Gut microbiota dynamics in weaner pigs in response to experimental ETEC challenge

Jolinda Pollock[1,2]
David Gally[2], Raksha Tiwari[3], Mike Hutchings[1] & Jos Houdijk[1]

[1]Animal and Veterinary Sciences, SRUC, Edinburgh, UK
[2]Infection and Immunity, Roslin Institute, UoE, Edinburgh, UK
[3]Zoetis, Kalamazoo, Michigan, USA

*Leading the way in Agriculture and Rural Research, Education and Consulting*

Project background

- Weaning exerts nutritional, social and immunological stressors

- Enteric disorders common –
  e.g. post-weaning colibacillosis

- Economic impact – reduction in welfare, health and performance.

Project background

- Gut microbiota composition dynamic during this phase – linked to enteric disease?

- Published studies – utilise agar plating and early molecular methodologies

- 16S rRNA gene sequencing – phylogenetic & relative abundance information.

PhD aims
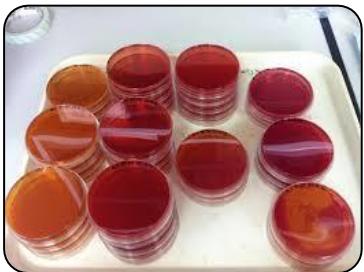
- Develop a 16S rRNA sequencing method to study gut microbiota dynamics

- Study the pathogenesis of ETEC by measuring adherence and shedding

- Assess if and how ETEC challenge and dietary manipulation influences microbiota composition and pig performance.

PhD structure

Expt. 1 – Pilot Sequencing Study

Expt. 2 – Faecal Microbiota Study

Expt. 3 – Infection Model Study

**Expt. 4 – Ileal Microbiota Study**

- **Development of 16S rRNA sequencing method**

- **Study of ETEC shedding and adhesion**

- **Measurement of pig performance**

- **Study of gut microbiota dynamics (rectum and ileum)**

PhD structure

SRUC

Expt. 1 – Pilot Sequencing Study

Expt. 2 – Faecal Microbiota Study

Expt. 3 – Infection Model Study

Expt. 4 – Ileal Microbiota Study

- o **Development of 16S rRNA sequencing method**

- o **Study of ETEC shedding and adhesion**

- o Measurement of pig performance

- o **Study of gut microbiota dynamics (rectum and ileum)**

The 16S rRNA gene

- The **16S rRNA gene** is present in *all* bacteria – exploited for bacterial ID.
- We can also use this gene for targeted study of **complex microbial communities**.
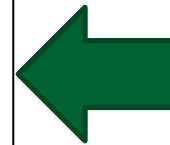- Whole gene too large for sequencing platform – so we utilise **hypervariable regions**.

Flanking Primers

V1 V2 V3 V4 V5 V6 V7 V8 V9
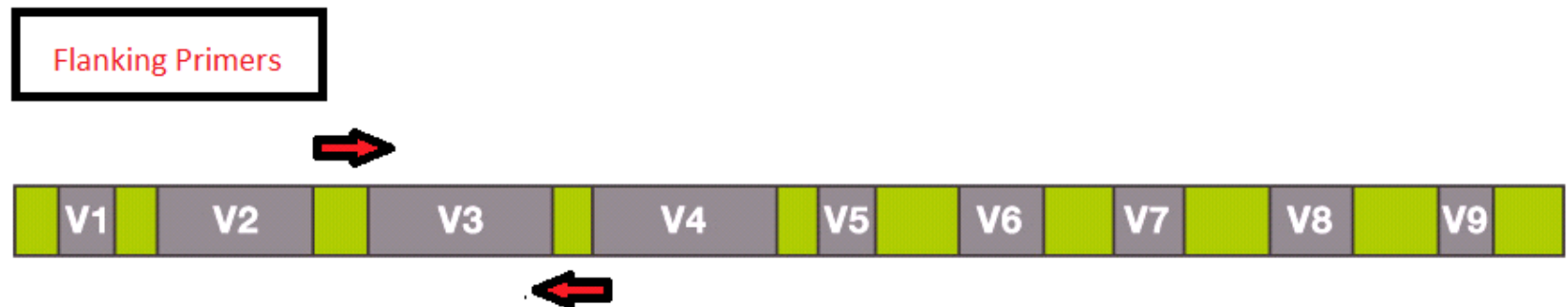
16S rRNA metabarcoding
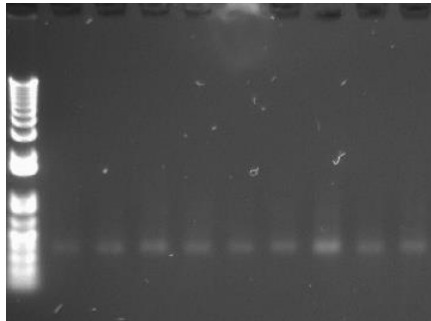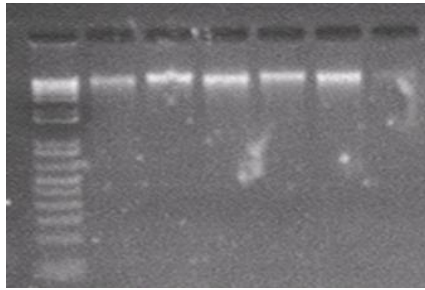
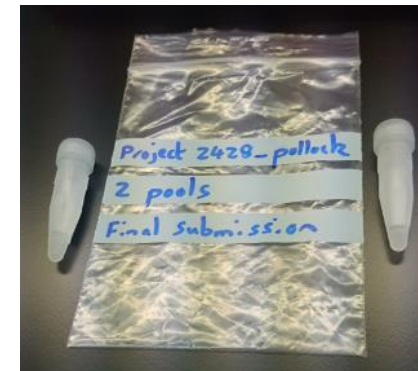**DNA extraction:**
MoBio Powersoil kit

