Swine plasma immunoglobulins for treatment of post-weaning diarrhoea

by Chris Juul Hedegaard, Postdoc

Technical University of Denmark
National Veterinary Institute
Division of Vaccinology and Immunology

Peter M. H. Heegaard
Innate immunology Group

DTU Vet
National Veterinary Institute
Contents

• Postweaning diarrhoea and antimicrobials

• Passive immunisation with porcine plasma purified immunoglobulins (as an sustainable alternative to antibiotics and dietary zinc)

• In vitro and in vivo studies
Postweaning Diarrhoea

- Common condition in weaner piglets (~3 days after weaning)
- In EU: up to 23% of piglets are estimated to be affected
- Usually caused by intestinal colonisation of F4+/F18+ Enterotoxigenic E. coli (ETEC)
- Results in decreased average daily weight gain
- Mortality up to 25% if untreated

**Common treatment/prevention:** orally administered antibiotics and dietary zinc-oxide containing feed

Diarrhoea
Antibiotics
Antibiotics
Antibiotics
Threat of microbial antibiotic resistance

Europe (ECDC/EMEA 2009):

25,000 people die each year in the EU from infections caused by bacteria resistant to antimicrobials

USA (CDC 2013):

Estimated minimum number of illnesses and deaths caused by antibiotic resistance*:

At least 2,049,442 illnesses, 23,000 deaths

*bacteria and fungus included in this report

Global information is insufficient to show complete disease burden impact and costs
Sales of veterinary antimicrobial agents in 26 EU/EEA countries

PCU = animal population correction unit

5th ESVAC report 2013

From: WHO 2012
Antimicrobial consumption in Denmark
Antimicrobial consumption in Denmark

30% of total Danish antibiotics goes to PWD treatment
Dietary zinc oxide has a negative effect on the environment

High dietary zinc supplementation increases the occurrence of tetracycline and sulfonamide resistance genes in the intestine of weaned pigs

Willfried Vahjen¹, Dominika Pietruszyńska, Ingo C. Starke and Jürgen Zentek

Effects of manure and mineral fertilization strategies on soil antibiotic resistance gene levels and microbial community in a paddy–upland rotation system

Hui Lin⁴*, Wanchun Sun⁴, Zulin Zhang⁵, Stephen J. Chapman⁷, Thomas E. Freitag⁷, Jianrong Fu⁵, Xin Zhang⁴, Junwei Ma⁴

¹The Institute of Environmental Resource and Soil Fertilizers, Zhejiang Academy of Agricultural Sciences, Hangzhou 310002, P.R. China
²The James Hutton Institute, CRAIG-STEWARD, Aberdeen AB5 3RY, Scotland, United Kingdom
³College of Resource and Environmental Sciences, Zhejiang Agricultural and Forestry University, Zhejiang, P.R. China

Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential

Chandan Pal¹, Johan Bengtsson-Palme¹, Erik Kristiansson² and D. G. Joakim Larsson¹*
Alternatives to antibiotics are (also) needed in swine production
Active immunisation (Vaccination)

- Leads to future protection (memory)

But may not be applicable for preventing enteric diseases:

1. As weaner piglets have immature immune system
2. Because of too short time to develop an immune response/protection after weaning
3. Due to interfering (lactogenic) maternal antibodies
4. As live oral vaccine can not be combined with antibiotics
5. If multifactorial
Passive immunisation

- Gives immediate protection/alleviation
- Sow lactogenic immunity surrogate

But no long-term protection (no memory)
Acquired immunity

Infection agents → Invasion → Antibodies → Protection

Immune-responses

Infectious agents: Bacteria, viruses, fungi, parasites

Protection: Antigen presentation, T cell activation, B cell activation, antibody production

Antibodies: Specific proteins that defend against infection
Slaughter

Blood is slaughterhouse waste

Inexpensively, untapped and renewable resources
Purification

Expanded Bed

DTU Vet
National Veterinary Institute
Natural antibodies against Postweaning diarrhoea

Purified Pig IgG = ppIgG

Immunoglobulin

ppIgG for prevention and treatment of PWD
Reactivity against bacteria

Indirect (whole-cell) ELISA

Competitive ELISA

Swine IgG (mg/ml)

% Inhibition

E. coli

S. Diarizonae


11/01/2017
Reactivity against bacteria

Indirect ELISA

\[ \text{TMB} = \text{ppIgG} \]
\[ \text{HRP:anti-pig} = \text{IgG antibody} \]
\[ \star = \text{Bacteria lysate} \]

PWD: experimental model

Litter mix

ppIgG (4 gr/day)

Weaning

0 1 2

Inoculation

Infected

Infected + ppIgG

Noninfected

Faecal sampling

Autopsy

11/01/2017
Shedding of ETEC (faeces) (p=0.0017)

Enterobacteriaceae in ileum (p=0.001)

Pre-clinical trial (Feacal score)

1. Infected + Zink
   N = 6

2. Infected
   N = 6

3. Infected + ppIgG
   N = 6 (5)

Litter mix
Day -28

ppIgG (2x2 gr/day)

