



# HoloRuminant

Understanding microbiomes of the ruminant holobiont

## Effect of colostrum source and calf breed on diarrhoea incidents in pre-weaned dairy calves

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EAAP Congress 2023 – EAAP, Lyon, France

*30 August 2023*





# Calf health and Diarrhoea

- Diarrheal disease
  - 40% of mortality in Ireland d0-5mo
  - Short- and long-term economic impacts
  - Immediate and long-term impacts on animal welfare

*Predominant causes of death in calves from Birth to 1 yr. of age.  
DAFM All Island report 2020<sup>1</sup>*

| Neonatal<br>(birth to 1mo)         | Calfhood<br>(1mo-5mo)              | Weanling<br>(6mo – 1yr)            |
|------------------------------------|------------------------------------|------------------------------------|
| <i>GIT Infections: 26%</i>         | <i>Respiratory Infections: 33%</i> | <i>Respiratory Infections: 40%</i> |
| Systemic Infections:<br>21%        | <i>GIT Infections: 14%</i>         | <i>GIT Infections: 18%</i>         |
| <i>Respiratory Infections: 10%</i> | Systemic Infections:<br>11%        | Systemic Infections:<br>12%        |

<sup>1</sup> Irish Department of Agriculture, Food and the Marine. 2021. All-Island Animal Disease Surveillance Report, 2020.





Dysbiosis: loss of commensal microbes accompanied by proliferation of harmful pathogens

(Chase & Kaushik, 2019)

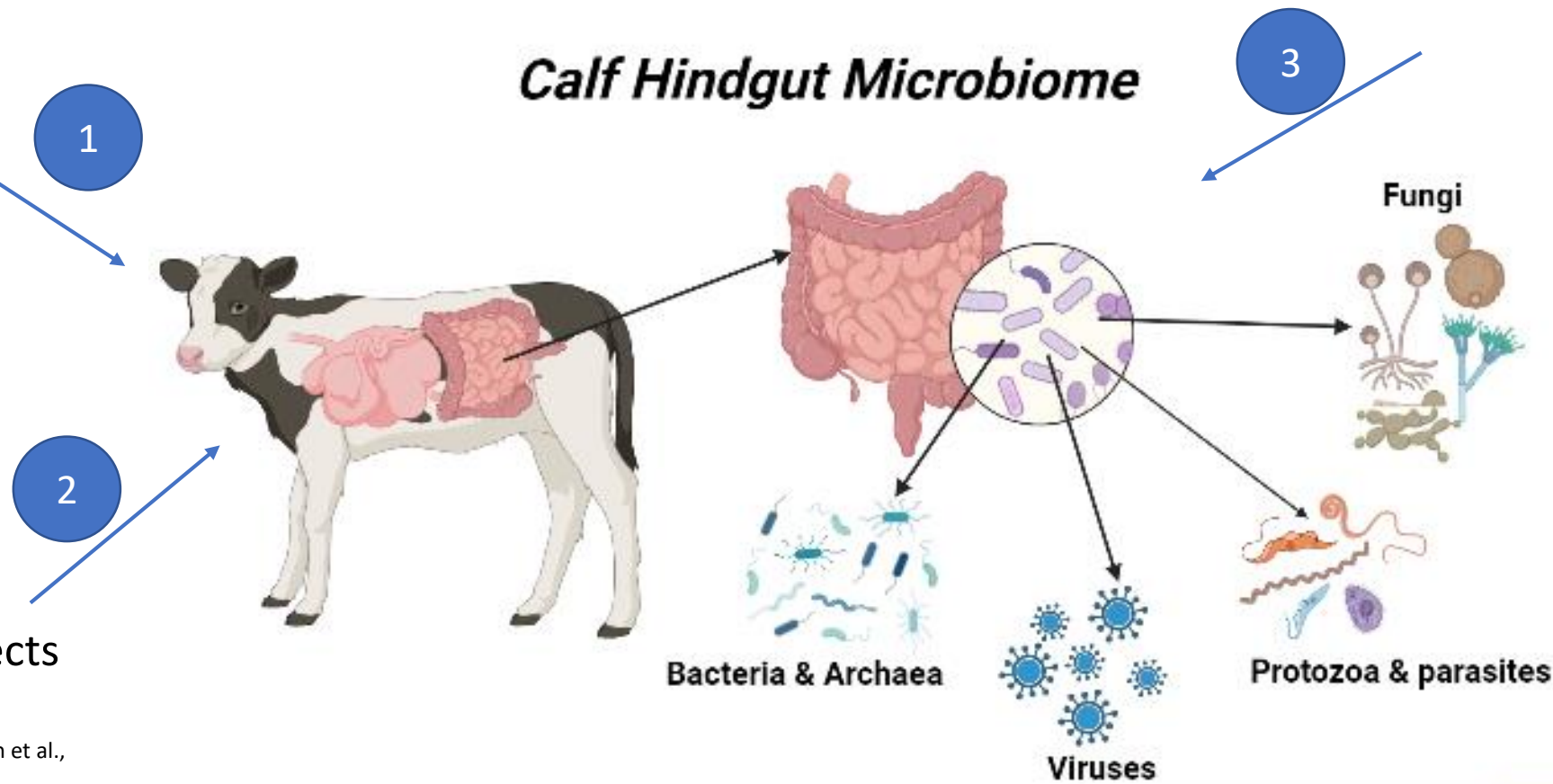
Other Factors:  
Breed  
Disease severity  
(Slanzon et al., 2022)