Reagent-only controls

Library preparation:
V3 hypervariable region PCR

Mock bacterial community

Sequencing & analysis
Illumina MiSeq/mothur software

Project 2428_pollock
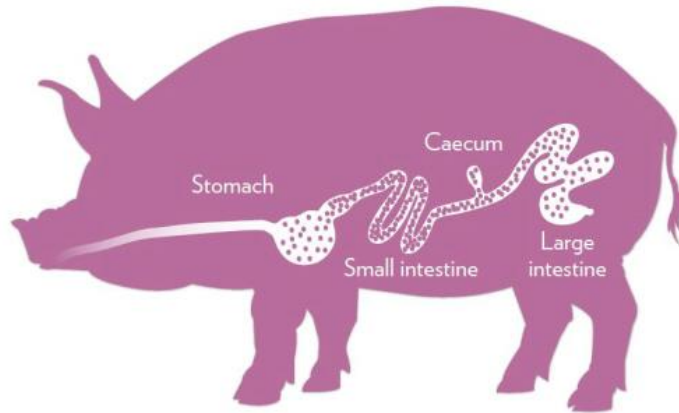2 pools
Final Submission

Expt. 2 – Faecal microbiota

1. Does the faecal microbiota composition change over the post-weaning period?

2. Will experimental ETEC challenge have an effect on faecal microbiota composition?

Experimental design

Sequence analysis

16S data

Total composition (structure)

Specific shifts (relative abundances)

Other
Proteobacteria
Spirochaetes
Unclassified
Bacteroidetes
Firmicutes

Statistical Tests
AMOVA
HOMOVA

Data visualisation
e.g. NMDS, PCoA
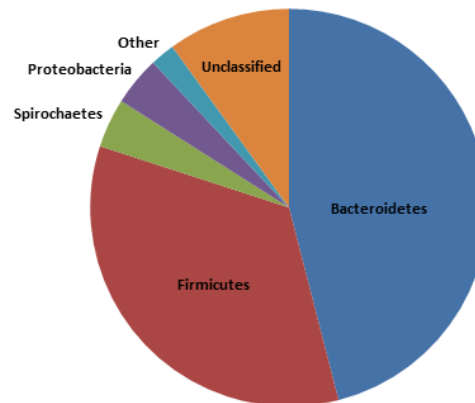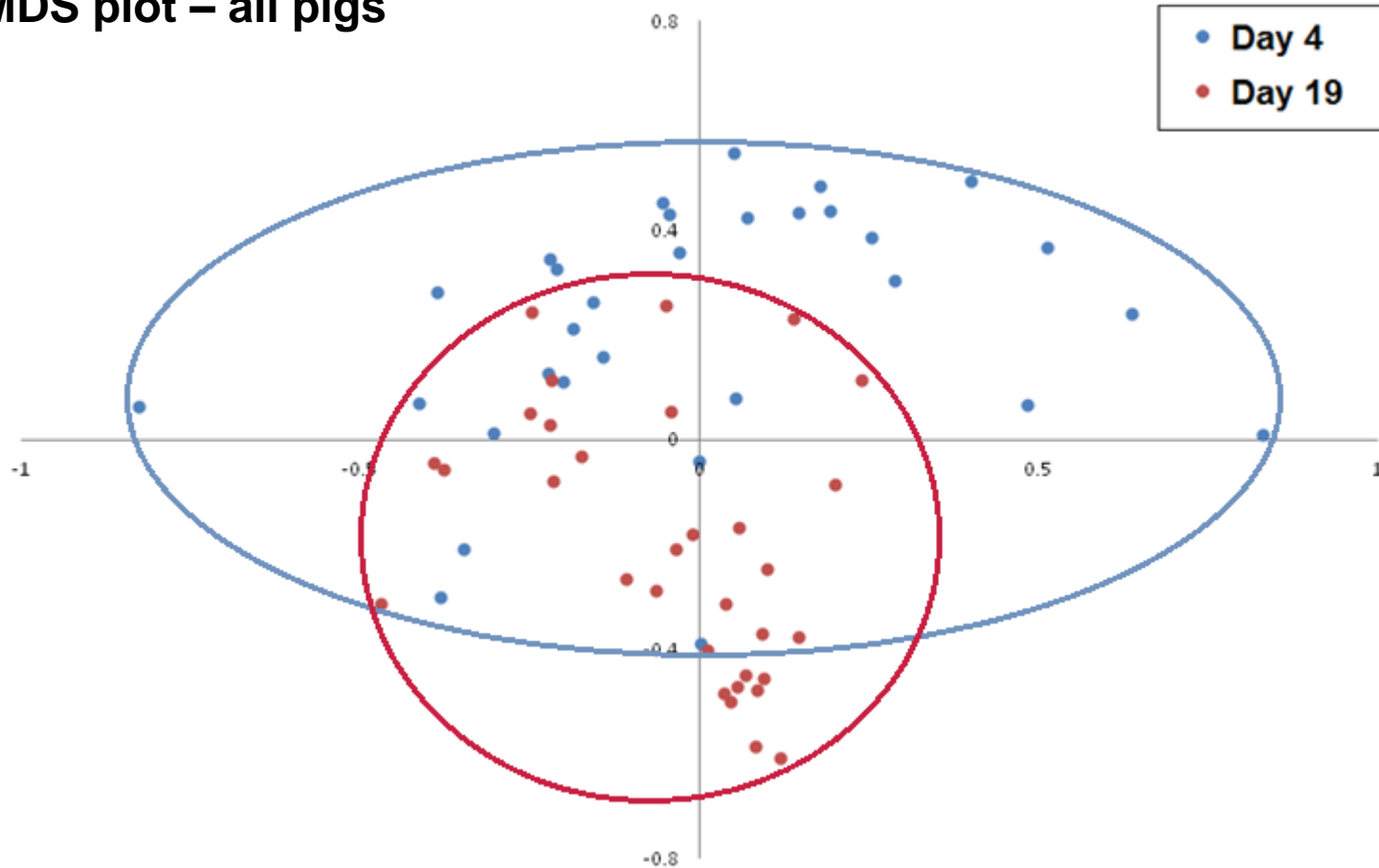
Statistical Tests
Metastats

