Phytobiomes Alliance Webinar

Understanding Ruminant Microbiomes to Improve Animal Health, Productivity, and Sustainability

Dr. Sinéad M. Waters
School of Biological and Chemical Sciences
University of Galway
Ireland

12th Nov 2025

Institiúid Uí Riain
Ryan Institute

University *of* Galway.ie

Outline

Ollscoil na Gaillimhe
University of Galway

1. Environment: Understanding the rumen microbiome to enhance nutrient utilization from feed and reduce methane emissions from agriculture

J. Dairy Sci. TBC
https://doi.org/10.3168/jds.2024-25778

The role of rumen microbiome in the development of methane mitigation strategies for ruminant livestock

S. M. Waters,[1] E. Roskam,[1,2*] P. E. Smith,[2] D. A. Kenny,[2] M. Popova,[3] M. Eugène,[3] and D. P. Morgavi[3]
[1]School of Biological and Chemical Sciences and Ryan Institute, University of Galway, University Road, Galway
[2]Animal and Bioscience Department, Teagasc Grange, Dunsany, Co. Meath, Ireland
[3]Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, Saint-Genes-Champanelle, France

- Development of methane mitigation strategies
- Mode of action of feed additives
- Improving accuracy of selection for low methane emitting cattle

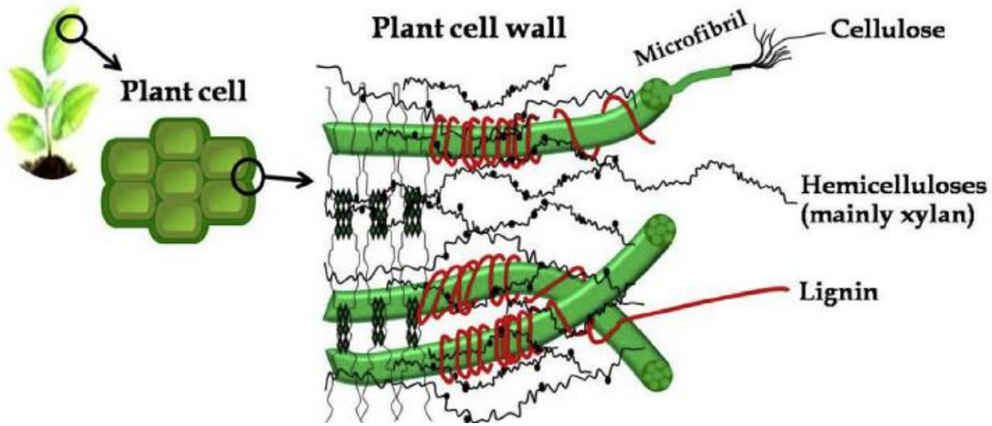
2. Health: Understanding the ruminant microbiome to improve animal health

- Bovine respiratory disease
- Diarrhoea in calves
- Colostrum

Role of ruminant livestock in global food security

Ollscoil na Gaillimhe
University of Galway

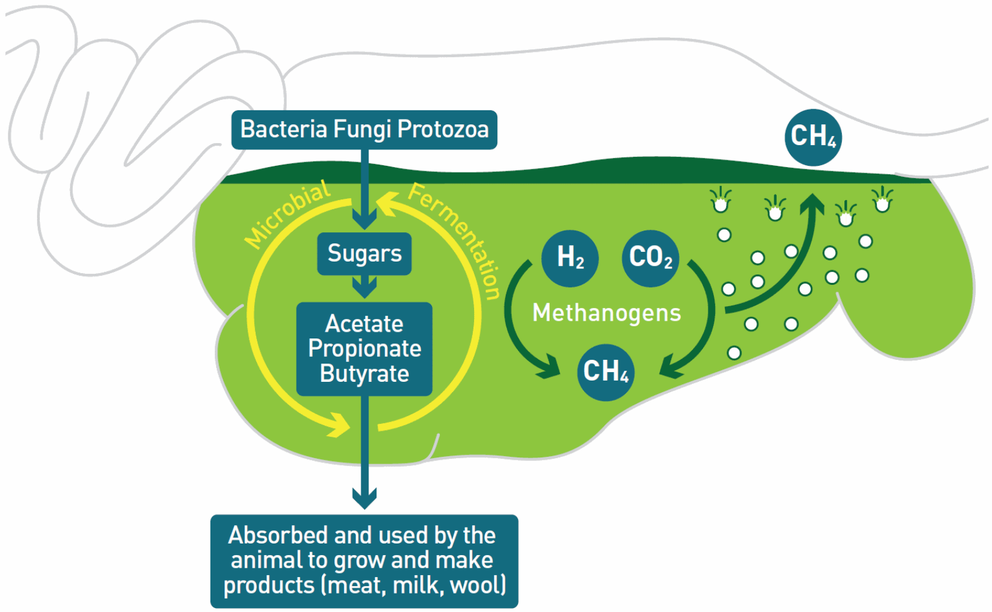
Ruminants - unique in their ability to convert plant material into high quality meat and milk protein for humans