Inoculations
0 1 2 7 14 21
Stop IgG
Stop zinc

Weaning

Faecal score

Dry Matter (%)

a: p<0.03; IgG vs. Zinc
b: p<0.02; IgG vs. Ctrl
c: p<0.02; IgG vs. Zinc
d: p<0.01; IgG vs. Ctrl
e: p<0.02; Zinc vs. Ctrl
Pre-clinical trial (Feacal bacteria)

1. Infected + Zink
   N = 6
2. Infected
   N = 6
3. Infected + ppIgG
   N = 6 (5)

ppIgG (2x2 gr/day)

Inoculations
0 1 2 7 14 21
Weaning
Stop IgG
Stop Zinc

Haemolytic bacteria

Non-haemolytic bacteria

log CFU vs. Days after weaning

a: p<0.02; IgG vs. Zinc
The impact of dietary swine plasma immunoglobulins on intestinal microbiota and general health in weaner piglets
Safety: Infectability

On-column virus deactivation

Model viruses

<table>
<thead>
<tr>
<th>Env. DNA</th>
<th>Non-env. DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Env. RNA</td>
<td>Non-env. RNA</td>
</tr>
</tbody>
</table>

Removal
Deactivation
Stability
Activity

Expanded Bed

Post production virus deactivation
Safety: Infectability (work in progress)

Porcine Parvovirus (PPV)

<table>
<thead>
<tr>
<th>Env. DNA</th>
<th>Non-env. DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Env. RNA</td>
<td>Non-env. RNA</td>
</tr>
</tbody>
</table>

9.52 x 10^6

On-column deactivation with Na-caprylate

Infectability

Env. DNA Non-env. DNA

Indirect ELISA (E. coli)

Activity

Removal

Fraction  Titre  Recovery (%) ^1

<table>
<thead>
<tr>
<th>Fibrinogen precipitate</th>
<th>6.85 x 10^6</th>
<th>71.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant</td>
<td>3.49 x 10^4</td>
<td>0.3666</td>
</tr>
<tr>
<td>Run through</td>
<td>2.28 x 10^4</td>
<td>0.2391</td>
</tr>
<tr>
<td>1st Wash</td>
<td>2.78 x 10^3</td>
<td>0.0292</td>
</tr>
<tr>
<td>2nd Wash (caprylate)</td>
<td>1.06 x 10^2</td>
<td>0.0011</td>
</tr>
<tr>
<td>3rd Wash (50° C)</td>
<td>3.07 x 10^2</td>
<td>0.0032</td>
</tr>
<tr>
<td>Eluate (NaOH)</td>
<td>3.04 x 10^2</td>
<td>0.0032</td>
</tr>
<tr>
<td>Regeneration</td>
<td>1.37 x 10^2</td>
<td>0.0014</td>
</tr>
</tbody>
</table>

^1Adjusted for sample volume

Caprylate deactivates PPV

Sample | Infectability lvl

| Neg. Ctrl              | – |
| Pos. Ctrl (low PPV lvl)| + |
| Pos. Ctrl (med PPV lvl)| ++ |
| Pos. Ctrl (high PPV lvl)| +++ |
| IgG+PPV                 | +++ |
| IgG+PPV+caprylate pH 4.5| – |
| IgG+PPV+caprylate pH 5.1| – |
Summary

• Swine production consume large amounts of antimicrobials (e.g. antibiotics and dietary zinc oxide) especially for treating postweaning diarrhoea that
  – pollute the environment
  – develop microbial antibiotic resistance

• Natural porcine plasma immunoglobulins harbour anti-E. coli activity that
  – in vivo can reduce induced diarrhoea in weaner piglets
  – by reducing faecal haemolytic bacteria (incl. E. coli)
  – do not alter the intestinal microbiota in healthy weaner piglets

• Natural porcine plasma immunoglobulins exerts similar effects as dietary zinc oxide on reducing diarrhoeagenic E. coli in weaner piglets

• These results indicate that purified porcine immunoglobulins is a sustainable alternative to antibiotics and zinc
Acknowledgements

Peter M. H. Heegaard
Henriette T. Vorsholt
Heidi G. Andersen
Sophia Rasmussen
Mikael Lenz Strube
Mette Boye
Tim Kaare Jensen
Annie Ravn
Hans Skaaning
Maja Rosendahl
Nanna B. Jensen
Lars E. Larsen
Lise K. Kvisgaard
Hue 3xT

Chromatographies A/S

Allan Lihme
Marie Bendix Hansen
Bodil Kjær Lindved
Kenneth Harlow
Michael Pålsson

Sponsor:

DTU VET

VET

(SEGES)

(Knowledge Centre for Agriculture and Pig Research)

Poul Bækbo
Claus Hansen

Foulum

DANISH CENTRE FOR FOOD AND AGRICULTURE

Charlotte Lauridsen
Inger Marie Jepsen
& staff

upfront