

# Supplementary data

**Table S1.** The Anti-recombinant protective antigen (rPA) and anti-formalin inactivated spores (FIS) IgG titres ( $\log_{10}$ ) in cattle vaccinated at week 0 and 3 and measured at week0, 3 and 5 (with means and standard deviations). Cattle were vaccinated twice (week 0 and3) with purified rPA(PrPA)+FIS+Pen-G (penicillin-G), crude rPA (CrPA)+FIS+Pen-G, SLSV+FIS+Pen-G, SLSV and NegCtl+Pen G (vaccinated with Emulsigen-D®/Alhydrogel® plus Pen-G).

| Serology                                                   | Vaccine type   | Week 0          | Week 3           | Week 5             |
|------------------------------------------------------------|----------------|-----------------|------------------|--------------------|
| Anti-rPA IgG ELISA titre ( $\log_{10}$ )                   | NegCtl+Pen-G   | 1,25 $\pm$ 0,32 | 1,36 $\pm$ 0,34  | 1,54 $\pm$ 0,30    |
|                                                            | PrPA+FIS+Pen-G | 1,17 $\pm$ 0,29 | 3,30 $\pm$ 0,34* | 4,45 $\pm$ 0,33*** |
|                                                            | CrPA+FIS+Pen-G | 1,25 $\pm$ 0,40 | 2,93 $\pm$ 0,40* | 4,30 $\pm$ 0,75*** |
|                                                            | SLSV+Pen-G     | 1,17 $\pm$ 0,22 | 1,64 $\pm$ 0,41  | 2,18 $\pm$ 0,41    |
|                                                            | SLSV           | 1,22 $\pm$ 0,21 | 2,88 $\pm$ 0,38  | 4,29 $\pm$ 0,40*** |
| Anti-FIS IgG ELISA titre ( $\log_{10}$ )                   | NegCtl+Pen-G   | 1,14 $\pm$ 0,05 | 1,32 $\pm$ 0,34  | 1,53 $\pm$ 0,40    |
|                                                            | PrPA+FIS+Pen-G | 1,21 $\pm$ 0,25 | 3,36 $\pm$ 0,09* | 4,58 $\pm$ 0,36*** |
|                                                            | CrPA+FIS+Pen-G | 1,21 $\pm$ 0,29 | 3,68 $\pm$ 0,38* | 4,48 $\pm$ 0,45*** |
|                                                            | SLSV+Pen-G     | 1,14 $\pm$ 0,18 | 3,11 $\pm$ 0,38* | 4,46 $\pm$ 0,40*** |
|                                                            | SLSV           | 1,27 $\pm$ 0,20 | 3,37 $\pm$ 0,35* | 4,53 $\pm$ 0,29*** |
| TNA <sup>c</sup> titre (NT <sub>50</sub> ) ( $\log_{10}$ ) | NegCtl+Pen-G   | ND              | 1,38 $\pm$ 0,31  | 1,40 $\pm$ 0,36    |
|                                                            | PrPA+FIS+Pen-G | ND              | 2,43 $\pm$ 0,40  | 3,46 $\pm$ 0,38*** |
|                                                            | CrPA+FIS+Pen-G | ND              | 2,15 $\pm$ 0,19  | 3,30 $\pm$ 0,49*** |
|                                                            | SLSV+Pen-G     | ND              | 1,48 $\pm$ 0,22  | 1,56 $\pm$ 0,36    |
|                                                            | SLSV           | ND              | 2,26 $\pm$ 0,27  | 3,40 $\pm$ 0,43*** |

<sup>a</sup>Mean  $\log_{10}$  titres  $\pm$  95% confidence interval, <sup>b</sup>Titres were compared to the respective pre-vaccination titres (\*\*\*\* $p$  < 0.0001, \*\*\* $p$  < 0.001, \*\* $p$  < 0.01, \* $p$   $\leq$  0.05), <sup>c</sup>TNA; Lethal toxin neutralization titres, rPA; Recombinant protective antigen, FIS; Formaldehyde inactivated spore, ND; Not detected, ns; Not significant, PrPA; Purified recombinant protective antigen, CrPA; Crude recombinant protective antigen, Pen-G; Penicillin-G, SLSV; Sterne live spore vaccine, NegCtl; Negative control

