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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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).

| 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           |

(continued on next page)
Table 1 (continued)

| Accession no. | Accession name | Description name | Theoretical MW | Theoretical pI | MASCOT score | Matched peptides | Coverage (%) |
|---------------|----------------|------------------|----------------|---------------|--------------|------------------|--------------|
| P68056        | HBBC_SHEEP     | Haemoglobin subunit beta-A | 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.

* 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).

| Accession no. | Accession name | Description name | No. ewes | Theoretical MW | Theoretical pI | MASCOT score | Matched peptides | Coverage (%) |
|---------------|----------------|------------------|---------|----------------|----------------|--------------|----------------|--------------|
| P60713        | ACTB_SHEEP     | Actin, cytoplasmic | 1       | 42052          | 5.18           | 74           | 11/79        | 45           |
| P63258        | ACTG_BOVIN     | Actin, cytoplasmic | 1       | 42108          | 5.20           | 75           | 11/72        | 37           |
| P12725        | A1AT_SHEEP     | Alpha-1-antiprotease | 1       | 46298          | 5.80           | 127          | 15/60        | 34           |
| P29701        | FETUA_SHEEP    | Alpha-2-HS-glycoprotein | 1       | 39511          | 5.10           | 58           | 8/58         | 31           |
| P09462        | ALBA_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-1| 1       | 30258          | 5.60           | 148          | 18/89        | 57           |
| Q32P2J        | APOA4_BOVIN    | Apolipoprotein A-IV| 1       | 42991          | 5.20           | 66           | 11/84        | 37           |
| P00828        | 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           |
| Q1KV70        | 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           |
| Q55154        | CAH3_PIG       | Carbonic anhydrase 3 | 1       | 29678          | 8.67           | 72           | 44/177       | 55           |
| A2VD01        | BREA1_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           |
| P02012        | 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     | Intersitial 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        | TREFL_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       | Rhodokinase      | 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           |

(continued on next page)
Table 2 (continued)

