TRFE: Serotransferrin, TRFL: Lactotransferrin, TTHY: Transthyretin, VTDB: Vitamin D-binding protein.

Table 3

Details of all proteins identified in a milk whey sample from the inoculated side of the udder of one ewe, 12 h after deposition of *M. haemolytica* into the ipsilateral teat of that animal (identification by MALDI-TOF MS)^a.

Accession no.	Accession name	Description name	Theoretical MW	Theoretical pI	MASCOT score	Matched peptides	Coverage (%)
Q0VCX2	GRP78_BOVIN	78 kDa glucose-regulated protein	72470	4.90	62	8/32	19
P60713	ACTB_SHEEP	Actin, cytoplasmic 1	42052	5.20	127	13/47	45
P63258	ACTG_BOVIN	Actin, cytoplasmic 2	42108	5.20	127	13/47	46
ASD7D1	ACTN4_BOVIN	Alpha-actinin-4	105319	5.20	95	16/55	22
Q9XSJ4	ENOA_BOVIN	Alpha-enolase	47639	6.40	181	21/68	60
P09462	LALBA_SHEEP	Alpha-lactalbumin	16761	4.66	135	10/37	45
P04653	CASA1_SHEEP	Alpha-S1-casein	24347	5.20	66	6/27	29
P04654	CASA2_SHEEP	Alpha-S2-casein	26486	8.70	112	10/30	38
P15497	APOA1_BOVIN	Apolipoprotein A-I	30258	5.60	118	14/65	46
Q32PJ2	APOA4_BOVIN	Apolipoprotein A-IV	42991	5.20	115	15/66	41
Q6QAT4	B2MG_SHEEP	Beta-2-microglobulin	13570	6.10	50	4/24	35
Q3ZC09	ENOB_BOVIN	Beta-enolase	47409	8.63	61	21/68	29
P67976	LACB_SHEEP	Beta-lactoglobulin-1/B	20308	5.34	137	13/65	85
E1BFE9	BI2L2_BOVIN	Brain-specific angiogenesis inhibitor 1-associated protein 2	59153	10.10	51	5/21	13
P54230	CTHL1_SHEEP	Cathelicidin-1	18036	9.30	136	11/51	55
P79362	CTHL2_SHEEP	Cathelicidin-2	20057	10.89	76	6/24	34
P49929	SC52_SHEEP	Cathelin-related peptide SC5	17959	10.10	50	5/26	25
Q6B7M7	COF1_SHEEP	Cofilin-1	18792	9.13	83	7/22	51
P31976	EZRI_BOVIN	Ezrin	68832	6.00	50	9/34	12
A4FU48	CAZA1_BOVIN	F-actin-capping protein subunit alpha-1	33082	5.50	98	9/37	48
P02676	FIBB_BOVIN	Fibrinogen beta chain	53933	9.20	60	13/67	24
Q9ITY8	GSTP1_CAPHI	Glutathione S-transferase P	23843	8.80	82	6/20	47
Q28554	G3P_SHEEP	Glyceraldehyde-3-phosphate dehydrogenase (fragment)	36073	9.30	72	8/39	30
P02075	HBB_SHEEP	Haemoglobin subunit beta	16120	6.90	54	4/19	39
B6E141	HPT_CAPIB	Haptoglobin	45411	9.10	53	7/39	19
P19120	HSP7C_BOVIN	Heat shock cognate 71 kDa protein	71082	5.24	55	10/59	23
Q3T149	HSPB1_BOVIN	Heat shock protein beta-1	22436	6.00	97	8/29	44
Q76LV2	HS90A_BOVIN	Heat shock protein HSP 90-alpha	85077	4.78	94	20/70	27
Q9GKX8	HS90B_HORSE	Heat shock protein HSP 90-beta	83527	4.82	77	17/70	26
P13943	MMP1_RABIT	Interstitial collagenase	53877	6.30	52	8/50	17
Q6XUZ5	IDHC_SHEEP	Isocitrate dehydrogenase (NADP) cytoplasmic	47153	6.40	54	7/32	19
P02669	CASK_SHEEP	Kappa-casein	21596	5.80	68	8/47	35
Q6EIZ0	K1C10_CANFA	Keratin, type I cytoskeletal 10	57847	4.90	78	15/85	23
A5JUY8	PERL_BUBBU	Lactoperoxidase	81559	9.70	52	10/47	18
Q29477	TRFL_CAPHI	Lactotransferrin	79361	9.50	120	18/70	31
Q50KA9	NDKA_CANFA	Nucleoside diphosphate kinase A	17283	5.70	53	4/14	31
Q3T0Q4	NDKB_BOVIN	Nucleoside diphosphate kinase B	17419	9.00	55	5/28	36
O77834	PRDX6_BOVIN	Peroxiredoxin-6	25108	6.00	85	7/21	31
P13696	PEBP1_BOVIN	Phosphatidylethanolamine-binding protein 1	21087	7.70	52	5/35	39
Q3T0P6	PGK1_BOVIN	Phosphoglycerate kinase 1	44973	9.50	50	6/29	22
Q3S262	PGAM1_BOVIN	Phosphoglycerate mutase 1	28948	6.80	113	9/24	54
Q3SZJ9	PMM2_BOVIN	Phosphomannomutase 2	28435	6.00	52	5/26	25
P05307	PDIA1_BOVIN	Protein disulfide-isomerase	57629	4.70	64	10/54	24
P38657	PDIA3_BOVIN	Protein disulfide-isomerase A3	57293	6.20	141	18/52	35
P28783	S10A9_BOVIN	Protein S100-A9	17160	6.30	50	6/57	44
P80601	UK114_CAPHI	Ribonuclease	14347	7.10	81	7/52	82
Q29443	TRFE_BOVIN	Serotransferrin	79870	6.90	89	19/103	31
P14639	ALBU_SHEEP	Serum albumin	71139	5.80	205	23/49	40
P42819	SAA_SHEEP	Serum amyloid A protein	12680	6.10	105	9/45	58
Q3ZBH0	TCPB_BOVIN	T-complex protein 1 subunit beta	57781	6.20	64	8/35	25
O19011	TGFB1_HORSE	Transforming growth factor beta-1	44631	9.72	50	14/39	18
Q5E956	TPIS_BOVIN	Triosephosphate isomerase	26901	6.50	181	6/39	78
Q5KR47	TPM3_BOVIN	Tropomyosin alpha-3 chain	32856	4.53	69	11/58	28

