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Safety and Preliminary results

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Swine plasma immunoglobulins for prevention and treatment of post-weaning diarrhoea: Safety and Preliminary results

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Background

Post-weaning diarrhoea (PWD) is a common condition in intensive swine production, resulting in reduced welfare of weaners, high consumption of antibiotics and zinc oxide, and economic losses for the farmer as a result of pig disease and death, and associated treatment costs.

Aim

To develop an antibiotic alternative, based on natural antibodies (immunoglobulins) derived directly from inexpensive raw material (swine blood plasma) for oral provision, and protection against PWD.

Conclusions

- Purified porcine immunoglobulin (pplgG) binds PWD-inducing Enterotoxigenic Escherichia coli (ETEC), and inhibits ETEC adhesion to porcine intestinal epithelial cells *in vitro*.
- Experimental ETEC infection was cleared significantly faster in weaner piglets given a pplgG feed supplement.
- Based on next-generation sequencing data, pplgG inhibits ileal adhesion of bacteria from the family *Enterobacteriaceae*.
- No adverse side effects were observed by using pplgG as a feed supplement.
- These results suggest that pplgG could be used for treatment of PWD and reduce antibiotic consumption.

Figure 1: Purified porcine IgG (pplgG)

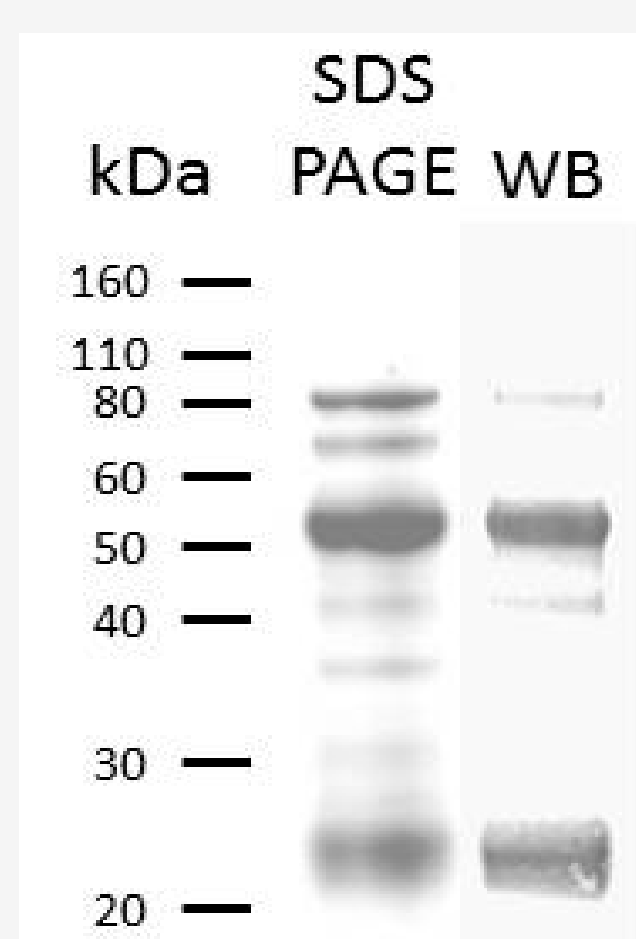
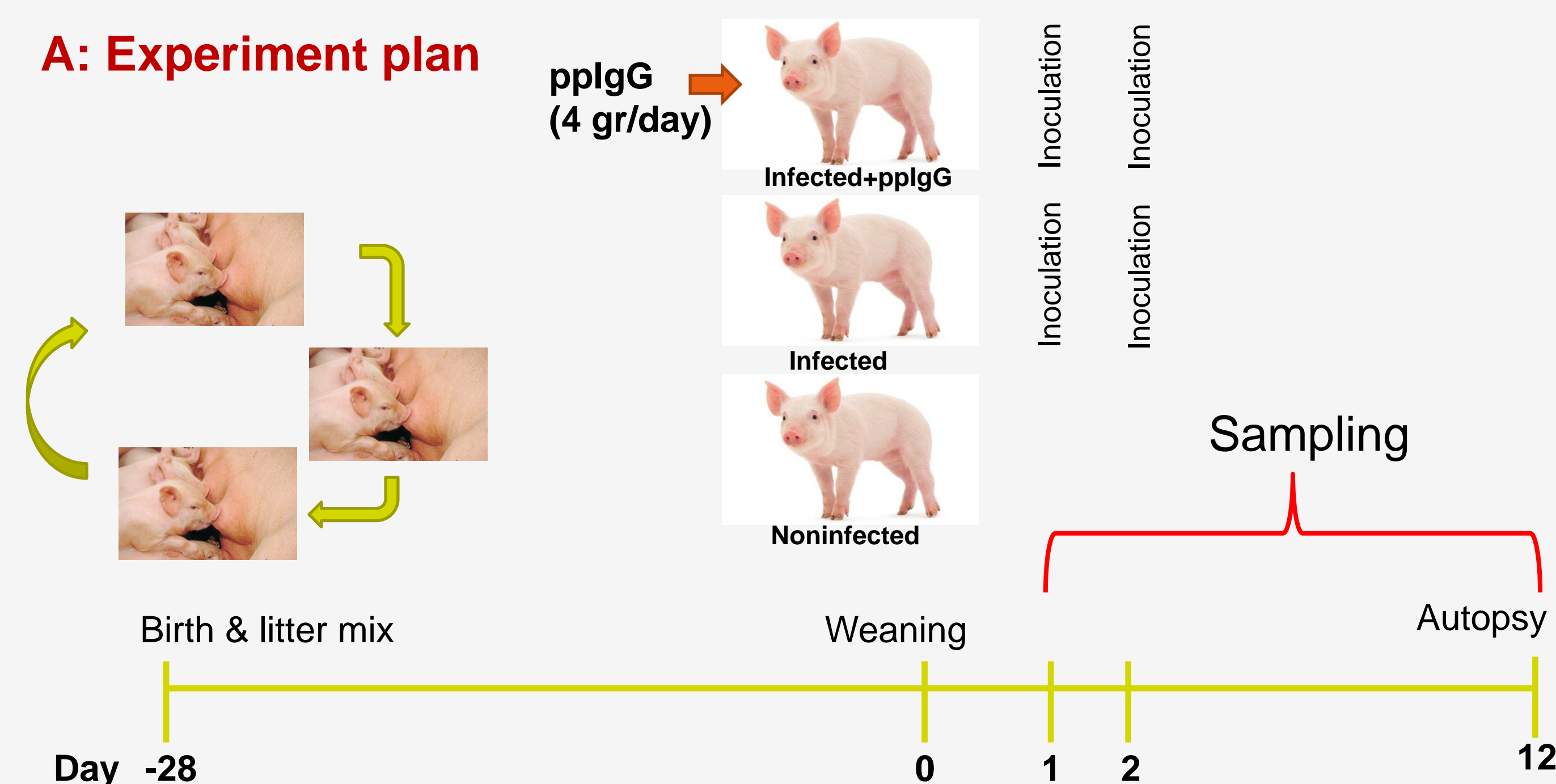