| Accession no. | Accession name | Description name | No. ewes\textsuperscript{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.
\textsuperscript{a} Combined details of ‘full protein map’ performed in the sample are presented.
\textsuperscript{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 \textit{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          |
| A5D7D1        | ACTN4_BOVIN    | Alpha-actinin-4    | 105319         | 5.20         | 95           | 16/55           | 22          |
| Q9XSJ4        | ANOA_BOVIN     | Alpha-enolase      | 47639          | 6.40         | 181          | 21/68           | 60          |
| P09462        | ALBA_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        | APA1_BOVIN     | Apolipoprotein A-I | 30258          | 5.60         | 118          | 14/65           | 46          |
| Q32PJ2        | APA4_BOVIN     | Apolipoprotein A-IV| 42991          | 6.10         | 115          | 15/66           | 41          |
| Q6QAT4        | B2MG_SHEEP     | Beta-2-microglobulin| 13570          | 6.10         | 50           | 4/24            | 35          |
| Q3ZC09        | ENOB_SHEEP     | Beta-enolase       | 47409          | 8.63         | 61           | 21/68           | 29          |
| P67976        | LACB_SHEEP     | Beta-lactoglobulin-1/B | 20308          | 5.34         | 137          | 13/65           | 85          |
| E1BFE9        | B2MG_SHEEP     | 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          |
| P97362        | CTHL2_SHEEP    | Cathelicidin-2     | 20057          | 10.89        | 76           | 6/24            | 34          |
| P49929        | SC52_SHEEP     | Cathelin-related peptide SC5 | 17595 | 10.10 | 50 | 5/26 | 25 |
| Q6B7M7        | CF1_SHEEP      | Coflin-1           | 18792          | 9.13         | 83           | 7/22            | 51          |
| P31976        | EZRI_BOVIN     | Ezrin              | 68832          | 6.00         | 50           | 9/34            | 12          |
| P948A8        | 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 |
| Q9RTY8        | GSTP1_CAPI     | Glutathione S-transferase P | 23843 | 8.80 | 82 | 6/20 | 47 |
| Q28S54        | G3P_SHEEP      | Glyceraldehyde-3-phosphate dehydrogenase (fragment) | 36073 | 9.30 | 72 | 8/39 | 30 |
| P02075        | HBP_SHEEP      | Haemoglobin subunit beta | 16120 | 6.90 | 54 | 4/19 | 39 |
| B6E141        | HPT_CAPI       | Haptoglobin        | 45411          | 9.10         | 53           | 7/39            | 19          |
| P19120        | HSP1_BOVIN     | Heat shock cognate 71 kDa protein | 71082 | 5.24 | 55 | 10/59 | 23 |
| Q3T140        | HSPB1_BOVIN    | Heat shock protein beta-1 | 22436 | 6.00 | 97 | 8/29 | 44 |
| Q76LV2        | HSP9A_BOVIN    | Heat shock protein HSP 90-alpha | 85077 | 4.78 | 94 | 20/70 | 27 |
| Q9GKX8        | HSP9B_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 |
| Q6UX5D        | IDHC_SHEEP     | Isocitrate dehydrogenase (NADP+) cytoplasmic | 47153 | 6.40 | 54 | 7/32 | 19 |
| P02669        | CARK_SHEEP     | Kappa-casein       | 21596          | 5.80         | 68           | 8/47            | 35          |
| Q29487        | TRF_CAPI       | Keratin, type 1 cytoskeletal 10 | 57847 | 4.90 | 78 | 15/85 | 23 |
| A5JUY8        | LCHR_BOVIN     | Lactocephalin      | 81559          | 9.70         | 52           | 10/47           | 18          |
| P28783        | PRDX6_BOVIN    | Lactoperoxidase    | 25108          | 6.00         | 85           | 7/21            | 31          |
| P13696        | MSPEB1_BOVIN   | Lactotransferrin   | 21087          | 7.70         | 52           | 5/35            | 39          |
| Q3Q196        | PGD_BOVIN      | Lactocephalin      | 44973          | 9.50         | 50           | 6/29            | 22          |
| Q07285        | PMM2_BOVIN     | Lactocephalin      | 28435          | 6.00         | 52           | 5/26            | 25          |
| P05017        | PDI1_BOVIN     | Protein disulfide-isomerase | 57629 | 4.70 | 64 | 10/54 | 24 |
| P36587        | PDI2_BOVIN     | Protein disulfide-isomerase | 57293 | 6.20 | 141 | 18/52 | 35 |
| P28783        | PDI3_BOVIN     | Protein disulfide-isomerase A3 | 17160 | 6.30 | 50 | 6/57 | 44 |
| P80601        | PRDX6_BOVIN    | Peroxiredoxin-6    | 21087          | 7.70         | 52           | 5/35            | 39          |
| Q07285        | PGD_BOVIN      | Phosphatidylethanolamine-binding protein 1 | 44973 | 9.50 | 50 | 6/29 | 22 |
| Q07285        | PGD_BOVIN      | Phosphatidylethanolamine-binding protein 1 | 28435 | 6.00 | 52 | 5/26 | 25 |
| Q07285        | PGD_BOVIN      | Phosphatidylethanolamine-binding protein 1 | 57629 | 4.70 | 64 | 10/54 | 24 |
| Q07285        | PGD_BOVIN      | Phosphatidylethanolamine-binding protein 1 | 57293 | 6.20 | 141 | 18/52 | 35 |

(continued on next page)
**Table 3 (continued)**

| Accession no. | Accession name | Description name | Theoretical MW | Theoretical pl | MASCOT score | Matched peptides | Coverage (%) |
|---------------|----------------|-------------------|----------------|---------------|--------------|-----------------|-------------|
| P81947        | TB1A1_BOVIN    | Tubulin alpha-1B chain | 50804          | 4.81          | 81           | 12/43           | 40          |
| Q2HJ86        | TB1A1D_BOVIN   | Tubulin alpha-1D chain | 50935          | 4.77          | 61           | 12/67           | 34          |
| Q32KN8        | TB1A3_BOVIN    | Tubulin alpha-3 chain | 50578          | 4.84          | 60           | 10/67           | 33          |
| P81948        | TB1A4A_BOVIN   | Tubulin alpha-4A chain | 50634          | 4.79          | 69           | 11/67           | 36          |
| P02554        | TB1B_PIG       | Tubulin beta chain   | 50285          | 4.64          | 185          | 19/60           | 55          |
| Q6B856        | TB2B2_BOVIN    | Tubulin beta-2B chain | 50377          | 4.64          | 173          | 17/60           | 53          |
| Q3MHH5        | TB2B4_BOVIN    | Tubulin beta-4B chain | 50255          | 4.65          | 153          | 14/60           | 45          |
| Q2KJD0        | TB2B5_BOVIN    | Tubulin beta-5 chain | 50095          | 4.60          | 214          | 25/65           | 61          |
| Q2HJ81        | TB2B6_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, pl: isoelectric point.