Data visualisation
Stacked barplots
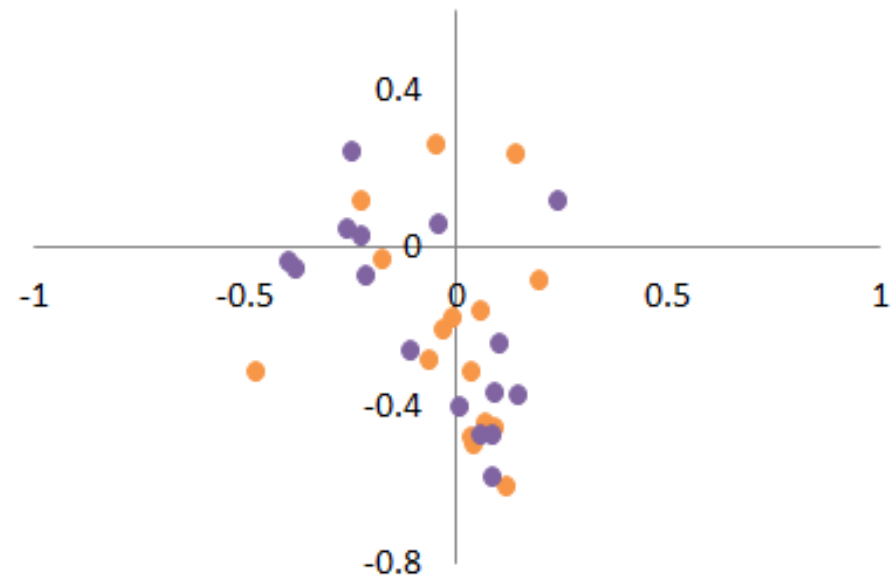Piecharts
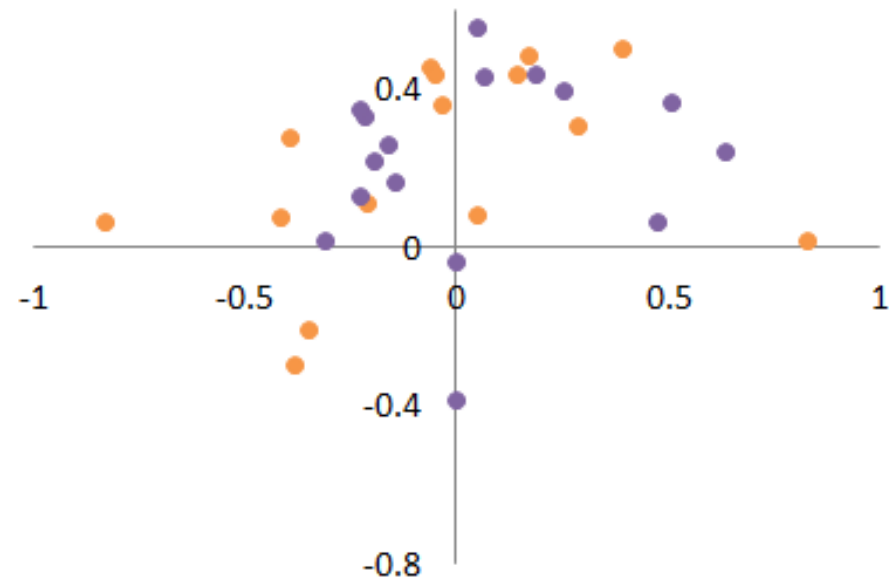
Temporal shifts