Composition  
varies across  
farms

(Gomez et al., 2017)

Within farm:  
presence of  
diarrhoea affects  
composition

(Gomez et al., 2017; Slanzon et al., 2022)



Created in BioRender.com bio



# Objective

*To examine the effect of **colostrum source**, **calf breed**, and **health status** on the faecal microbiome of Jersey and Holstein heifer calves **throughout the pre-weaning period**.*





# Animal Model

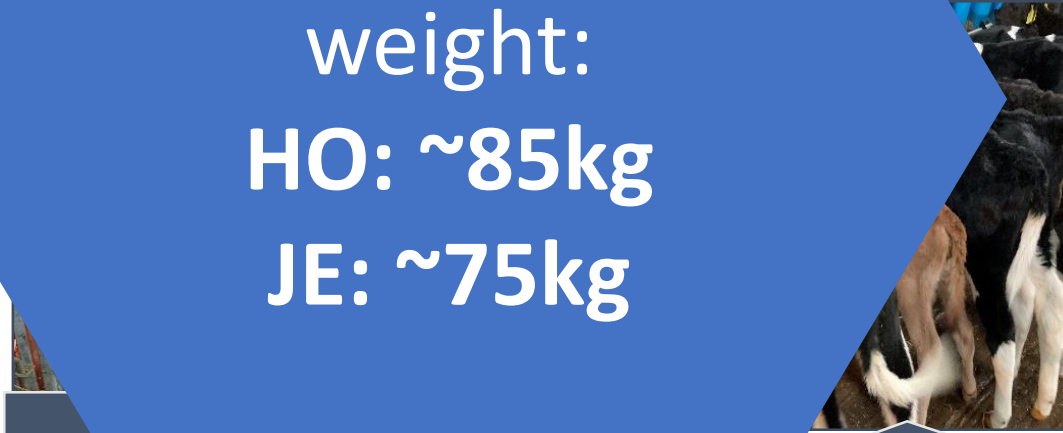
## Calves

51 spring born **Holstein (HO)** and **Jersey (JE)** cross heifers were fed 8.5% of birth weight (bw) in **colostrum from their dam** (n = 23) within 2 hours of birth. Health status: **Diarrhoeic calves** (n = 24)