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Table 3 (continued)

Accession no.	Accession name	Description name	Theoretical MW	Theoretical pI	MASCOT score	Matched peptides	Coverage (%)
P81947	TBA1B_BOVIN	Tubulin alpha-1B chain	50804	4.81	81	12/43	40
Q2HJ86	TBA1D_BOVIN	Tubulin alpha-1D chain	50935	4.77	61	12/67	34
Q32KN8	TBA3_BOVIN	Tubulin alpha-3 chain	50578	4.84	60	10/67	33
P81948	TBA4A_BOVIN	Tubulin alpha-4A chain	50634	4.79	69	11/67	36
P02554	TBB_PIG	Tubulin beta chain	50285	4.64	185	19/60	55
Q6B856	TBB2B_BOVIN	Tubulin beta-2B chain	50377	4.64	173	17/60	53
Q3MHM5	TBB4B_BOVIN	Tubulin beta-4B chain	50255	4.65	153	14/60	45
Q2KJD0	TBB5_BOVIN	Tubulin beta-5 chain	50095	4.60	214	25/65	61
Q2HJ81	TBB6_BOVIN	Tubulin beta-6 chain	50324	4.60	61	11/61	22
A4UMC	TFP11_RABIT	Tuftelin-interacting protein 11	96645	5.60	50	13/71	18
P26234	VINC_PIG	Vinculin	124437	5.50	79	14/47	19
Q3MHN5	VTDB_BOVIN	Vitamin D-binding protein	54904	5.20	70	10/56	31

MS: mass spectrometre, MW: molecular weight, pI: isoelectric point.

^a Details of 'full protein map' performed in the sample are presented.