**Table S2.** The anti-recombinant protective antigen (rPA) and anti-formalin inactivate *Bacillus anthracis* 34F2 spores (FIS) titres (log<sub>10</sub>) of different isotypes in vaccinated cattle (with means and standard deviations) of Immunoglobulin isotypes titre of vaccinated cattle measured at weeks 0, 3 (two weeks after first vaccination) and 5 (two weeks after second vaccination).

| Serology                                                  | Vaccine types  | IgM       |             |              | IgG1      |             |              | IgG2       |             |              |
|-----------------------------------------------------------|----------------|-----------|-------------|--------------|-----------|-------------|--------------|------------|-------------|--------------|
|                                                           |                | Week 0    | Week 3      | Week 5       | Week 0    | Week 3      | Week 5       | Week 0     | Week 3      | Week 5       |
| Anti-rPA Igs<br>ELISA<br>titre<br>(log <sub>10</sub> )    | NegCtl+Pen-G   | 1,17±0,23 | 1,51±0,14   | 1,34±0,36    | 1,50±0,35 | 1,52±0,35   | 1,50±0,33    | 1,65±0,49  | 1,51±0,35   | 1,50±0,32    |
|                                                           | PrPA+FIS+Pen-G | 1,62±0,24 | 3,05±0,36** | 3,19±0,35**  | 1,55±0,33 | 2,98±0,45*  | 3,26±0,31*** | 1,50±0,29  | 2,79±0,12** | 3,39±0,48*** |
|                                                           | CrPA+FIS+Pen-G | 1,57±0,32 | 2,94±0,38** | 3,06±0,45**  | 1,52±0,32 | 2,89±0,51** | 3,07±0,22**  | 1,58±0,23  | 2,65±0,18*  | 3,40±0,38*** |
|                                                           | SLSV+Pen-G     | 1,56±0,32 | 1,70±0,28   | 1,76±0,21    | 1,51±0,28 | 1,61±0,22   | 1,83±0,30    | 1,58±0,22  | 1,69±0,77   | 1,93±0,41    |
|                                                           | SLSV           | 1,64±0,23 | 2,84±0,39** | 3,02±0,45**  | 1,51±0,28 | 2,81±0,27** | 2,93±0,23**  | 1,54±0,24  | 2,66±0,21*  | 3,56±0,32*** |
| Anti-FIS<br>Igs<br>ELISA<br>titre<br>(log <sub>10</sub> ) | NegCtl+Pen-G   | 1,62±0,37 | 1,65±0,31   | 1,62±0,43    | 1,73±0,09 | 1,60±0,30   | 1,72±0,17    | 1,68±0,136 | 1,57±0,36   | 1,53±0,37    |
|                                                           | PrPA+FIS+Pen-G | 1,64±0,15 | 2,95±0,23** | 3,09±0,27**  | 1,62±0,26 | 2,98±0,38** | 3,11±0,37*** | 1,61±0,23  | 2,71±0,31*  | 3,24±0,32*** |
|                                                           | CrPA+FIS+Pen-G | 1,52±0,30 | 2,89±0,23** | 3,06±0,37**  | 1,57±0,21 | 2,86±0,24*  | 2,98±0,19**  | 1,60±0,23  | 2,75±0,30*  | 3,13±0,44*** |
|                                                           | SLSV+Pen-G     | 1,58±0,22 | 2,74±0,23*  | 2,90±0,24**  | 1,62±0,21 | 1,85±0,37** | 2,29±0,46**  | 1,57±0,21  | 1,97±0,30*  | 2,00±0,41**  |
|                                                           | SLSV           | 1,51±0,27 | 2,91±0,20** | 3,08±0,27*** | 1,63±0,23 | 2,74±0,29*  | 2,94±0,11**  | 1,66±0,15  | 2,60±0,24*  | 2,96±0,35**  |

<sup>a</sup>Mean log<sub>10</sub> titres ± 95% confidence interval, <sup>b</sup>Titres were compared to the respective pre-vaccination titres (\*\*\**p* < 0.001, \*\**p* < 0.01, \**p* ≤ 0.05). rPA83; Recombinant protective antigen 83, PrPA; Purified rPA83, CrPA; Crude rPA83, Pen-G; Penicillin-G, SLSV; Sterne live spore vaccine, NegCtl; Negative control (Emulsigen-D®/Alhydrogel® and Pen-G).