NMDS plot – all pigs

- Change in community structure - **AMOVA P < 0.05**
- Decrease in genetic diversity - **HOMOVA P < 0.05**

ETEC vs Sham
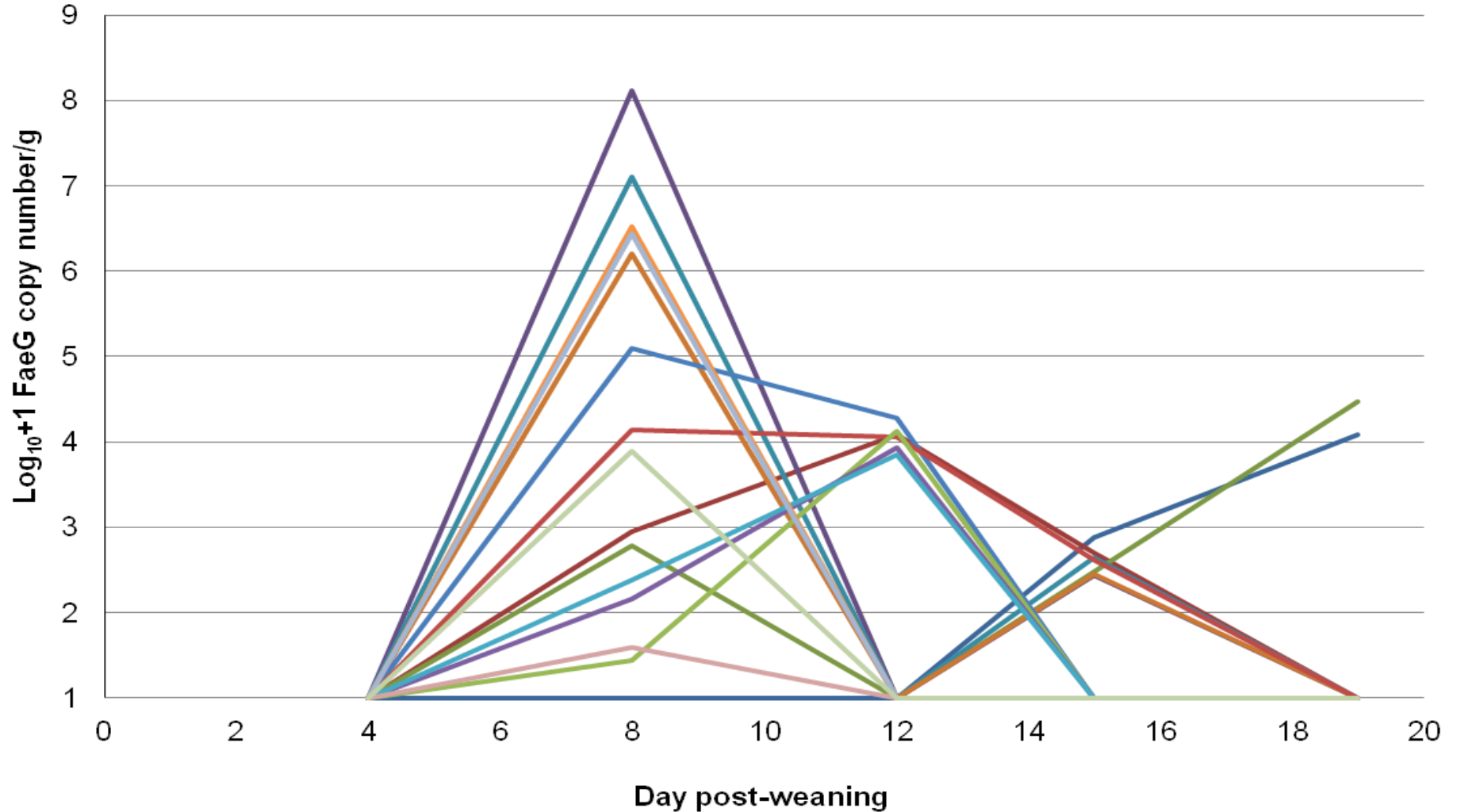

Day 4 (pre-challenge)

Day 19

No differences in community structure or relative abundances
AMOVA P > 0.05
Metastats P > 0.05