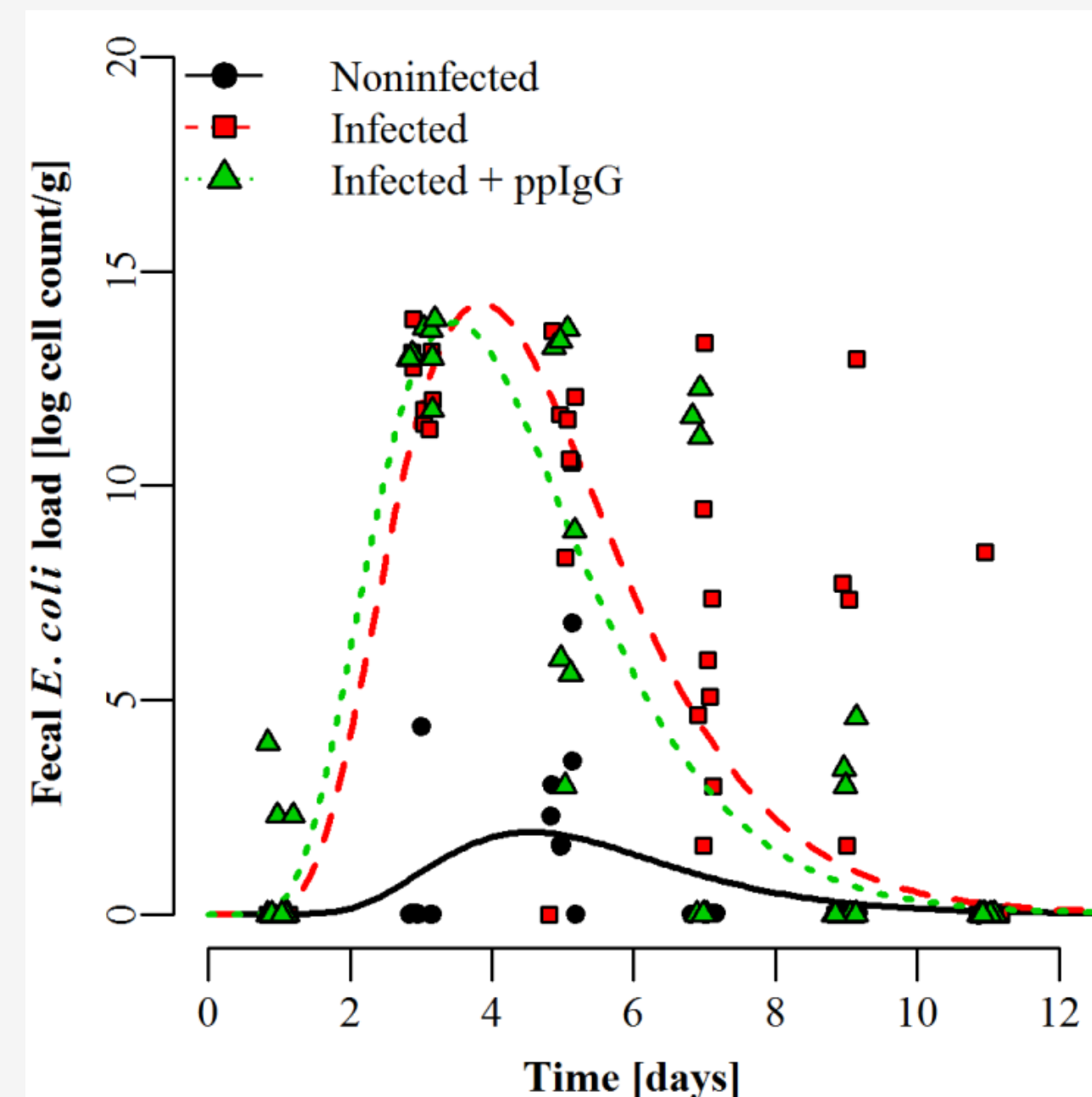
Figure 1: Pig plasma IgG (pplgG) was purified from blood plasma at UpFront Chromatography A/S (Copenhagen) by high-volume Expanded Bed Adsorption with a proprietary adsorbent. Plasma was obtained from a DAKA A/S (Lunderskov, Denmark). pplgG (1 mg/ml) was run under reducing conditions on 12% SDS PAGE. In parallel, Western blotting (WB) was performed on the same sample. The blot was developed with biotinylated rabbit anti-pig IgG F(ab)2 antibody, followed by alkaline phosphatase-coupled streptavidin. By SDS PAGE and Western blotting analysis the pplgG was estimated to consist of approximately 85% pure immunoglobulin.

