Swine plasma immunoglobulins for prevention and treatment of post-weaning diarrhoea

Safety and Preliminary results

Hedegaard, Chris Juul; Strube, Mikael Lenz; Bendix Hansen, Marie; Kjær Lindved, Bodil; Larsen, Lars Erik; Lihme, Allan; Boye, Mette; Heegaard, Peter M. H.

Publication date:
2015

DTU Vet
National Veterinary Institute

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

Chris J. Hedegaard[1], Mikael L. Strube[1], Marie B. Hansen[2], Bodil K. Lindved[2], Lars E. Larsen[1], Allan Lihme[2], Mette Boye[1], and Peter M.H. Heegaard[1]*

1. National Veterinary Institute, Technical University of Denmark, Frederiksberg, Denmark. 2. Upfront Chromatography A/S, Copenhagen, Denmark. *contact: PMHH@vet.dtu.dk

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

Figure 1: Purified porcine IgG (ppIgG)

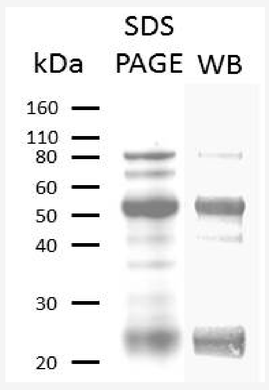


Figure 1: Pig plasma IgG (ppIgG) was purified from blood plasma at UpFront Chromatography A/S (Copenhagen) by high-volume Expanded Bed Adsorption with a proprietary adsorbent. Plasma was obtained from a DAKA A/S (Lunderskov, Denmark).
ppIgG (1 mg/ml) was run under reducing conditions on 12% SDS PAGE. 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 ppIgG was estimated to consist of approximately 85% pure immunoglobulin.