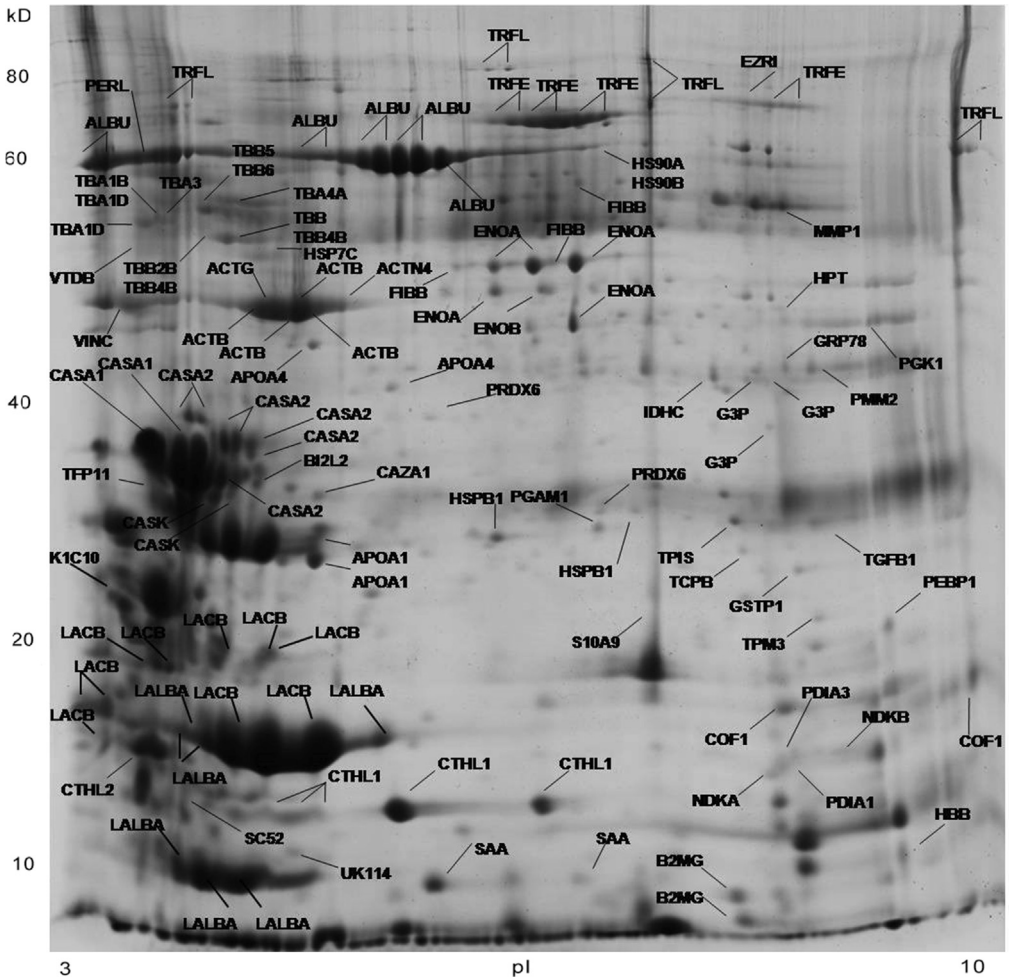


Table 4

Details of proteins observed with decrease, increase, new identification or varying abundance in blood plasma samples from five ewes, after deposition of *M. haemolytica* into one teat of each animal (identification by MALDI-TOF MS).

Accession no.	Accession name	Description name	Theoretical MW	Theoretical pI	MASCOT score	Matched peptides	Coverage (%)
Proteins that showed decrease (n = 6)							
P60713	ACTB_SHEEP	Actin, cytoplasmic 1	42052	5.18	96	14/91	50
P68240	HBA_SHEEP	Haemoglobin subunit alpha-1/2	15212	9.44	66	5/50	52
P02077	HBBA_CAPHI	Haemoglobin subunit beta-A	16068	6.91	80	5/31	48
P68056	HBBC_SHEEP	Haemoglobin subunit beta-C	15681	8.09	66	5/31	40
Q3SZV7	HEMO_BOVIN	Haemopexin	52974	8.90	66	10/56	16
A4UMC5	TFP11_RABIT	Tuftelin-interacting protein 11	96645	5.60	51	14/70	16
Proteins that showed new appearance (n = 13)							
P20757	ANGT_SHEEP	Angiotensinogen	51443	6.60	147	19/84	54
P32262	ANT3_SHEEP	Antithrombin-III	52979	6.50	92	14/64	36
P17690	APOH_BOVIN	Beta-2-glycoprotein 1	39538	9.70	59	8/69	37
Q2UUX4	CO3_BOVIN	Complement C3	188675	6.40	63	22/84	15
P81187	CFAB_BOVIN	Complement factor B	86737	8.80	72	17/97	20
Q29RL2	CHRD1_BOVIN	Cysteine and histidine-rich domain-containing protein 1	38144	8.69	51	8/97	30
Q58DT0	ELF5_BOVIN	ETS-related transcription factor Elf-5	30697	5.56	51	7/97	29
P12799	FIBG_BOVIN	Fibrinogen gamma-B chain	50839	5.50	63	11/82	24
P0CH25	HBA1_CAPHI	Haemoglobin subunit alpha-1	15212	9.40	66	5/25	52
B6E141	HPT_CAPIB	Haptoglobin	45411	9.10	116	15/61	30
P51744	IL4_CEREL	Interleucin-4	15602	10.60	51	6/78	42
Q9BE40	MYH1_BOVIN	Myosin-1	223772	5.50	55	15/69	14
Q9T5X9	PRDX6_PIG	Peroxiredoxin-6	25078	5.66	51	28/138	48
Proteins that showed varying abundance (n = 14)							
P12725	A1AT_SHEEP	Alpha-1-antitrypsin	46298	5.80	147	17/66	40
Q7SIH1	A2MG_BOVIN	Alpha-2-macroglobulin	168953	5.70	65	19/86	19
P15497	APOA1_BOVIN	Apolipoprotein A-I	30258	5.60	168	20/102	59
Q32PJ2	APOA4_BOVIN	Apolipoprotein A-IV	42991	5.20	134	20/102	47
Q9XT27	CERU_SHEEP	Ceruloplasmin	120020	5.40	122	19/60	26
P02676	FIBB_BOVIN	Fibrinogen beta chain	53933	9.20	76	21/103	31
Q3SX14	GELS_BOVIN	Gelsolin	80966	5.50	109	11/64	29
P02075	HBB_SHEEP	Haemoglobin subunit beta	16120	6.90	114	9/39	69
P81286	PLMN_SHEEP	Plasminogen (fragment)	38664	8.80	85	14/106	45
P18902	RET4_BOVIN	Retinol-binding protein 4	21397	5.30	100	10/60	67
Q29443	TRFE_BOVIN	Serotransferrin	79870	6.90	112	14/42	23
P14639	ALBU_SHEEP	Serum albumin	71139	5.80	244	31/82	48
P12303	TTHY_SHEEP	Transthyretin	15875	5.50	107	10/74	55
Q3MHNS	VTDB_BOVIN	Vitamin D-binding protein	54904	5.20	87	10/37	31

MS: mass spectrometry, MW: molecular weight, pI: isoelectric point.

