Development of host-microbiome interactions in the pig: current understanding and future perspectives

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Bristol University
Changes in technologies used to analyse microbiomes

Percent of total microbiome publications

- DGGE
- Culture
- Microarray
- 16S rRNA
- Metagenome

Graph showing the percent of total microbiome publications over time from 1990 to 2020.
The human microbiome project: Microbial diversity increases with age in human populations.

doi:10.1038/nature11053
Diversity and richness increase with age through a series of transitions between ecosystems
Is the type of colonisation important?

Segmented filamentous bacteria (SFB) upregulate a wide range of gut helper T-cells

Gaboriau-Routhiau et al., 2009, Immunity 31:677
Genetically identical mice from different suppliers have different immune systems dependant on their microbiota.
Rates of Cure without Relapse for Recurrent *Clostridium difficile* Infection.
<table>
<thead>
<tr>
<th>Outcome</th>
<th>Placebo (n = 37)</th>
<th>FMT (n = 38)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical remission, n (%)</td>
<td>2 (5)</td>
<td>9 (24)</td>
<td>.03</td>
</tr>
<tr>
<td>Clinical response, n (%)</td>
<td>9 (24)</td>
<td>15 (39)</td>
<td>.16</td>
</tr>
<tr>
<td>Full Mayo score</td>
<td>6.34</td>
<td>6.09</td>
<td>.42</td>
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<tr>
<td>IBDQ score</td>
<td>149.38</td>
<td>152.13</td>
<td>.44</td>
</tr>
<tr>
<td>EQ-5D score</td>
<td>70.07</td>
<td>68.52</td>
<td>.99</td>
</tr>
<tr>
<td>CRP, mg/L (n = 17 placebo, n = 15 FMT)</td>
<td>3.3 ± 3.4</td>
<td>4.9 ± 5.9</td>
<td>.38</td>
</tr>
</tbody>
</table>

Potential relevance of pig gut content transplantation for production and research

Nuria Canibe¹, Mark O’Dea² and Sam Abraham²

https://doi.org/10.1186/s40104-019-0363-4
Stages in the development of the mucosal immune system of the pig

1. Rudimentary Peyers patches, essentially no mucosal T cells. Limited B-cell repertoire. Few dendritic cells but MHCII on endothelial cells. The newborn pig.

2. Non-specific expansion of B-cells and Peyers patches. Appearance of early, activated T-cells, influx of MHCII+ cells. 1 days to 2 weeks.

3. Appearance of CD4+ T cells. 2 weeks to 4 weeks.

4. Antigen-specific B-cell responses. Appearance of CD8+ T cells. 4 weeks to 6 weeks.
Most of this expansion of the mucosal immune system is driven by microbiota.

Colonisation of newborn, gnotobiotic piglets with defined microbiota results in expansion of the mucosal immune system which replicates, approximately, that in conventional pigs.

Defined colonisation of gnotobiotic piglets expands mucosal SIRPα⁺ DC first, then CD4⁺ T-cells (Inman, 2012, PLoS One)

- Colonised
- Germ-free
The studies described so far have been *causal* – direct interventions targeted at the microbiome which result in effects on the host.

- These kinds of studies demonstrate mechanisms from which likely interventions can be inferred
- They are usually undertaken in highly controlled environments

Much of the literature is *observational*, and describes associations

- These studies are much harder to infer interventions from
- They are usually undertaken in much more ‘field-relevant’ conditions
• One of the problems is that our understanding of the way the gut microbiome works is at a very early stage.

• We are currently focusing on **lists**:
  - Lists of organisms
  - Lists of genes
  - Diversity, richness, evenness
  - Ratio of Firmicutes to Bacteroidetes

• We need to concentrate on **function**
  - Ecosystem services
  - Nutrient cycles and food webs
  - Predator-prey relationships (phages and protists)
  - Ecological guilds
Understanding the function of microbial ecosystems will allow rational design of interventions.