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

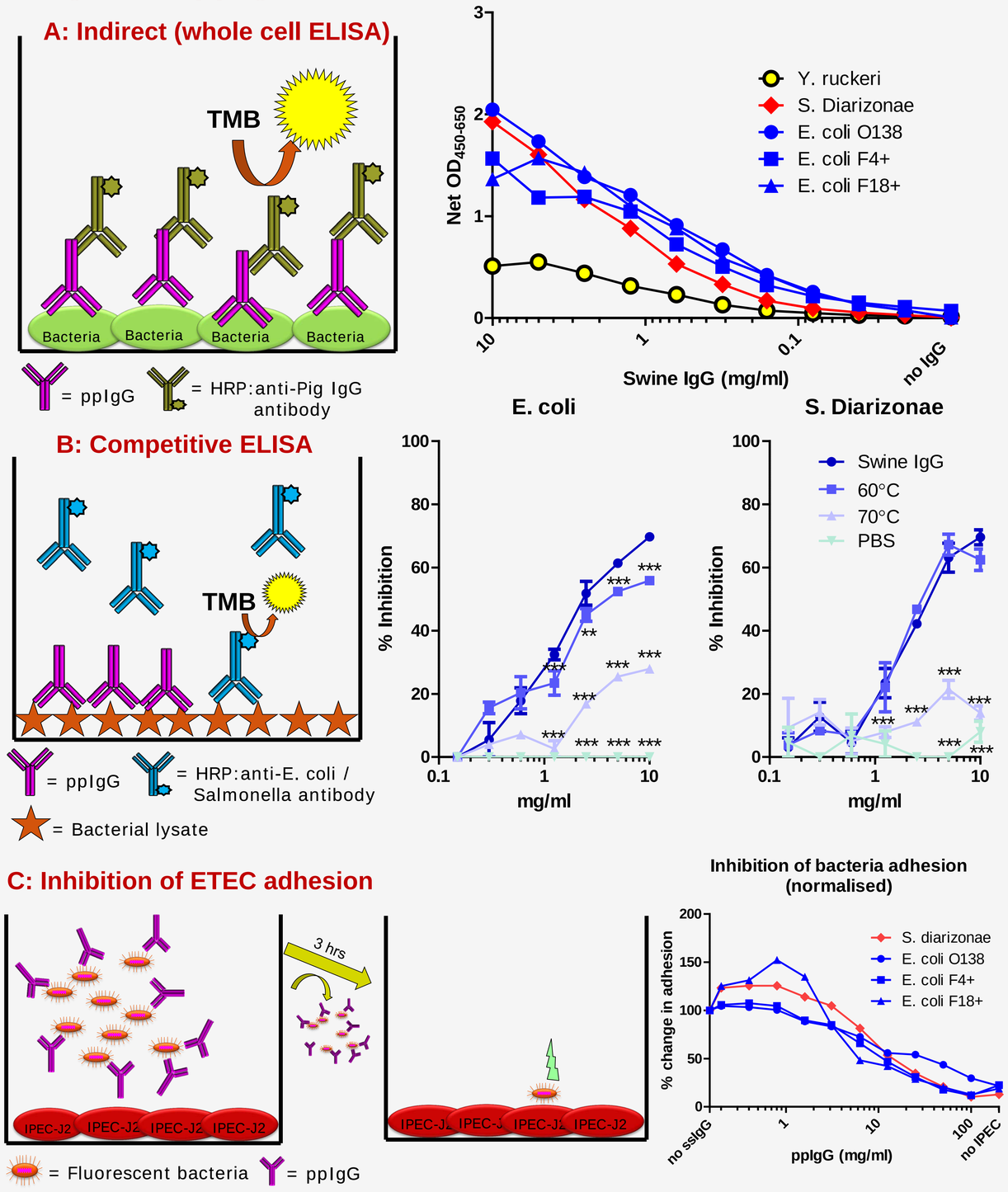
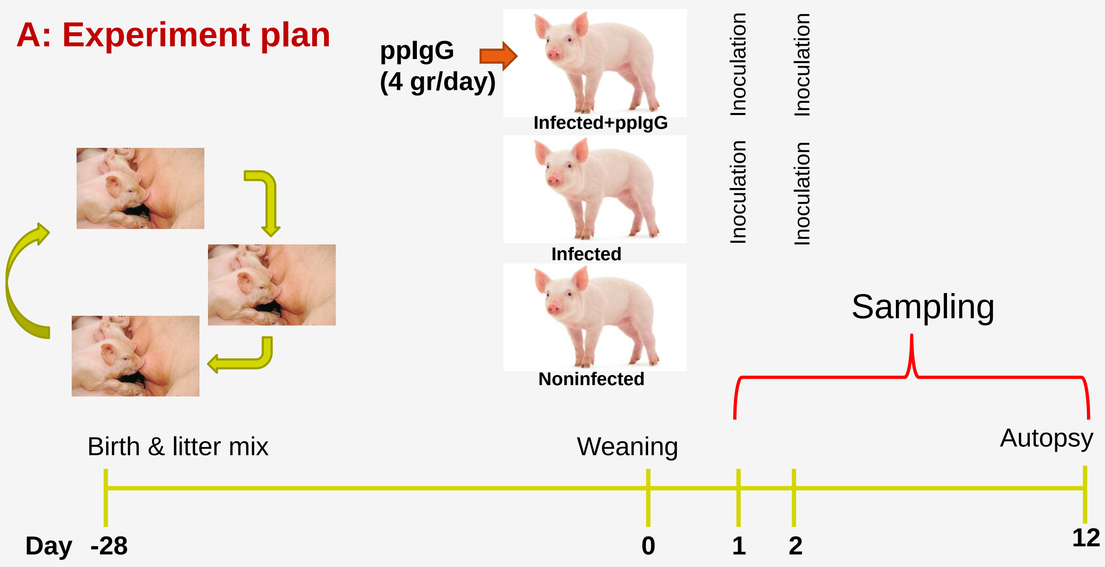
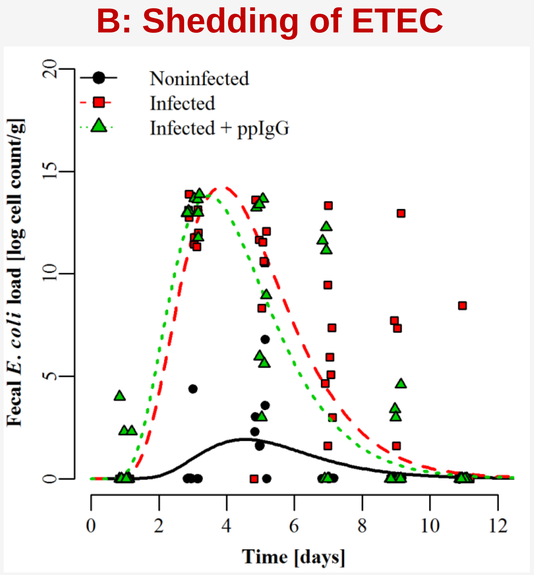