Figure 3: pplgG *in vivo* (model of PWD)

A: Experiment plan



B: Shedding of ETEC



C: Enterobacteriaceae in ileum

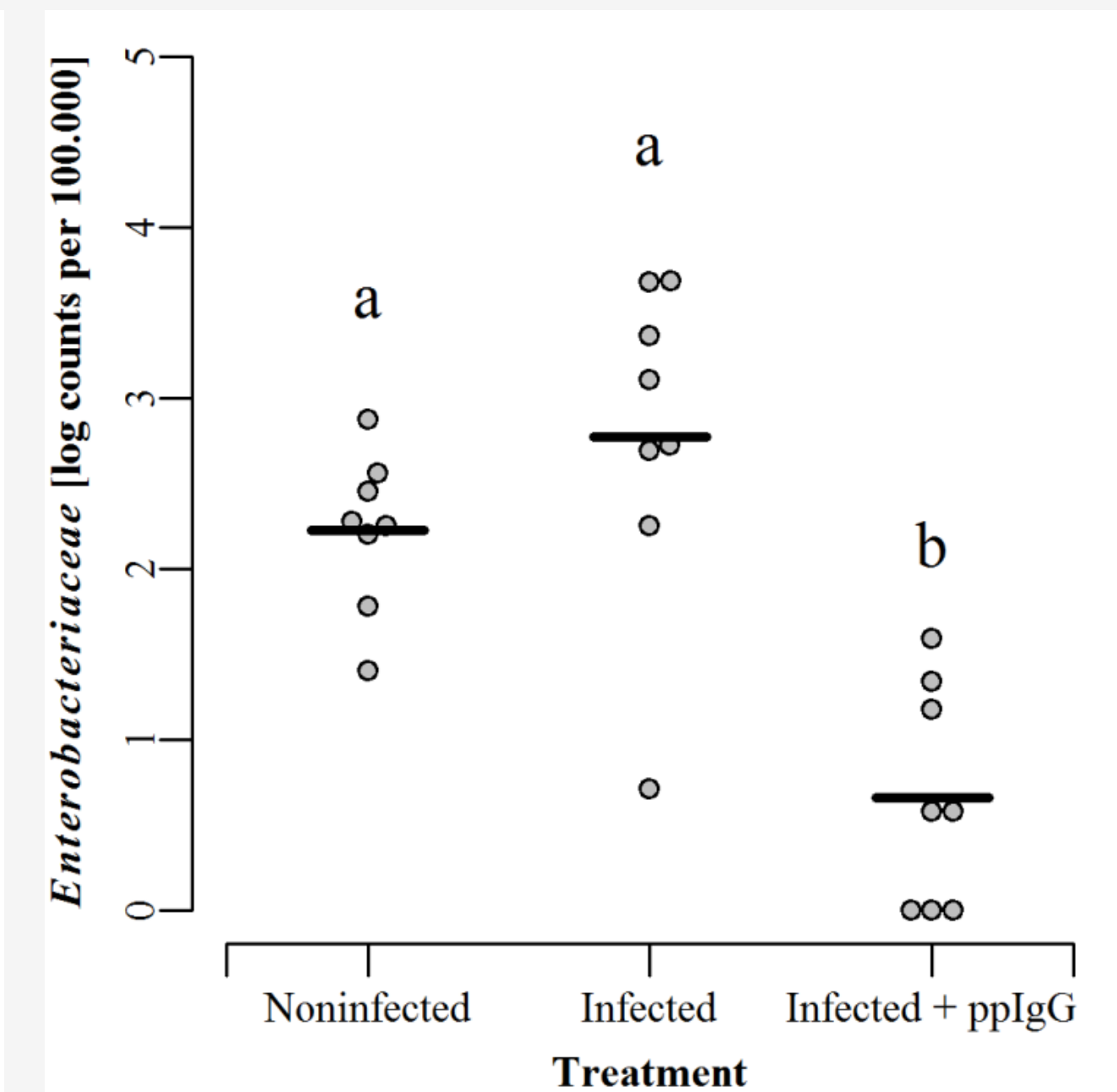
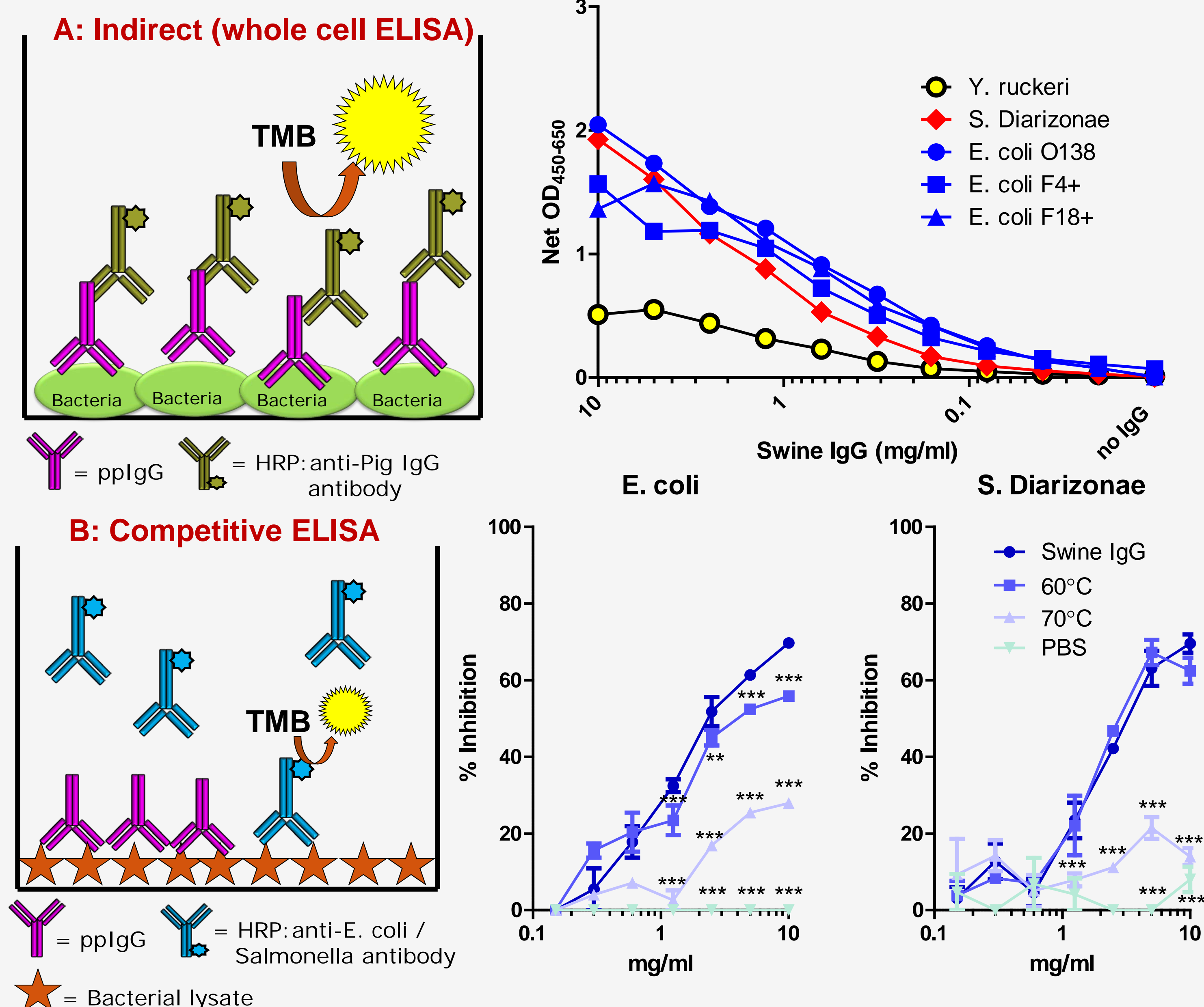