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 (%) |
|---------------|----------------|------------------|----------------|----------------|---------------|------------------|--------------|
| P02757 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 |
| Q35ZV7 HEMO_BOVIN Haemopexin | 52974 | 8.90 | 66 | 10/56 | 16 |
| A4UMC5 TFP11_RABIT Tuffelin-interacting protein 11 | 96645 | 5.60 | 51 | 14/70 | 16 |

**Proteins that showed decrease (n = 6)**

| Accession no. | Accession name | Description name | Theoretical MW | Theoretical pI | MASCOT score | Matched peptides | Coverage (%) |
|---------------|----------------|------------------|----------------|----------------|---------------|------------------|--------------|
| 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 |
| Q2UVX4 CO3_SHEEP Complement C3 | 188675 | 6.40 | 63 | 22/84 | 15 |
| P81187 CFAB_BOVIN Complement factor B | 86737 | 8.80 | 72 | 17/97 | 20 |
| Q29R12 CHRD1_BOVIN Cysteine and histidine-rich domain-containing protein 1 | 38144 | 6.95 | 51 | 8/97 | 30 |

**Proteins that showed new appearance (n = 13)**

| Accession no. | Accession name | Description name | Theoretical MW | Theoretical pI | MASCOT score | Matched peptides | Coverage (%) |
|---------------|----------------|------------------|----------------|----------------|---------------|------------------|--------------|
| P12725 ANGT_SHEEP Angiotensinogen | 46298 | 5.80 | 147 | 17/66 | 40 |
| Q7SH1 A2MG_BOVIN Alpha-2-macroglobulin | 168553 | 5.70 | 65 | 19/86 | 19 |
| P15497 APOA1_BOVIN Apolipoprotein A-I | 30258 | 5.60 | 168 | 20/102 | 59 |
| Q32P2 JPOA4_BOVIN Apolipoprotein A-IV | 42991 | 5.20 | 134 | 20/102 | 47 |
| Q9X777 CERU_SHEEP Ceruloplasmin | 120020 | 5.40 | 122 | 19/60 | 26 |
| P02076 FBB_BOVIN Fibrinogen beta chain | 53933 | 9.20 | 76 | 21/103 | 31 |
| Q35K14 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_BOVIN 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_BOVIN Serum albumin | 71139 | 5.80 | 244 | 31/82 | 48 |
| P12303 TTHY_BOVIN Transthyretin | 15875 | 5.50 | 107 | 10/74 | 55 |
| Q3MHN5 VTD_BOB Vinmin D-binding protein | 54904 | 5.20 | 87 | 10/37 | 31 |

**Proteins that showed varying abundance (n = 14)**

| Accession no. | Accession name | Description name | Theoretical MW | Theoretical pI | MASCOT score | Matched peptides | Coverage (%) |
|---------------|----------------|------------------|----------------|----------------|---------------|------------------|--------------|
| P12725 A1AT_SHEEP Alpha-1-antiproteinase | 46298 | 5.80 | 147 | 17/66 | 40 |
| Q7SH1 A2MG_BOVIN Alpha-2-macroglobulin | 168553 | 5.70 | 65 | 19/86 | 19 |
| P15497 APOA1_BOVIN Apolipoprotein A-I | 30258 | 5.60 | 168 | 20/102 | 59 |
| Q32P2 JPOA4_BOVIN Apolipoprotein A-IV | 42991 | 5.20 | 134 | 20/102 | 47 |
| Q9X777 CERU_SHEEP Ceruloplasmin | 120020 | 5.40 | 122 | 19/60 | 26 |
| P02076 FBB_BOVIN Fibrinogen beta chain | 53933 | 9.20 | 76 | 21/103 | 31 |
| Q35K14 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_BOVIN 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_BOVIN Serum albumin | 71139 | 5.80 | 244 | 31/82 | 48 |
| P12303 TTHY_BOVIN Transthyretin | 15875 | 5.50 | 107 | 10/74 | 55 |
| Q3MHN5 VTD_BOB Vinmin D-binding protein | 54904 | 5.20 | 87 | 10/37 | 31 |