Legend:
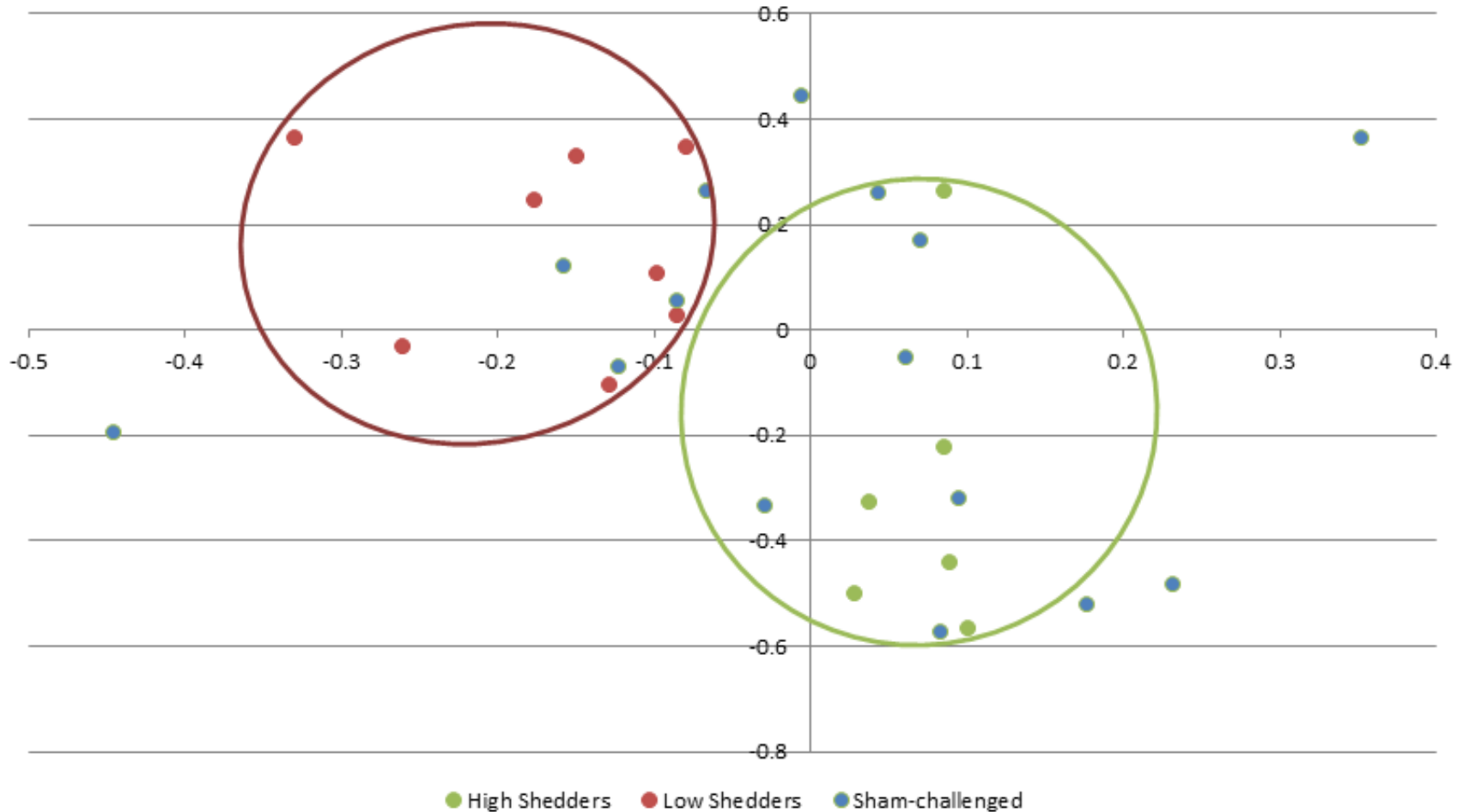● ETEC-challenged
● Sham-challenged

ETEC shedding

ETEC shedding

"Clearers" vs "Shedders"