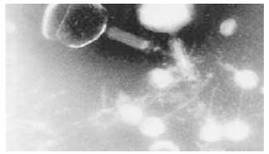
University *of* Galway.ie

A symbiotic relationship exists between host animal and rumen microbial community

- **Rumen microbes** - **fermentation** of cellulose, hemicellulose and fiber to end products utilizable by the host
  - Short chain fatty acids, acetate, propionate, butyrate
- **Host animal** - provides substrate and a suitable **anaerobic** environment

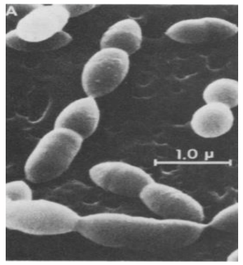


Ollscoil na Gaillimhe
University of Galway



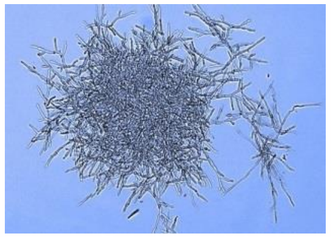
Viruses:
Phage numbers 10^9 and 10^{10} particles ml

Bacteria
10^{10} to 10^{11} cells/ml



Archaea
10^6 to 10^8 cells/ml

Protozoa
$<10^5$ cells/ml



Anaerobic Fungi
$<10^5$ cells/ml

Ruminant derived methane emissions

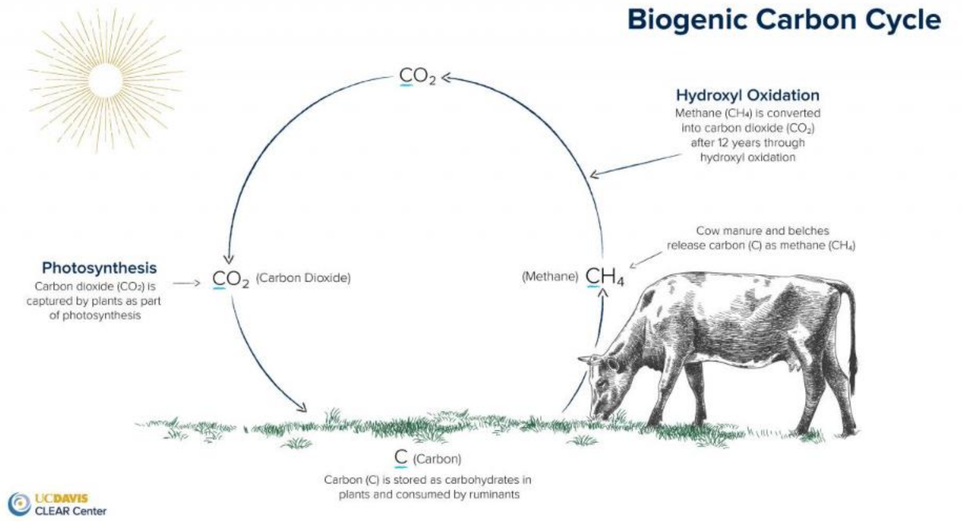
2nd most important GHG implicated in global warming