MS: mass spectrometre, MW: molecular weight, pl: 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).
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 (%) |
|---------------|----------------|------------------|----------------|---------------|--------------|-----------------|--------------|
| P12725        | A1AT_SHEEP     | Alpha-1-antiproteinase | 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          | 3.54          | 137          | 15/65           | 85           |
| Q5S154        | 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        | HBO1_TACAC     | Haemoglobin subunit alpha-1 | 15509          | 9.50          | 51           | 5/58            | 46           |
| P02102        | HBO1_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           |
| A5JUY9        | PERL_BUBBU     | Lactoperoxidase | 81559          | 9.70          | 52           | 10/47           | 18           |
| Q8MVJ0        | MYH1_HORSE     | Myosin-1         | 223772         | 5.49          | 51           | 18/127          | 13           |
| P08049        | NEP_RABIT      | Neprilysin       | 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           |
| Q0VCX2        | GRP78_BOVIN    | 78 kDa glucose-regulated protein | 72470          | 4.90          | 62           | 8/32            | 19           |
| A5DFD1        | ACTN4_BOVIN    | Alpha-actinin-4 | 105319         | 5.20          | 95           | 16/55           | 22           |
| Q9X5J4        | ENO_BUBBU      | Alpha-eno-lase   | 47639          | 6.40          | 181          | 21/68           | 60           |
| Q3ZC09        | ENO_BUBBU      | Beta-eno-lase    | 47409          | 8.63          | 61           | 10/68           | 29           |
| E1BF65        | B2L2_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           |
| Q9TMG6        | CHS1_SHEEP     | Chitinase-3-like protein 1 | 43209         | 9.60          | 98           | 13/60           | 38           |
| Q8B7M7        | COF1_SHEEP     | Cofilin-1        | 18792          | 9.13          | 83           | 7/22            | 51           |
| P31976        | EZR1_BOVIN     | Ezrin            | 68832          | 6.00          | 50           | 9/34            | 12           |
| A4FAU8        | CAZA1_BOVIN    | F-actin-capping protein subunit alpha-1 | 33082          | 5.50          | 98           | 9/37            | 48           |
| Q0VCX4        | G3ST3_BOVIN    | Galactos-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           |
| Q0VCX4        | C1GLT_BOVIN    | Glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransfer | 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           |
| Q3T948        | 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        | NDCA_CANFA     | Nucleoside diphosphate kinase A | 17283          | 5.70          | 53           | 4/14            | 31           |
| Q3TOQ4        | 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    | Peroxosmal membrane protein 11B | 28773          | 11.34         | 50           | 7/51            | 28           |
| Q28177        | BESF2_BOVIN    | Phakinin         | 46150          | 5.54          | 50           | 19/115          | 29           |
| P13896        | PEPO1_BOVIN    | Phosphatidylethanolamine-binding protein 1 | 21087          | 7.70          | 52           | 5/35            | 39           |
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. Histo-pathological 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, pI 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 (Coomassie Blue staining kit; Thermo Fisher Scientific, Waltham, USA). Protein spots from all gels analysed were detected, aligned,