ETEC shedding

● High Shedders ● Low Shedders ● Sham-challenged
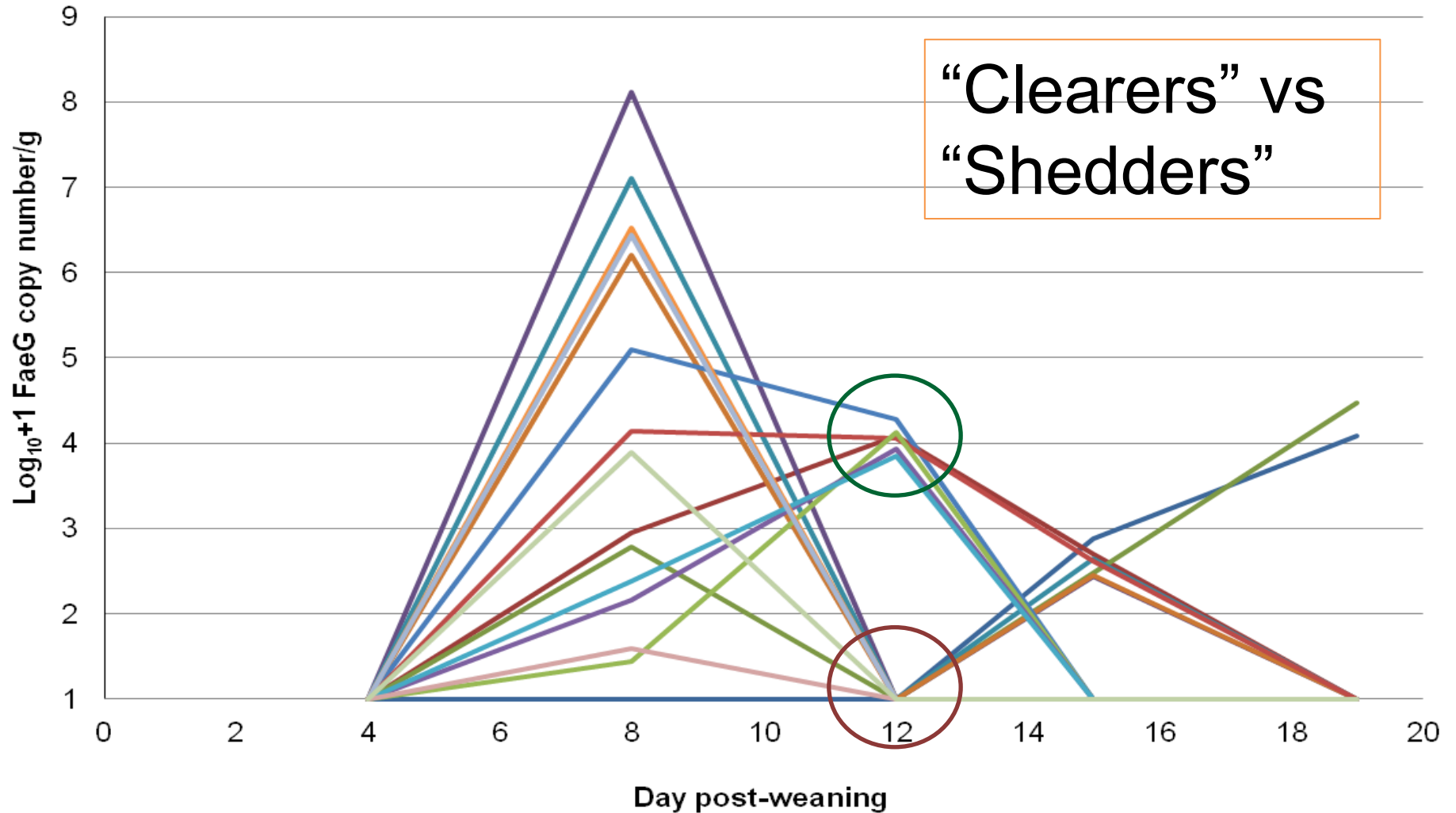
"Shedders" vs "Clearers" – structures significantly different (AMOVA: P = 0.029)

Conclusions

1. Community structure changed significantly over time and microbiota became significantly more stable/less diverse

2. ETEC challenge had no effect on faecal microbiota structure, phylotype relative abundances or stability when comparing ETEC- and sham-challenged pigs

3. Variations in community structure were observed when taking ETEC shedding level into account.

Expt. 4 – Protein x ETEC

- Previous work - ↓dietary protein, ↓ ETEC attachment/shedding
- Microbial fermentation of excess protein – production of ammonia and biogenic amines
- Lowering protein – impact on performance?
- Little understanding currently of how different levels of protein affect the ileal microbiota.

Expt. 4 - Aims

- Study gut microbiota dynamics over the immediate post-weaning period in sham- and ETEC challenged pigs
- Show whether dietary protein level has an impact on the ileal and faecal microbiotas
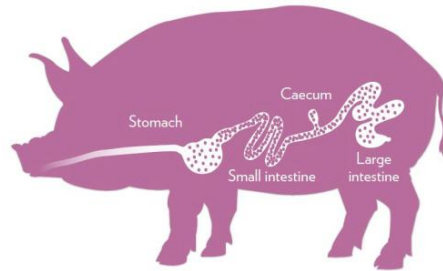- Investigate whether lowering dietary protein impacts host performance.