- GWP_{100} = 28

Atmospheric half-life 9-12 years

Ruminant methane:

- 32% of global GHG emissions



International GHG reduction commitments

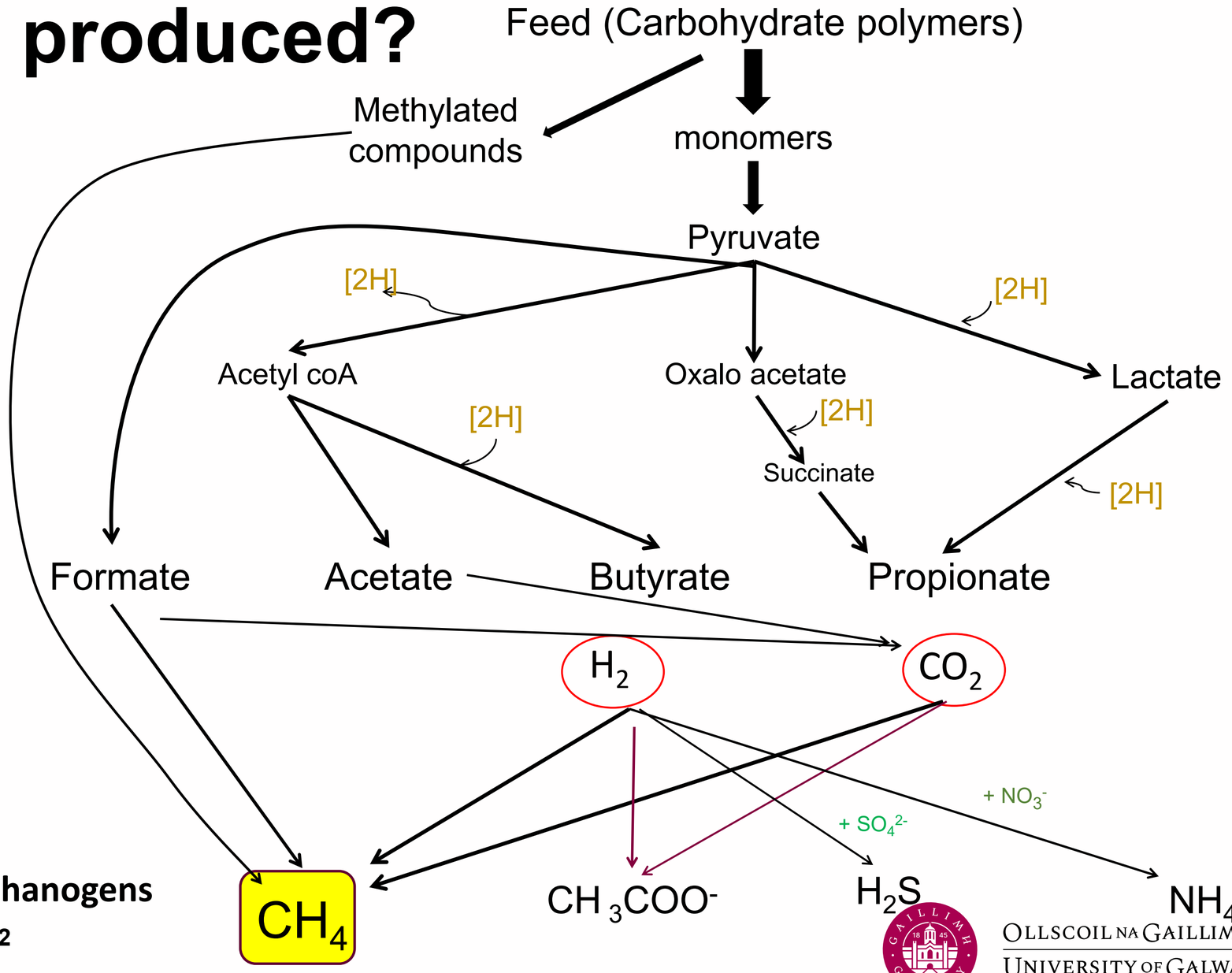
- COP 21 (UNFCCC Paris Agreement)
- EU 2030 – reduce GHG by 40% based on 1990 levels.
- Global methane pledge – 30% reduction by 2030

Global Methane Pledge

Ollscoil na Gaillimhe
University of Galway

How is methane produced?