Weaning by  
weight:  
HO: ~85kg  
JE: ~75kg



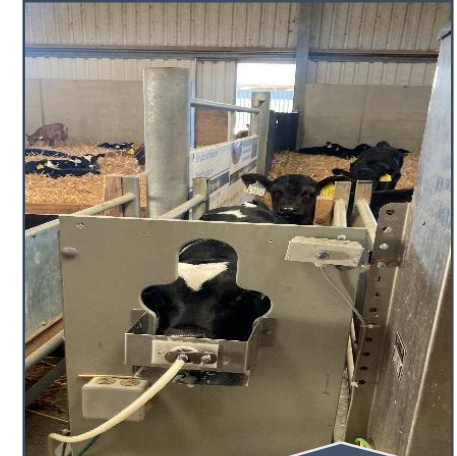
Dam groomed calf for ~ 30 min



Individual pens with straw up to 3d, group pens of 40 calves 3d to weaning



2<sup>nd</sup> feed-14d: whole milk via bucket with teat



14d + milk replacer via automated feeder





# Data Collection

- Clinical Assessment
- Sample collection: Faeces

Pre-diarrhoea  
d7 (A)

Diarrhoeal disease  
d21-22 (B)

- Clinical assessment (faecal score of 2-3 to qualify as diarrheic)
- Sample Collection: Faeces, weight

- Clinical Assessment
- Sample Collection: Faeces, weight

Post-diarrhoea  
d82 (C)

January 2022

June 2022

## Faecal Sample Storage

- At collection: Liquid Nitrogen, Dry Ice
- Storage: -80° C



## Statistical analysis

SAS 9.4- PROC Mixed & Univariate (Wilcoxon)

- Effects and interactions



# Clinical Assessment:

A

B

C

**Pre-disease**  
(d7)- all  
calves  
healthy

**Day of Disease (d21-22)**

Faecal Scores:

***Diarrheic:*** median 3 (2-3)

***Healthy:*** median 0 (0-1)

**Rectal Temperature:**

Elevated in diarrheic calves  
(+0.37°C (SE 0.01);  $P < 0.05$ )

**Post-  
disease**  
(d82): all  
calves  
recovered

0.90

1.00





# Faecal microbial DNA extraction and sequencing



150 faecal samples



Microbial DNA extraction

(Yu and Morrison, 2004)

Attachment of dual and Illumina sequencing adapters



DADA2 & SILVA



ZYMO RESEARCH

ZYMO DNA Reference Standard (DS)

PCR amplification of V4 region 16S rRNA gene

Illumina MiSeq (500 cycle V2 kit)