Fig. 4. 2-DE gel with annotation of representative protein spots, from a milk whey sample from a mammary gland of one ewe 12 h after intramammary inoculation with *M. haemolytica* (protein identification by MALDI-TOF MS). ACTB: Actin, cytoplasmic 1, ACTG: Actin, cytoplasmic 2, ACTN4: Alpha-actinin-4, ALBU: Serum albumin, APOA1: Apolipoprotein A-I, APOA4: Apolipoprotein A-IV, B2MG: Beta-2-microglobulin, BIZL2: Brain-specific angiogenesis inhibitor 1-associated protein 2, CASA1: Alpha-S1-casein, CASA2: Alpha-S2-casein, CASK: Kappa-casein, CAZA1: F-actin-capping protein subunit alpha-1, COF1: Cofilin-1, CTHL1: Cathelicidin-1, CTHL2: Cathelicidin-2, ENOA: Alpha-enolase, ENOB: Beta-enolase, EZRI: Ezrin, FIBB: Fibrinogen beta chain, G3P: Glyceraldehyde-3-phosphate dehydrogenase, GRP78: 78 kDa glucose-regulated protein, GSTP1: Glutathione S-transferase P, HBB: Haemoglobin subunit beta, HPT: Haptoglobin, HS90A: Heat shock protein HSP 90-alpha, HS90B: Heat shock protein HSP 90-beta, HSP7C: Heat shock cognate 71 kDa protein, HSPB1: Heat shock protein beta-1, IDHC: Isocitrate dehydrogenase (NADP) cytoplasmic, K1C10: Keratin, type I cytoskeletal 10, LACB: Beta-lactoglobulin-1/B, LALBA: Alpha-lactalbumin, MMP1: Interstitial collagenase, NDKA: Nucleoside diphosphate kinase A, NDKB: Nucleoside diphosphate kinase B, PDIA1: Protein disulfide-isomerase, PDIA3: Protein disulfide-isomerase A3, PEBP1: Phosphatidylethanolamine-binding protein 1, PERL: Lactoperoxidase, PGAM1: Phosphoglycerate mutase 1, PGK1: Phosphoglycerate kinase 1, PMM2: Phosphomannomutase 2, PRDX6: Peroxiredoxin-6, S10A9: Protein S100-A9, SAA: Serum amyloid A protein, SC52: Cathelin-related peptide SC5, TBA1B: Tubulin alpha-1B chain, TBA1D: Tubulin alpha-1D chain, TBA3: Tubulin alpha-3 chain, TBA4A: Tubulin alpha-4A chain, TBB: Tubulin beta chain, TBB2B: Tubulin beta-2B chain, TBB4B: Tubulin beta-4B chain, TBB5: Tubulin beta-5 chain, TBB6: Tubulin beta-6 chain, TCPB: T-complex protein 1 subunit beta, TFP11: Tuftelin-interacting protein 11, TGFB1: Transforming growth factor beta-1, TPIS: Triosephosphate isomerase, TPM3: Tropomyosin alpha-3 chain, TRFE: Serotransferrin, TRFL: Lactotransferrin, UK114: Ribonuclease, VINC: Vinculin, VTDB: Vitamin D-binding protein.

Table 5

Details of proteins observed with decrease, new appearance, increase or varying abundance in milk whey samples from the inoculated side of the udder of five ewes, after deposition of *M. haemolytica* into the teat of each animal (identification by MALDI-TOF MS).