### Table 5 (continued)

| Accession no. | Accession name | Description name | Theoretical MW | Theoretical pl | MASCOT score | Matched peptides | Coverage (%) |
|---------------|----------------|------------------|----------------|---------------|--------------|------------------|--------------|
| Q3TP62        | PK1_BOVIN      | Phosphoglycerate kinase 1 | 44973          | 9.50          | 50           | 6/29             | 22           |
| Q3SZ62        | PCAM1_BOVIN    | Phosphoglycerate mutase 1 | 28948          | 6.80          | 113          | 9/24             | 54           |
| Q3SZ9         | PMM2_BOVIN     | Phosphomannomutase 2  | 28435          | 6.00          | 52           | 5/26             | 25           |
| P05307        | PDA1_BOVIN     | Protein disulde-isomerase | 57629          | 4.70          | 64           | 10/54            | 24           |
| P38657        | PDA3_BOVIN     | Protein disulde-isomerase A3 | 57293          | 6.20          | 141          | 18/52            | 35           |
| P28783        | S100A9_BOVIN   | Protein S100-A9 | 17160          | 6.30          | 50           | 7/57             | 44           |
| P86061        | UK114_CAPI     | Ribonucleose      | 14437          | 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        | TGFBI_HORSE    | Transforming growth factor beta-1 | 44631       | 9.72          | 50           | 8/39             | 18           |
| Q5E596        | TPS_BOVIN      | Triosephosphate isomerase | 26901          | 6.10          | 180          | 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           |
| P91848        | TBA4A_BOVIN    | Tubulin alpha-4A chain | 50634          | 4.79          | 69           | 10/67            | 36           |
| P02554        | TB8_PIG        | Tubulin beta chain | 50285          | 4.64          | 185          | 19/60            | 55           |
| Q68658        | TBB2R_BOVIN    | Tubulin beta-2B chain | 50377          | 4.64          | 173          | 17/60            | 53           |
| Q3HMH5        | TBB4R_BOVIN    | Tubulin beta-4B chain | 50255          | 4.65          | 153          | 16/60            | 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           | 11/41            | 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)**

| Accession no. | Accession name | Description name | Theoretical MW | Theoretical pl | MASCOT score | Matched peptides | Coverage (%) |
|---------------|----------------|------------------|----------------|---------------|--------------|------------------|--------------|
| 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)**

| Accession no. | Accession name | Description name | Theoretical MW | Theoretical pl | MASCOT score | Matched peptides | Coverage (%) |
|---------------|----------------|------------------|----------------|---------------|--------------|------------------|--------------|
| 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           |
| P6QAT4        | 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        | TRLF_CAPI     | 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           | 7/37             | 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.
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 v.8.0 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 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 v.8.0 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 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 v.8.0 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).
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_CAPI     | Beta-casein                     | ✓               | -                   |
| ENOB_BOVIN     | Beta-enolase                    | ✓               | -                   |
| BZLI2_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     | Collin-1                        | ✓               | -                   |
| BRE1A_BOVIN    | E3 ubiquitin-protein ligase     | ✓               | -                   |
| EZRI_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_CAPI     | 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_CAPI      | Haemoglobin subunit epsilon-1   | ✓               | -                   |
| HPT_CAPI       | 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      | Nepriysin                       | ✓               | -                   |
| ND2A_CANFA     | Nucleoside diphosphate kinase A | ✓               | -                   |
| ND2B_BOVIN     | Nucleoside diphosphate kinase B | ✓               | -                   |
| PRDX6_BOVIN    | Peroxiredoxin-6                 | ✓               | -                   |
| PX11B_BOVIN    | Peroxisomal membrane protein 11B | ✓ | - |
| BFS2_BOVIN     | Phakinin                        | ✓               | -                   |
| PEPB1_BOVIN    | Phosphatidylethanolamine-binding protein 1 | ✓ | - |
| PGK1_BOVIN     | Phosphoglycerate kinase 1       | ✓               | -                   |
| PGAM1_BOVIN    | Phosphoglycerate mutase 1       | ✓               | -                   |
| PMM2_BOVIN     | Phosphomannomutase 2            | ✓               | -                   |
| PIGR_BOVIN     | Polymeric immunoglobulin receptor | ✓ | - |
| PDI1A_BOVIN    | Protein disulfide-isomerase     | ✓               | -                   |
| PDI3A_BOVIN    | Protein disulfide-isomerase A3  | ✓               | -                   |
| S10A9_BOVIN    | Protein S100-A9                  | ✓               | -                   |
| KPYM_FELCA     | Pyruvate kinase                 | ✓               | -                   |
| RK_BOVIN       | Rhodopsin kinase                | ✓               | -                   |
| UK114_CAPI     | Ribonuclease                     | ✓               | -                   |
| TCPB_BOVIN     | T-complex protein 1 subunit beta | ✓ | - |

(continued on next page)
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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