Fermentative anaerobes (primary & secondary fermenters)



Methanogens

- **Hydrogenotrophic reduction of CO_2**
  - $4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$
  - Methyl-coenzyme reductase (mcr) enzyme

Ollscoil na Gaillimhe
University of Galway

Modified from Jeyanathan, 2014

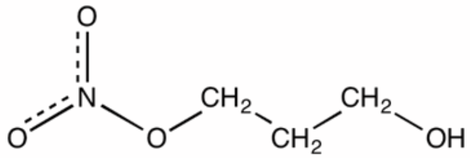
Ollscoil na Gaillimhe
University of Galway

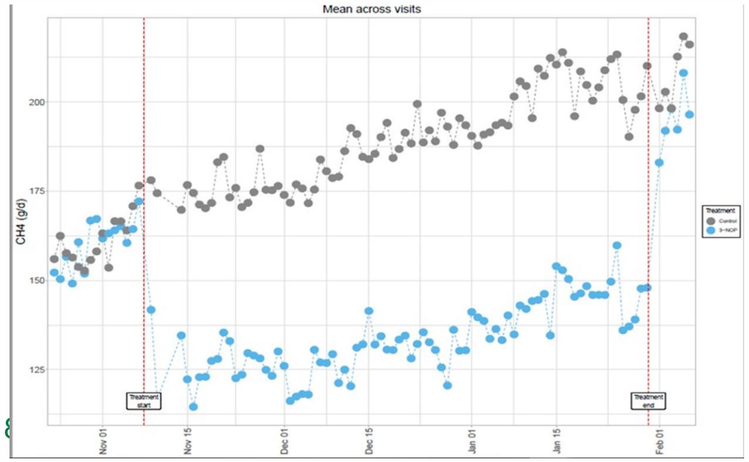
Understanding the rumen microbiome supports the:
1. Design of methane mitigating feed additives and
2. Elucidation of their mode of action

Bovaer (3-NOP)

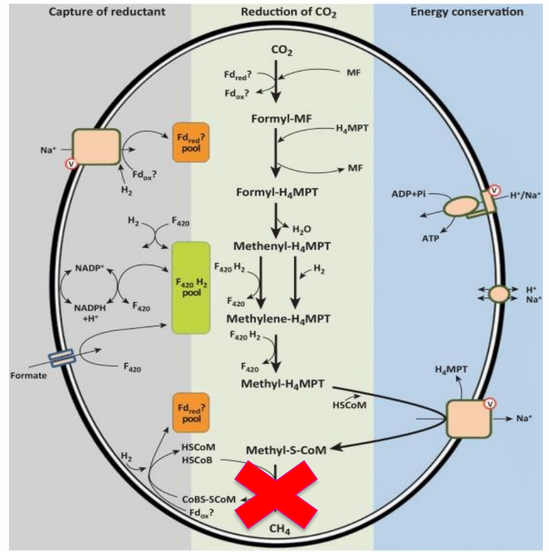
- Synthetic non-toxic compound, 3-nitrooxypropanol
- Mean reduction in methane emissions of ~30%
- Mode of action – limits the last step of the methanogenesis cycle (MCR)
- Immediate reduction to CH_4 once fed but emissions will increase once feeding stopped

3-nitrooxypropanol





- No effect on DMI, ADG, feed efficiency
- Methane data 25-30%
- Kirwan et al., 2024 JAS



Dietary supplementation with Feed additives (CaO_2) Rumen Microbial Community Analysis