# Sequencing results

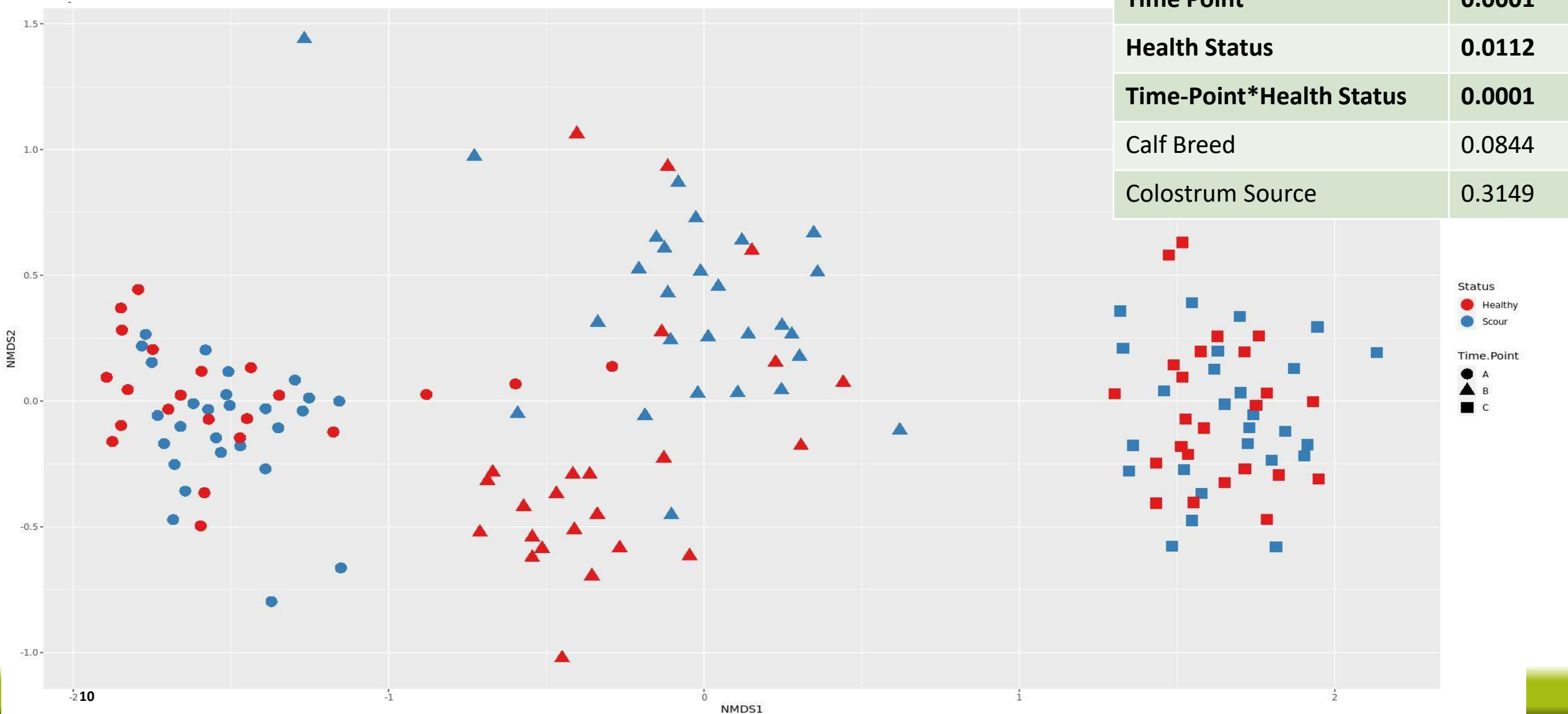
- 3 time points (**A**, **B**, and **C**):
  - 396 genera detected as significant
  - 4,638 species
- 4 Phyla dominated:
  - *Firmicutes*: 71%
  - *Bacteroidota*: 15%
  - *Actinobacteria*: 5%
  - *Proteobacteria*: 4%

95% of R.A.

Number of ASVs significantly associated with Health Status across the pre-weaning period

|         | <b>A</b> | <b>B</b>  | <b>C</b> |
|---------|----------|-----------|----------|
| Genera  | <b>0</b> | <b>24</b> | <b>0</b> |
| Species | <b>0</b> | <b>43</b> | <b>0</b> |