Experimental design

ETEC-challenged
N = 64

| Low Protein N = 32 | High Protein N = 32 |

Baseline
N = 16

Sham-challenged
N = 64

| Low Protein N = 32 | High Protein N = 32 |

Post-mortem sampling (faeces/digesta)

Inoculation
10^8 cfu ETEC or PBS (by mouth)

"Performance pigs"

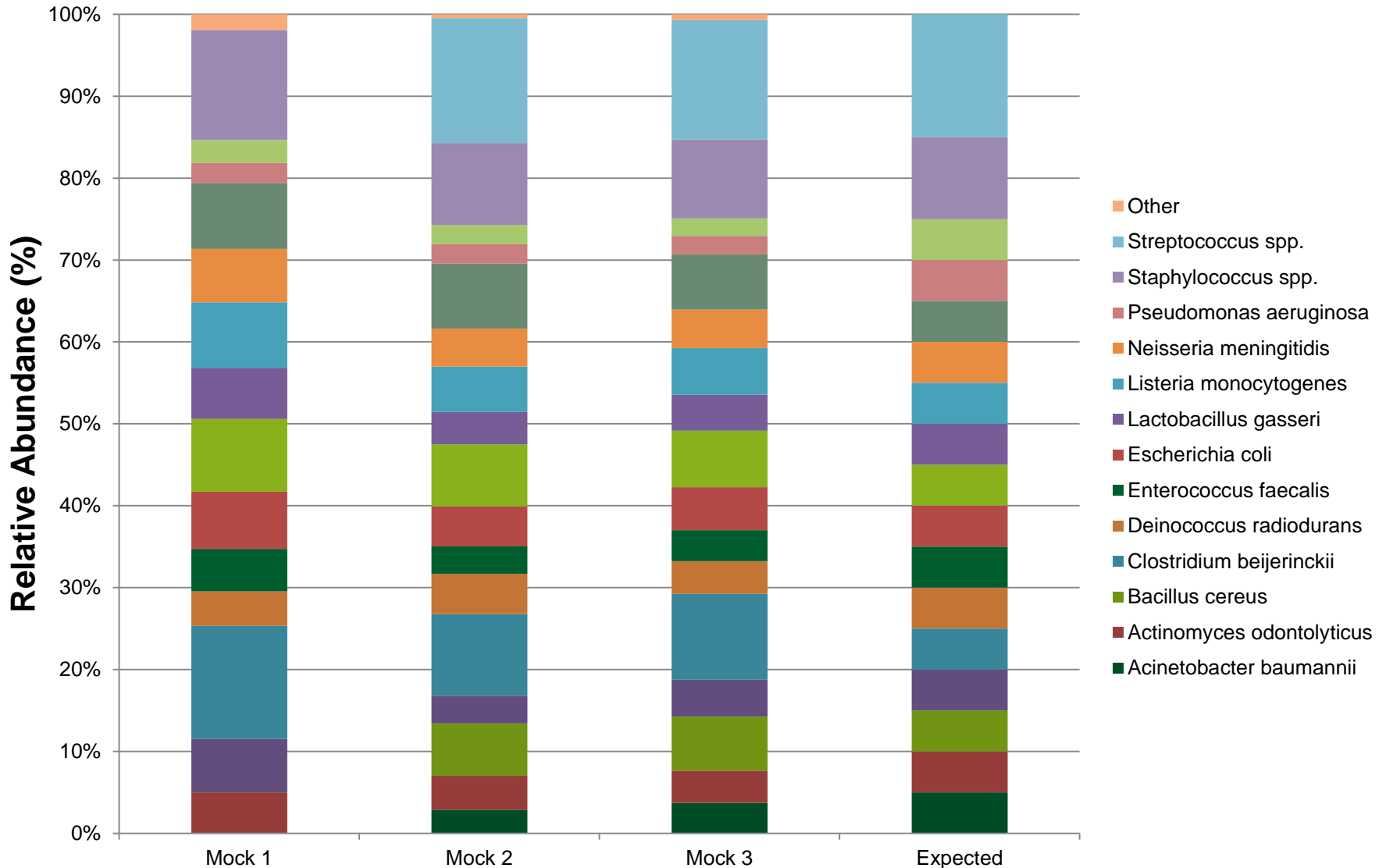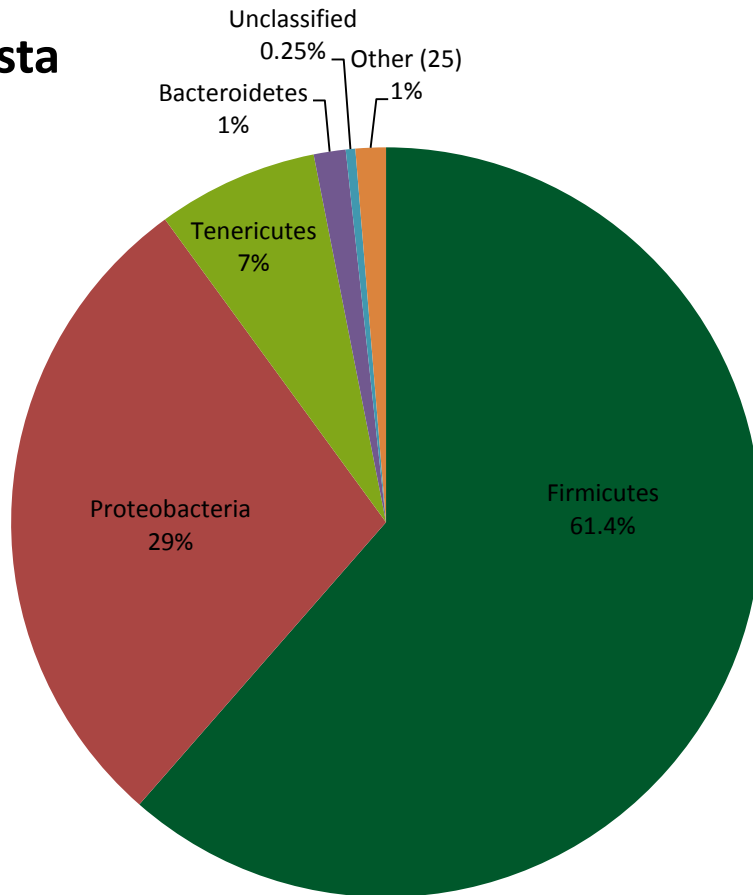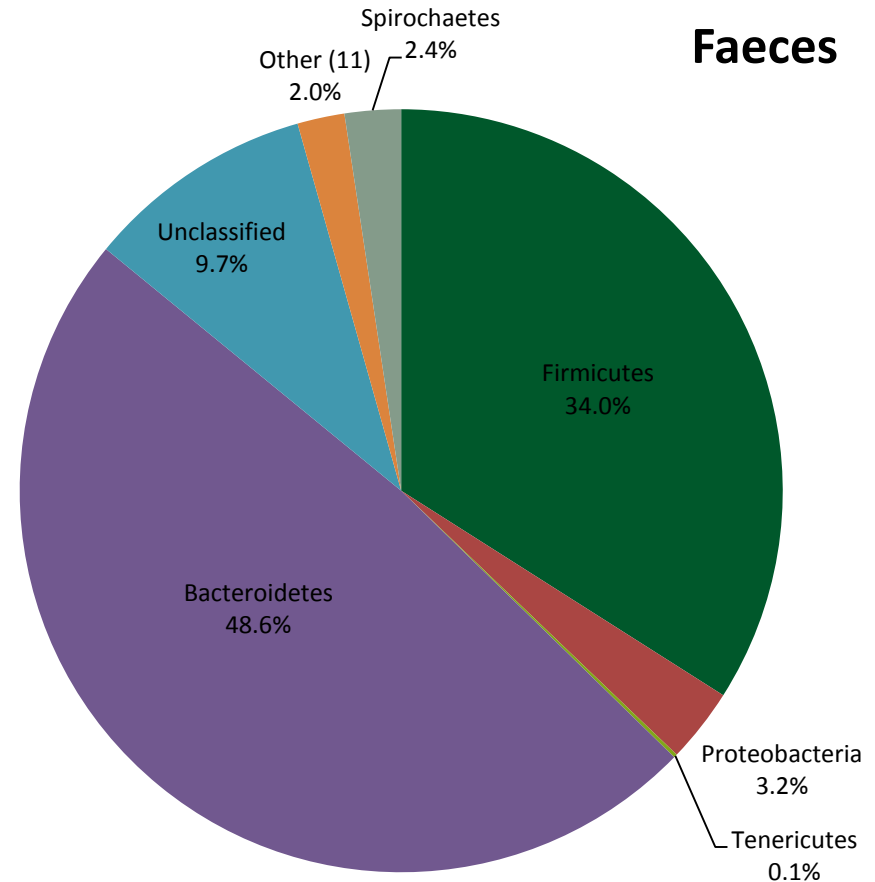| -1 | 0 | 5 | 9 | 13 |