Accession no.	Accession name	Description name	Theoretical MW	Theoretical pI	MASCOT score	Matched peptides	Coverage (%)
Proteins that showed decrease (n = 18)							
P12725	A1AT_SHEEP	Alpha-1-antitrypsin	46298	5.80	92	13/76	32
P29701	FETUA_SHEEP	Alpha-2-HS-glycoprotein	39511	5.10	58	15/60	31
P00829	ATPB_BOVIN	ATP synthase subunit beta, mitochondrial	56249	5.00	51	10/87	25
P33048	CASB_CAPHI	Beta-casein	24906	5.10	54	8/67	34
P67976	LACB_SHEEP	Beta-lactoglobulin-1/B	20308	5.34	137	13/65	85
Q55154	CAH3_PIG	Carbonic anhydrase 3	29678	8.76	62	44/177	55
A2VDP1	BRE1A_BOVIN	E3 ubiquitin-protein ligase BRE1A	114272	5.60	60	15/86	21
P10790	FABPH_BOVIN	Fatty acid-binding protein, heart	14827	7.66	57	11/57	41
P00883	ALDOA_RABIT	Fructose-bisphosphate aldolase A	39774	9.20	50	19/96	36
O18751	PYGM_SHEEP	Glycogen phosphorylase, muscle form	97702	6.70	99	31/196	44
P01977	HBA1_TACAC	Haemoglobin subunit alpha-1	15509	9.50	51	5/58	46
P02102	HBE1_CAPHI	Haemoglobin subunit epsilon-1	16117	9.50	52	5/61	41
P00339	LDHA_PIG	L-lactate dehydrogenase A chain	36880	9.10	65	32/127	43
A5JUY8	PERL_BUBBU	Lactoperoxidase	81559	9.70	52	10/47	18
Q8MJV0	MYH1_HORSE	Myosin-1	223772	5.49	51	18/127	13
P08049	NEP_RABIT	Nephrilysin	86212	5.20	50	9/58	15
P11979	KPYM_FELCA	Pyruvate kinase	58522	7.90	79	38/154	40
P28327	RK_BOVIN	Rhodopsin kinase	63464	5.90	50	9/64	23
Proteins that showed new appearance (n = 53)							
Q0VCX2	GRP78_BOVIN	78 kDa glucose-regulated protein	72470	4.90	62	8/32	19
A5D7D1	ACTN4_BOVIN	Alpha-actinin-4	105319	5.20	95	16/55	22
Q9XSJ4	ENOA_BOVIN	Alpha-enolase	47639	6.40	181	21/68	60
Q3ZC09	ENOB_BOVIN	Beta-enolase	47409	8.63	61	10/68	29
E1BFE9	BI2L2_BOVIN	Brain-specific angiogenesis inhibitor 1-associated protein 2	59153	10.10	51	5/21	13
Q9TV13	CASP1_HORSE	Caspase-1	45815	6.06	50	9/63	27
P54230	CTHL1_SHEEP	Cathelicidin-1	18036	9.30	136	11/51	55
P79362	CTHL2_SHEEP	Cathelicidin-2	20057	10.89	76	6/24	34
P49929	SC52_SHEEP	Cathelin-related peptide SC5	17959	10.10	50	5/26	25
Q6TMG6	CH3L1_SHEEP	Chitinase-3-like protein 1	43209	9.60	98	13/60	38
Q6B7M7	COF1_SHEEP	Cofilin-1	18792	9.13	83	7/22	51
P31976	EZRI_BOVIN	Ezrin	68832	6.00	50	9/34	12
A4FUA8	CAZA1_BOVIN	F-actin-capping protein subunit alpha-1	33082	5.50	98	9/37	48
Q0VCH4	G3ST3_BOVIN	Galactose-3-O-sulfotransferase 3	49124	10.50	50	8/56	24
Q9TTY8	GSTP1_CAPHI	Glutathione S-transferase P	23843	8.80	82	6/20	47
Q28554	G3P_SHEEP	Glyceraldehyde-3-phosphate dehydrogenase (fragment)	36073	9.30	72	8/39	30
Q0VC84	C1GLT_BOVIN	Glycoprotein-N-acetylglucosamine 3-beta-galactosyltransferase	43469	6.00	50	6/29	22
P02075	HBB_SHEEP	Haemoglobin subunit beta	16120	6.90	54	4/19	39
B6E141	HPT_CAPIB	Haptoglobin	45411	9.10	54	7/39	22
Q3T149	HSPB1_BOVIN	Heat shock protein beta-1	22436	6.00	97	8/29	44
Q76LV2	HS90A_BOVIN	Heat shock protein HSP 90-alpha	85077	4.78	94	20/70	27
Q9GKX8	HS90B_HORSE	Heat shock protein HSP 90-beta	83527	4.82	77	17/70	26
Q6XUZ5	IDHC_SHEEP	Isocitrate dehydrogenase (NADP) cytoplasmic	47153	6.40	54	7/32	19
Q9BE39	MYH7_BOVIN	Myosin-7	85835	6.00	50	9/73	18
Q50KA9	NDKA_CANFA	Nucleoside diphosphate kinase A	17283	5.70	53	4/14	31
Q3T0Q4	NDKB_BOVIN	Nucleoside diphosphate kinase B	17419	9.00	55	5/28	36
O77834	PRDX6_BOVIN	Peroxiredoxin-6	25108	6.00	85	7/21	31
Q148K5	PX11B_BOVIN	Peroxisomal membrane protein 11B	28773	11.34	50	7/51	28
Q28177	BFSP2_BOVIN	Phakinin	46150	5.54	50	19/115	29
P13696	PEBP1_BOVIN	Phosphatidylethanolamine-binding protein 1	21087	7.70	52	5/35	39

Table 5 (continued)