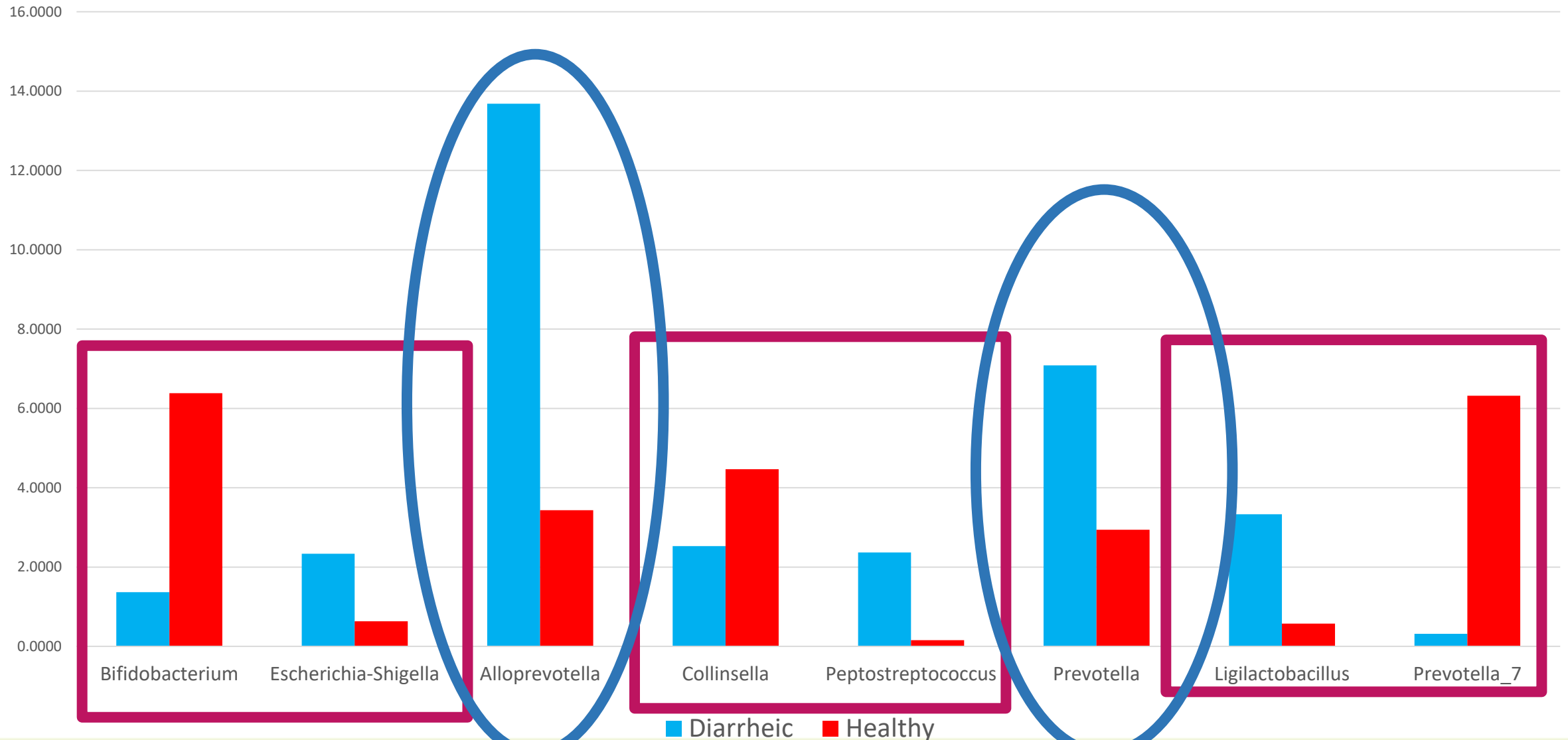
# Temporal changes in beta diversity of the faecal microbiome from birth to weaning