Figure 2: pplgG reacts with relevant bacteria *in vitro*



C: Inhibition of ETEC adhesion

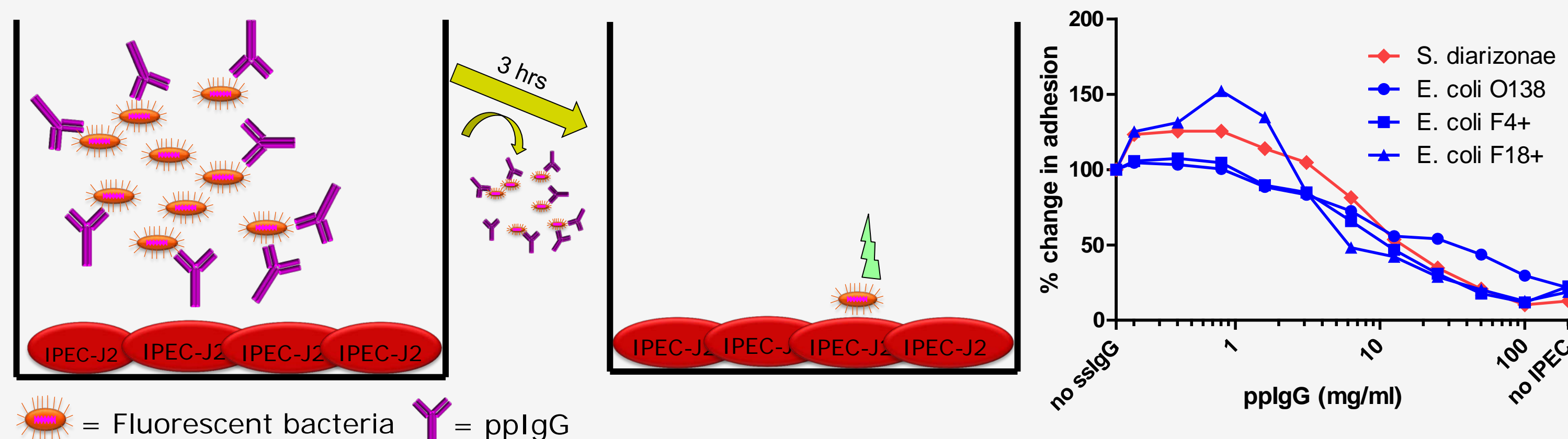


Figure 2: pplgG reacts with relevant bacteria *in vitro*

Three different *in vitro* assays were applied (see below) and it was observed that pplgG could selective bind to relevant pig-pathogenic bacteria.

Indirect: pplgG was added in 2-fold dilution series from 10 to 0.02 mg/ml. After 1 hour of incubation and 3 washes in PBS-Tween, detection antibody (HRP-conjugated rabbit anti-porcine Ig (DAKO)) was added and detected pplgG bound to relevant bacteria (i.e. ETEC and *S. enterica*) but not to the fish-pathogen *Y. ruckeri*.

Competitive ELISA: Along with pplgG either Genway Biotech's anti-E. coli (18-511-245057) or anti-salmonella (18-511-245055) HRP-conjugated antibodies were used. The read out was dependent on the ability of the pplgG to inhibit the signal by interfering with the binding of conjugated antibody to its ligands. Denaturing pplgG (60-70°C) significantly reduced the ability of the pplgG to inhibit the binding of the detection antibody to the bacterial antigens.

Adhesion-inhibition assay: Fluorescently stained bacteria were pre-incubated at 4°C with various amounts of pplgG (from 100 mg/ml to 0.2 mg/ml, plus controls with no pplgG) before added to 15.000 neonatal porcine jejunum derived IPEC-J2 cells/well (DSMZ, Braunschweig, Germany) for additional 3 hrs of incubation at 4°C. After adhesion, plates were washed thrice and buffer was added before reading fluorescence at 485/528 nm by means of Synergy HT (BioTek) using Gen5 software (BioTek). pplgG inhibits the binding of the four pig-relevant bacteria to IPEC-J2 cells.

Figure 3: pplgG reduces ETEC infection in PWD model

A: Offspring of 11 sows were randomly mixed after farrowing (day -28), to avoid confounding treatment effect with the genetic background. At 28 days of age, 24 piglets were randomly selected, weaned and distributed according to their experimental group (day 0). On day 1 two groups (Infected+pplgG and Infected) were given 2×10^{10} CFU of ETEC (*E. coli* F4+O149). The group 'Infected+pplgG' was provided every day with oat/wheat-feed mixed with 160 ml (32 grams) of pplgG. After 12 days the weaner piglets were killed and inspected. **B:** Faecal samples were collected on day 1, 3, 5, 7, 9 and 11 post infection and analysed by F4-specific qPCR for shedding of the ETEC. The infection was cleared significantly faster in the 'infection+pplgG' group compared to the infection control group ($p=0.0007$), even though onset of infection was faster in infection+pplgG than in the infection control group ($p=0.0017$). **C:** DNA was purified from the ileum samples (taken at autopsy). V1-V2 regions of 16S rRNA gene was amplified, and amplicons were sequenced on MiSeq platform (Illumina Inc. San Diego, USA). The resulting read counts were then analysed on the family level, which showed a significantly lowered ($p<0.001$) colonisation by the family *Enterobacteriaceae* in the ileum as compared to both the non-infected control and the infected control group. **Collectively the data presented in Figure 2+3 suggest that pplgG inhibits ileal/intestinal adhesion of bacteria from this family.**