Accession no.	Accession name	Description name	Theoretical MW	Theoretical pl	MASCOT score	Matched peptides	Coverage (%)
Q3T0P6	PGK1_BOVIN	Phosphoglycerate kinase 1	44973	9.50	50	6/29	22
Q3SZ62	PGAM1_BOVIN	Phosphoglycerate mutase 1	28948	6.80	113	9/24	54
Q3SZJ9	PMM2_BOVIN	Phosphomannomutase 2	28435	6.00	52	5/26	25
P05307	PDIA1_BOVIN	Protein disulfide-isomerase	57629	4.70	64	10/54	24
P38657	PDIA3_BOVIN	Protein disulfide-isomerase A3	57293	6.20	141	18/52	35
P28783	S10A9_BOVIN	Protein S100-A9	17160	6.30	50	7/57	44
P80601	UK114_CAPHI	Ribonuclease	14347	7.10	81	7/52	82
P42819	SAA_SHEEP	Serum amyloid A protein	12680	6.10	105	9/45	58
Q3ZBH0	TCPB_BOVIN	T-complex protein 1 subunit beta	57781	6.20	64	8/35	25
O19011	TGFB1_HORSE	Transforming growth factor beta-1	44631	9.72	50	8/39	18
Q5E956	TPIS_BOVIN	Triosephosphate isomerase	26901	6.50	181	14/39	78
Q5KR47	TPM3_BOVIN	Tropomyosin alpha-3 chain	32856	4.53	69	11/58	28
P81947	TBA1B_BOVIN	Tubulin alpha-1B chain	50804	4.81	81	12/43	40
Q2HJ86	TBA1D_BOVIN	Tubulin alpha-1D chain	50935	4.77	61	12/67	34
Q32KN8	TBA3_BOVIN	Tubulin alpha-3 chain	50578	4.84	60	11/67	33
P81948	TBA4A_BOVIN	Tubulin alpha-4A chain	50634	4.79	69	10/67	36
P02554	TBB_PIG	Tubulin beta chain	50285	4.64	185	19/60	55
Q6B856	TBB2B_BOVIN	Tubulin beta-2B chain	50377	4.64	173	17/60	53
Q3MHH5	TBB4B_BOVIN	Tubulin beta-4B chain	50255	4.65	153	16/60	45
Q2KJ0	TBB5_BOVIN	Tubulin beta-5 chain	50095	4.60	214	25/65	61
Q2HJ81	TBB6_BOVIN	Tubulin beta-6 chain	50324	4.60	61	11/41	22
P26234	VINC_PIG	Vinculin	124437	5.50	79	14/47	19
Q3MHN5	VTDB_BOVIN	Vitamin D-binding protein	54904	5.20	70	10/56	31
Proteins that showed increase (n = 3)							
P60713	ACTB_SHEEP	Actin, cytoplasmic 1	42052	5.18	146	17/72	53
P15497	APOA1_BOVIN	Apolipoprotein A-I	30258	5.60	200	24/118	63
P02669	CASK_SHEEP	Kappa-casein	21596	5.80	68	8/47	35
Proteins that showed varying abundance (n = 15)							
P63258	ACTG_BOVIN	Actin, cytoplasmic 2	42108	5.20	146	17/72	53
P09462	LALBA_SHEEP	Alpha-lactalbumin	16761	4.66	135	10/37	45
P04653	CASA1_SHEEP	Alpha-S1-casein	24347	5.20	66	6/27	29
P04654	CASA2_SHEEP	Alpha-S2-casein	26486	8.70	112	10/30	38
Q32PJ2	APOA4_BOVIN	Apolipoprotein A-IV	42991	5.20	125	18/94	48
Q6QAT4	B2MG_SHEEP	Beta-2-microglobulin	13570	6.10	52	5/43	40
P02676	FIBB_BOVIN	Fibrinogen beta chain	53933	9.20	72	11/52	26
P19120	HSP7C_BOVIN	Heat shock cognate 71 kDa protein	71082	5.24	55	10/39	23
P13943	MMP1_RABIT	Interstitial collagenase	53877	6.30	52	8/50	17
Q29477	TRFL_CAPHI	Lactotransferrin	79361	9.50	120	18/70	31
P81265	PIGR_BOVIN	Polymeric immunoglobulin receptor	83695	7.70	50	11/72	15
Q29443	TRFE_BOVIN	Serotransferrin	79870	6.90	112	24/107	33
P14639	ALBU_SHEEP	Serum albumin	71139	5.80	205	23/49	40
P12303	TTHY_SHEEP	Transthyretin	15831	5.90	79	7/57	63
A4UMC5	TFP11_RABIT	Tuftelin-interacting protein 11	96645	5.60	50	13/71	18
P26234	VINC_PIG	Vinculin	124437	5.50	79	14/47	19

MS: mass spectrometre, MW: molecular weight, pl: isoelectric point.

microbiological, cytological and histopathological methods. The uninoculated mammary gland (contralateral) was used as uninfected control. Before challenge (on D0), as well as sequentially after that (on D0 + 12 h, D1, D2, D3, D4), animals were sampled. In milk samples collected from all ewes on D1, increased somatic cell counts and *M. haemolytica* recovery were simultaneously recorded. Histopathological confirmation of mastitis was provided by histopathological examination of mammary biopsy tissue samples performed 3 or 4 days post-challenge. Blood plasma and milk whey prepared from the samples were processed for proteomics examination.

Two-dimensional gel electrophoresis was performed by using 18 cm, pl 3–10 NL, IPG strips (Bio-Rad, Hercules, USA) [2]. Second-dimensional electrophoresis was performed on non-gradient 12% SDS-polyacrylamide gels, which were stained by colloidal Coomassie blue dye (Colloidal Blue staining kit; Thermo Fisher Scientific, Waltham, USA). Protein spots from all gels analysed were detected, aligned,

Table 6

Details of proteins observed with decrease, new appearance, increase or varying abundance in milk whey samples from the contralateral side of the udder of five ewes, after deposition of *M. haemolytica* into the teat of each animal (identification by MALDI-TOF MS).