Number of days post-weaning

Mock bacterial community

Ileum vs Rectum - Taxonomy

Ileum - Temporal shifts

- Community structures change significantly over time (AMOVA: P = 0.046)

- Decreases in Veillonellaceae, *Oscillospira guilliermondii*, *Butyricimonas* spp, *Streptococcus minor* and *Providencia* spp (Metastats: q < 0.05)

- Increase in Desulfovibrionaceae (Metastats: q = 0.03).

| Baseline n=16 | Day 5 n=32 | Day 9 n=32 | Day 13 n = 64 |

Ileum - Diet x ETEC

- No differences in community structure on day 5 and day 9 (AMOVA: P > 0.05)

- Differences in community structure on day 13 (AMOVA: P = 0.013)

- An effect of ETEC challenge on microbiota structure observed on day 13 in diet A (low protein diet) (AMOVA: P = 0.016)

| Diet A ETEC | Diet A SHAM | Diet B ETEC | Diet B SHAM |

What's past is prologue

"...it would appear to be a pointless and doubtful exercise to examine and disentangle the apparently random appearing bacteria in normal faeces and the intestinal tract, a situation that seems controlled by a thousand coincidences... Yet I have nevertheless devoted myself now for a year virtually exclusively to this special study, it was with the conviction that the accurate knowledge of these conditions is essential, for the understanding of not only the physiology of digestion..., but also the pathology and therapy of microbial intestinal diseases."

Theodor Escherich (1888)

Thank you for listening!