# At Disease Manifestation



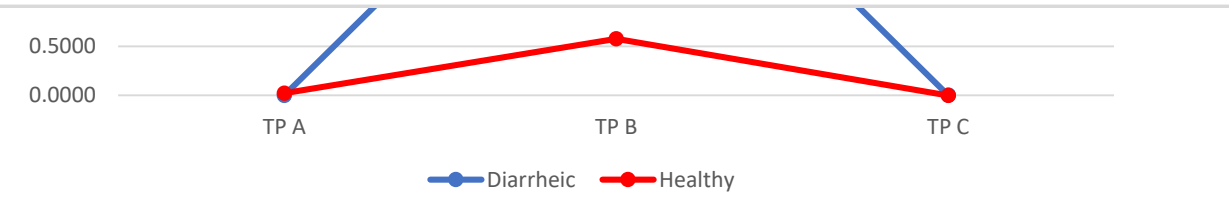
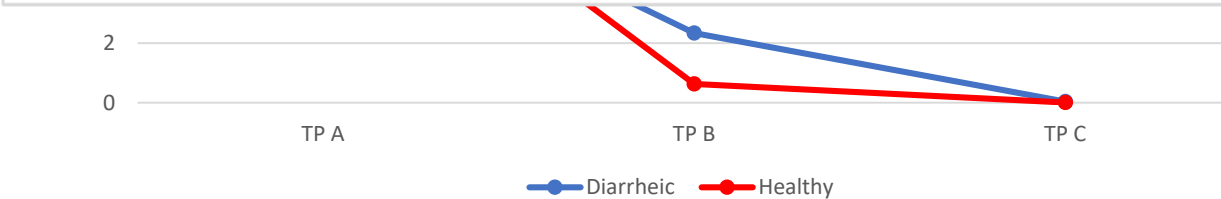
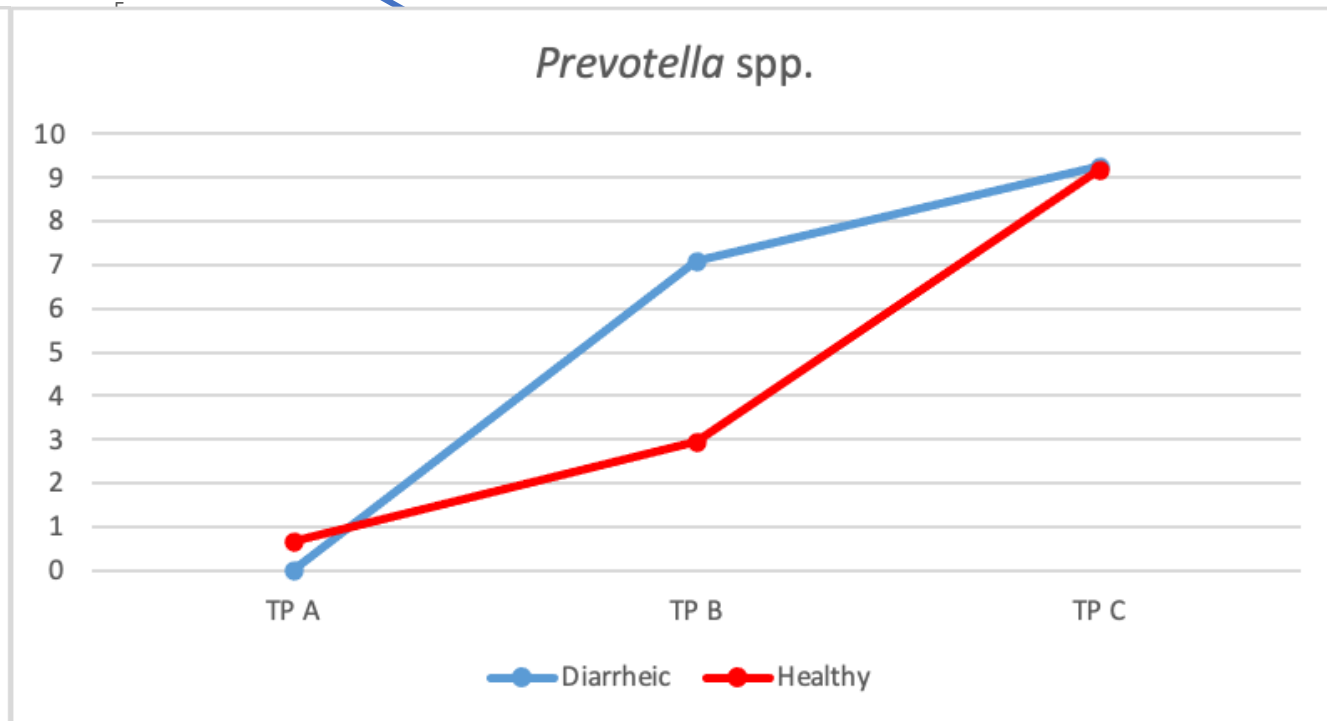
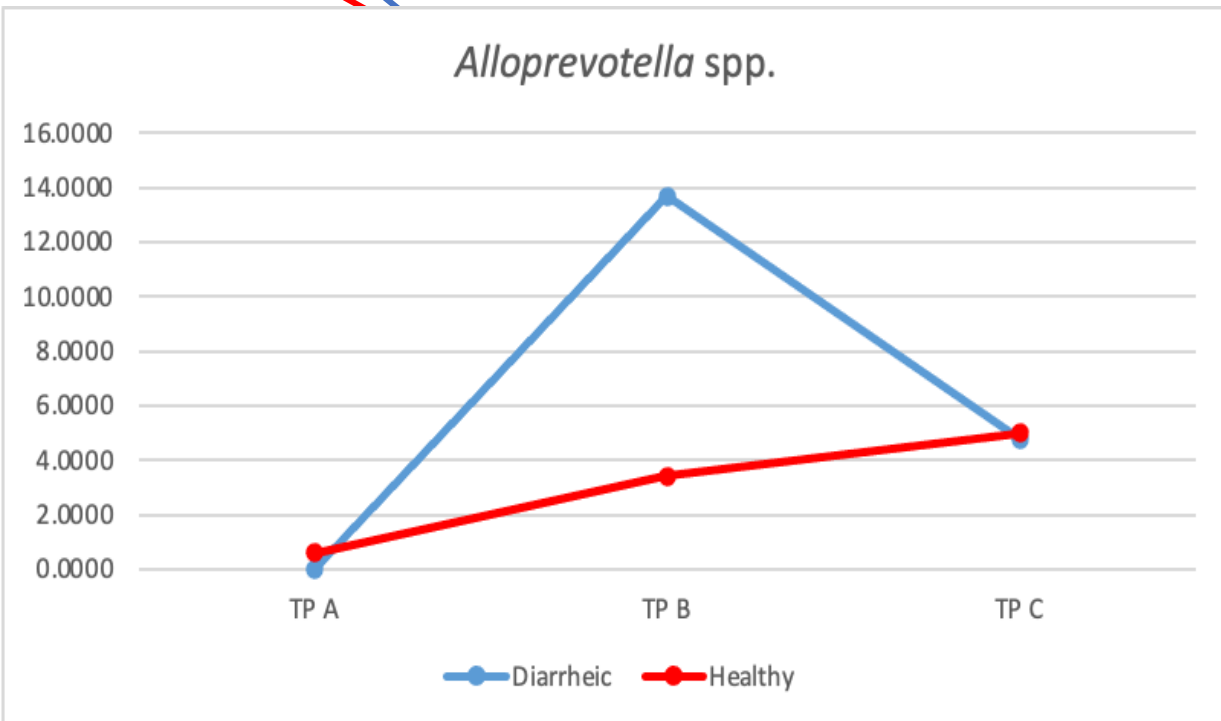
Relative Abundance at Disease Manifestation (B)



# Progression of relative abundance overtime in diarrheic and healthy calves



Unexpected: Relative abundance of known commensals is higher in diarrheic calves





## Conclusions

- $\alpha$ -diversity increased with age
- Interaction between **time\*health** was significant
- Changes in relative abundance of microbes during **diarrhoeal incidence** confirms microbial dysbiosis
- *Alloprevotella* dominated diarrheic calves
  - Has not been previously associated with diarrhoea in calves







# Acknowledgments

## Teagasc

Professor Sinead Waters  
Dr. Bernadette Earley  
Mr. Ricki Fitzgerald  
Dairygold Research Farm Staff

## UCD

Dr. Catherine McAloon  
Dr. Conor McAloon

## Funding

Horizon 2020 (*HoloRuminant*)  
Grant Agreement N° 101000213

## HoloRuminant PARTNERS





# Thank you for your time

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