Accession no.	Accession name	Description name	Theoretical MW	Theoretical pI	MASCOT score	Matched peptides	Coverage (%)
Proteins that showed decrease (n = 1)							
P09462	LALBA_SHEEP	Alpha-lactalbumin	16700	4.90	58	7/78	38
Proteins that showed new appearance (n = 8)							
Q767K7	ATAT_PIG	Alpha-tubulin N-acetyltransferase 1	34166	10.56	50	11/68	27
P54230	CTHL1_SHEEP	Cathelicidin-1	18036	9.30	55	6/62	44
Q3SX14	GELS_BOVIN	Gelsolin	80966	5.50	50	7/39	16
Q28641	MYH4_RABIT	Myosin-4	223841	5.50	50	18/84	13
Q9GL76	PVRL1_PIG	Nectin-1	57410	5.80	50	8/44	22
P00949	PGM1_RABIT	Phosphoglucosyltransferase-1	61805	6.65	50	6/44	20
P42819	SAA_SHEEP	Serum amyloid A protein	12680	6.10	69	6/35	43
A4UMC5	TFP11_RABIT	Tuftelin-interacting protein 11	96645	5.60	50	11/53	16
Proteins that showed varying abundance (n = 11)							
P12725	A1AT_SHEEP	Alpha-1-antitrypsin	46298	5.80	95	14/82	35
P29701	FETUA_SHEEP	Alpha-2-HS-glycoprotein	39511	5.10	56	8/62	32
P04654	CASA2_SHEEP	Alpha-S2-casein	26543	9.00	82	12/74	42
P15497	APOA1_BOVIN	Apolipoprotein A-I	30258	5.60	169	18/68	58
Q32PJ2	APOA4_BOVIN	Apolipoprotein A-IV	42991	5.20	50	8/59	25
Q6QAT4	B2MG_SHEEP	Beta-2-microglobulin	13570	6.10	52	5/43	40
P67976	LACB_SHEEP	Beta-lactoglobulin-1/B	20308	5.34	121	15/83	36
P02669	CASK_SHEEP	Kappa-casein	21596	5.76	51	7/78	86
Q29477	TRFL_CAPHI	Lactotransferrin	79361	9.50	191	27/85	43
Q29443	TRFE_BOVIN	Serotransferrin	79870	6.90	94	23/113	29
P14639	ALBU_SHEEP	Serum albumin	71139	5.80	162	24/79	43

MS: mass spectrometre, MW: molecular weight, pI: isoelectric point.

matched and quantified using the PD-Quest v8.0 image processing software (Bio-Rad). Manual inspection of the spots was used to verify the accuracy of matching.

In four gels, all spots on each gel were considered as protein spots of interest for Mass Spectrometry identification. These gels were produced from following samples: (i) blood plasma sample from one ewe collected before challenge (D0), (ii-iii) two milk whey samples from two ewes from the side of the udder that was scheduled for inoculation before challenge (D0), (iv) one milk whey from one ewe from the inoculated side of the udder immediately after challenge (D0 + 12 h). 'Full protein maps' were produced from these four samples.

In the remaining gels, differential proteomics evaluation was performed. Protein spots of interest were detected, aligned and matched between: (i) gels from sequential blood plasma samples from ewes, (ii) gels from milk whey samples from the two mammary glands of each ewe (inoculated side versus non-inoculated side) on the same sampling point and (iii) gels from sequential milk whey samples from the mammary glands of ewes using the PD Quest v8.0 image processing software (Bio-Rad). Differential abundance of proteins on each sampling time-point after challenge was evaluated in comparison with the respective protein before challenge (i.e., on D0). Protein decrease was defined when (i) proteins had been identified on D0, but not after challenge, or (ii) when protein spot densities after challenge were significantly lower than on D0. Protein new appearance was defined when proteins were detected only after challenge. Protein increase was defined when protein spot densities after challenge were significantly higher than on D0. Finally, protein varying abundance was defined when (i) proteins had been identified both on D0 and intermittently after challenge or (ii) protein spot densities post-challenge had not been significantly lower or higher than on D0.

Protein identification was performed by peptide mass fingerprinting. In the four gels in which all spots on each gel were considered as protein spots of interest, these were annotated by using the Melanie v.4.02 software (Swiss Institute of Bioinformatics, Lausanne, Switzerland). In the remaining

Table 7

List of proteins observed with differential abundance in milk whey samples of only one mammary gland of five ewes, after deposition of *M. haemolytica* into one teat of each animal (identification by MALDI-TOF MS).