We are probably at least twenty years from this depth of understanding.
In this model, manipulating the gut microbiome towards increased diversity provides performance benefits.
In this model, diversity is only a proxy for optimal gut microbiome and may not provide performance benefits.
Faecal microbiome is associated with performance in pigs

Ramayo-Caldas et al. Phylogenetic network analysis applied to pig gut microbiota identifies an ecosystem structure linked with growth traits. ISME Journal (2016) 10, 2973–2977

<table>
<thead>
<tr>
<th>Enterotypes</th>
<th>Weight (Kg) at 60 days</th>
<th>Daily gain (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruminococcus</td>
<td>25.8 ± 0.2</td>
<td>438.3 ± 6.7</td>
</tr>
<tr>
<td>Prevotella</td>
<td>26.7 ± 0.1**</td>
<td>456.3 ± 4.4*</td>
</tr>
</tbody>
</table>
Gut microbiome changes dramatically along the pig intestinal tract
Crespo-Piazuelo et al, 2018, Scientific Reports 8:12727

…and this doesn’t take into account the difference between lumen and mucosal microbiomes.

Faecal microbiomes are not representative of gut microbiomes.

Whether they can be deconvolved from faecal microbiomes is controversial
Faeces represents a specific microbial ecosystem associated with the terminal rectum, rather than the whole gastrointestinal tract.

Acquisition of a stable, mature gastrointestinal microbiome by young piglets is likely to occur through a succession of less complex microbial ecosystems.

Microbial ecosystems in the sow rectum are certainly not representative of the ecosystem which needs to establish in the intestinal compartments of the neonatal piglet.

Natural colonisation of piglets from sow faeces or by FMT is necessarily highly selective – relatively little of the transferred microbial ecosystem is likely to ‘take’ in the newborn intestine.

Critical points in development of these microbial ecosystems are likely to be birth and weaning.
Can we manipulate this directly in conventional animals with complex, highly variable microbiota?

And are the effects of such manipulation predictable?
Manipulation of the microbiota by rearing environment

<table>
<thead>
<tr>
<th>Origin</th>
<th>Indoor Farm</th>
<th>Outdoor Farm</th>
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</thead>
<tbody>
<tr>
<td>First</td>
<td>6 Sows</td>
<td>6 Sows</td>
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<tr>
<td>24 hrs</td>
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</tbody>
</table>

Rearing

- Farm: 3
- Isolator: 3
- Isolator+Ab: 3
- Farm: 3
- Isolator: 3
- Isolator+Ab: 3

**Time points**
- 5 days
- 28 days
- 56 days

**Tissues**
- Upper jejunum
- Middle jejunum
- Lower jejunum
- Colon
Hierarchical cluster analysis of faecal microbiota (DGGE)

Mucosal immune response genes are upregulated in isolator/antibiotic and indoor piglets vs outdoor (Mulder et al, 2009)

Most affected are type I interferon-related and MHC class I and II processing pathways
Isolator piglets: **more** mucosal MHCII+ DC in the first three weeks

Principal component 3 is determined by farm of origin, even eight weeks after piglets are moved to a control environment.

Major loadings are:
- CD16MHCII
- MIL11MHCII
- CD16MIL11MHCII
Multivariate statistical analysis of mucosal antigen-presenting micro-environments identifies effects associated with early rearing macro-environment (the first 24 hours, the first week).

These early effects persist through weaning until at least 8 weeks old.

**Increased** epithelial barrier function after *B. lactis* administration

![Bar graph showing increased epithelial barrier function after *B. lactis* administration compared to control.](image)

- **ZO-1**: Control: -1.0, *B. Lactis* from 1 day: -0.9, p < 0.001
- **E-cadherin**: Control: 0.6, *B. Lactis* from 1 day: 0.4, p = 0.015
- **CD45**: Control: 0.3, *B. Lactis* from 1 day: 0.3, p < 0.001
**Decreased** local IgA and IgM synthesis after *B. lactis* administration

MLN – mesenteric lymph node

JPP – jejunal Peyers patch

PSI – upper jejunum

DSI – lower jejunum

Cae – caecum

Col - colon

*(Lewis et al, 2013, BJN 110:1243)*
**B. lactis**, diet and Ig synthesis

Merrifield et al, Gut, in press

![Graph showing the effect of B. lactis on Ig synthesis in different diets.](graph.png)
Mucosal synthesis of immunoglobulins is linked both to probiotic and to weaning diet. The effect of probiotic is different between diets.