Figure 2: ppIgG reacts with relevant bacteria *in vitro*
Three different *in vitro* assays were applied (see below) and it was observed that ppIgG could selective bind to relevant pig-pathogenic bacteria.
Indirect: ppIgG was added in 2-fold dilution series from 10 to 0.02 mg/ml. After 1 hour of incubation and 3 washes in PBS-Tween, detection antibody (HRP-conjugated rabbit anti-porcine Ig (DAKO)) was added and detected ppIgG bound to relevant bacteria (i.e. ETEC and *S. enterica*) but not to the fish-pathogen *Y. ruckeri*.
Competitive ELISA: Along with ppIgG 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 ppIgG to inhibit the signal by interfering with the binding of conjugated antibody to its ligands. Denaturing ppIgG (60-70°C) significantly reduced the ability of the ppIgG 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 ppIgG (from 100 mg/ml to 0.2 mg/ml, plus controls with no ppIgG) 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). ppIgG inhibits the binding of the four pig-relevant bacteria to IPEC-J2 cells.

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





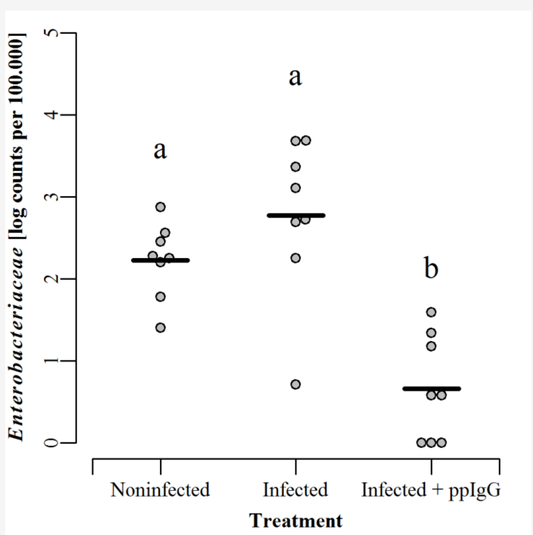


Figure 3: ppIgG reduces ETEC infection in PWD model
A: Offspring of 11 sows were randomly mixed after farrowing (day -28), to avoid confounding treatment effect with the genetic background. At 28 days of age, 24 piglets were randomly selected, weaned and distributed according to their experimental group (day 0). On day 1 two groups (Infected+ppIgG and Infected) were given $2x10^{10}$ CFU of ETEC (*E. coli* F4+O149). The group 'Infected+ppIgG was provided every day with oat/wheat-feed mixed with 160 ml (32 grams) of ppIgG. 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+ppIgG' group compared to the infection control group ($p=0.0007$), even though onset of infection was faster in infection+ppIgG 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 ppIgG inhibits ileal/intestinal adhesion of bacteria from this family.**

All animal procedures were approved by the Danish Animal Experiments Inspectorate under the Ministry of Justice (permit number: 2014-15-2934-01048) and animal experiments were conducted in strict accordance with their guidelines.