Accession name	Description name	Inoculated side	Non-inoculated side
GRP78_BOVIN	78 kDa glucose-regulated protein	✓	-
ACTB_SHEEP	Actin, cytoplasmic 1	✓	-
ACTG_BOVIN	Actin, cytoplasmic 2	✓	-
ACTN4_BOVIN	Alpha-actinin-4	✓	-
ENOA_BOVIN	Alpha-enolase	✓	-
CASA1_SHEEP	Alpha-S1-casein	✓	-
ATPB_BOVIN	ATP synthase subunit beta, mitochondrial	✓	-
CASB_CAPHI	Beta-casein	✓	-
ENOB_BOVIN	Beta-enolase	✓	-
BIZL2_BOVIN	Brain-specific angiogenesis inhibitor 1-associated protein 2	✓	-
CAH3_PIG	Carbonic anhydrase 3	✓	-
CASP1_HORSE	Caspase-1	✓	-
CTHL2_SHEEP	Cathelicidin-2	✓	-
SC52_SHEEP	Cathelin-related peptide	✓	-
CH3L1_SHEEP	Chitinase-3-like protein 1	✓	-
COF1_SHEEP	Cofilin-1	✓	-
BRE1A_BOVIN	E3 ubiquitin-protein ligase	✓	-
EZRL_BOVIN	Ezrin	✓	-
CAZA1_BOVIN	F-actin-capping protein subunit alpha-1	✓	-
FABPH_BOVIN	Fatty acid-binding protein, heart	✓	-
FIBB_BOVIN	Fibrinogen beta chain	✓	-
ALDOA_RABIT	Fructose-bisphosphate aldolase A	✓	-
G3ST3_BOVIN	Galactose-3-O-sulfotransferase 3	✓	-
GSTP1_CAPHI	Glutathione S-transferase P	✓	-
G3P_SHEEP	Glyceraldehyde-3-phosphate dehydrogenase (fragment)	✓	-
PYGM_SHEEP	Glycogen phosphorylase, muscle form	✓	-
C1GLT_BOVIN	Glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase 1	✓	-
HBA1_TACAC	Haemoglobin subunit alpha- 1	✓	-
HBB_SHEEP	Haemoglobin subunit beta	✓	-
HBE1_CAPHI	Haemoglobin subunit epsilon-1	✓	-
HPT_CAPHI	Haptoglobin	✓	-
HSP7C_BOVIN	Heat shock cognate 71 kDa protein	✓	-
HSPB1_BOVIN	Heat shock protein beta-1	✓	-
HS90A_BOVIN	Heat shock protein HSP 90-alpha	✓	-
HS90B_HORSE	Heat shock protein HSP 90-beta	✓	-
MMP1_RABIT	Interstitial collagenase	✓	-
IDHC_SHEEP	Isocitrate dehydrogenase (NADP) cytoplasmic	✓	-
PERL_BOVIN	Lactoperoxidase	✓	-
LDHA_PIG	L-lactate dehydrogenase A chain	✓	-
MYH1_HORSE	Myosin-1	✓	-
MYH7_BOVIN	Myosin-7	✓	-
NEP_RABIT	Neprilysin	✓	-
NDKA_CANFA	Nucleoside diphosphate kinase A	✓	-
NDKB_BOVIN	Nucleoside diphosphate kinase B	✓	-
PRDX6_BOVIN	Peroxioredoxin-6	✓	-
PX11B_BOVIN	Peroxisomal membrane protein 11B	✓	-
BFSP2_BOVIN	Phakinin	✓	-
PEBP1_BOVIN	Phosphatidylethanolamine-binding protein 1	✓	-
PGK1_BOVIN	Phosphoglycerate kinase 1	✓	-
PGAM1_BOVIN	Phosphoglycerate mutase 1	✓	-
PMM2_BOVIN	Phosphomannomutase 2	✓	-
PIGR_BOVIN	Polymeric immunoglobulin receptor	✓	-
PDIA1_BOVIN	Protein disulfide-isomerase	✓	-
PDIA3_BOVIN	Protein disulfide-isomerase A3	✓	-
S10A9_BOVIN	Protein S100-A9	✓	-
KPYM_FELCA	Pyruvate kinase	✓	-
RK_BOVIN	Rhodopsin kinase	✓	-
UK114_CAPHI	Ribonuclease	✓	-
TCPB_BOVIN	T-complex protein 1 subunit beta	✓	-

(continued on next page)

Table 7 (continued)

Accession name	Description name	Inoculated side	Non-inoculated side
TGFB1_HORSE	Transforming growth factor beta-1	✓	-
TTHY_SHEEP	Transthyretin	✓	-
TPIS_BOVIN	Triosephosphate isomerase	✓	-
TPM3_BOVIN	Tropomyosin alpha-3 chain	✓	-
TBA1B_BOVIN	Tubulin alpha-1B chain	✓	-
TBA1D_BOVIN	Tubulin alpha-1D chain	✓	-
TBA3_BOVIN	Tubulin alpha-3 chain	✓	-
TBA4A_BOVIN	Tubulin alpha-4A chain	✓	-
TBB_PIG	Tubulin beta chain	✓	-
TBB2B_BOVIN	Tubulin beta-2B chain	✓	-
TBB4B_BOVIN	Tubulin beta-4B chain	✓	-
TBB5_BOVIN	Tubulin beta-5 chain	✓	-
TBB6_BOVIN	Tubulin beta-6 chain	✓	-
VINC_PIG	Vinculin	✓	-
VTDB_BOVIN	Vitamin D-binding protein	✓	-
ATAT_PIG	Alpha-tubulin N-acetyltransferase	-	✓
GELS_BOVIN	Gelsolin	-	✓
MYH4_RABIT	Myosin-4	-	✓
PVRL1_PIG	Nectin-1	-	✓
PGM1_RABIT	Phosphoglucomutase-1	-	✓

✓: protein observed with differential abundance in milk sample from respective mammary gland from at least one ewe; -: protein not observed with differential abundance in milk sample from respective mammary gland in any ewe.

gels, protein spots of interest were annotated manually using the Melanie 4.02 software and were excised from 2D-gels by use of Proteiner SPII (Bruker Daltonics, Bremen, Germany).

All excised spots were subjected to tryptic digestion. Then, peptide mixtures were analysed in a MALDI-TOF mass spectrometre (Ultraflex, Bruker Daltonics) [3]. Matching of peptides and protein searches were carried out in the MASCOT Server 2 (Matrix Science, Boston, USA). Masses of peptides were searched under 'mammalia', but excluding '*Homo sapiens*' and 'rodents', in the UniProt Knowledge base database (UniProtKB/Swiss-Prot [release 2014 12]) [4].

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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