The effect of probiotic on urinary metabolites also differs between diets.

Probiotic supplementation changes the structure of immuno-metabolic correlations.
Administration of a probiotic to pigs has profound effects on the immune system and on metabolism.

Not surprisingly, the effect is at least partially dependant on diet.

The value of pre- and probiotics is likely to be dependent on diet and husbandry – what works in one system may not in others.

The effects are complex, and it’s not clear whether they are ‘good’ or ‘bad’.
Decreased local IgA and IgM synthesis after \textit{B. lactis} administration

MLN – mesenteric lymph node
JPP – jejunal Peyers patch
PSI – upper jejunum
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Cae – caecum
Col - colon

(Lewis et al, 2013, BJN 110:1243)
Increased local IgA after *B. cereus* (Toyo) administration

‘This effect could be related to a more successful mucosal defence’.

Increased IgA indicates that probiotic is a good thing

Published in: Lydia Scharek; Jana Guth; Matthias Filter; Prof. Michael F. G. Schmidt; *Archives of Animal Nutrition* **2007**, 61, 223-234.
DOI: 10.1080/17450390701431540
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Figure 2. IgA levels in faeces of control and probiotic piglets of the *B. cereus* trial. IgA levels were determined by ELISA technique. Values of optical density (OD, 492 nm) were corrected according to reference samples from the same microtest plates. Data are presented as box plots with number of samples on top of each box. Dots represent extreme values. Fat asterisks mark significant differences between groups.
The literature is extremely biased towards the idea that nutritional intervention is ‘a good thing’.

1. Probiotics (or prebiotics) are a good thing
2. If we give probiotic X, we see an effect Y
3. Because probiotics are a good thing, effect Y must be a good thing
4. Because effect Y is a good thing, probiotic X is a good thing.
5. Repeat.
Effects on microbiota, metabolism and immunity

‘Good bacteria’

Positive effects on health and productivity

‘Bad bacteria’

Effects on microbiota, metabolism and immunity

Negative effects on health and productivity
Effects on other microbiota: positive
Effects on host nutrients: negative
Effects on epithelial cells: neutral
Effects on dendritic cells: neutral
Effects on T-cells: positive
Effects on B-cells: negative
What is a ‘good’ microbiota and a ‘bad’ microbiota?

1. Understanding the function of the microbial ecosystem
   • Diversity. Measures of the species diversity, richness and evenness of intestinal microbiomes
   • Analysis of spatially distinct microbial ecosystems, food webs and nutrient flows

2. Empirical associations between microbiomes and health records

3. Resilience. How easy is a specific microbial ecosystem to manipulate?
‘Microbiome space’: the multidimensional space of all possible microbiotas, defined by composition.

‘Goodness’ and ‘badness’ defined against health records in a specific environment

‘Stability’ ‘resilience’ or ‘plasticity’: the effort needed to change the microbiota.
Empirical correlation between microbiomes and ‘health’

Colours indicate ‘goodness’ and ‘badness’, contours indicate ‘stability’
Replicate effects are common confounders of animal experiments and effect sizes can be as big as those of treatment.
Replicate effects were already apparent at 24 hours old, when the piglets were brought into the isolator.
In our hands, **replicate effects** are associated with differences in the composition of the microbiota determined by 16S rRNA sequencing. Piglets reared on formula and weaned onto soya or egg diets (Merrifield et al, ISME Journal (2016) 10, 145.)
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<tr>
<th>Bristol University</th>
<th>Imperial College, London</th>
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<td>Mick Bailey</td>
<td>Jeremy Nicholson</td>
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