Contents lists available at ScienceDirect

Animal

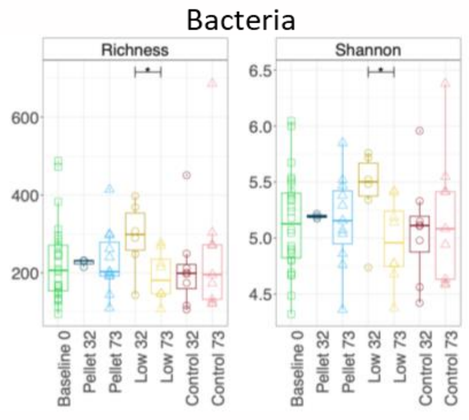
The international journal of animal biosciences

Dietary supplementation with calcium peroxide improves methane mitigation potential of finishing beef cattle

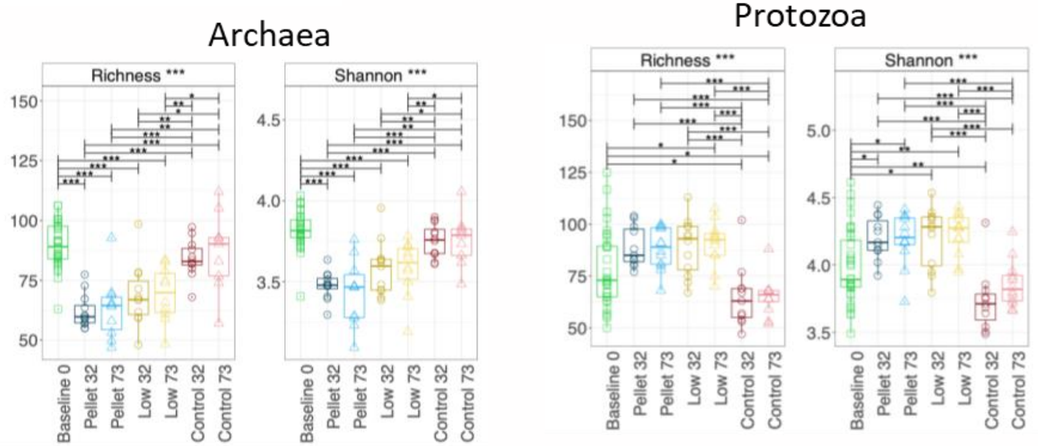
E. Roskam [a,b], D.A. Kenny [a,c], A.K. Kelly [c], V. O'Flaherty [b,d], S.M. Waters [b,*]

[a] Animal and Bioscience Research Department, Teagasc Grange, Co. Meath C15PW93, Ireland
[b] School of Biological and Chemical Sciences and Ryan Institute, University of Galway, Co. Galway H91TK33, Ireland
[c] School of Agriculture and Food Science, University College Dublin, Co. Dublin D04V1W8, Ireland
[d] GlasPort Bio Ltd, Unit 204, Business Innovation Centre, University of Galway, Co. Galway H91TK33, Ireland

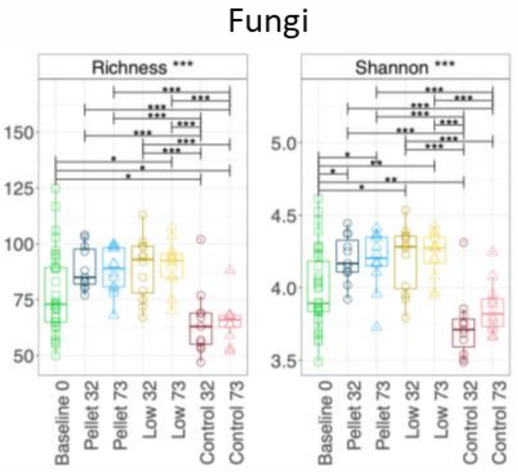
- **28% reduction in methane emissions**
- **No effect on feed intake or average daily gain**



Little significant changes in the bacterial community alpha diversity



Significant, concurrent reductions in archaeal and protozoal alpha diversity, which appears to be dose dependent.



Increased alpha diversity of fungal ASVs in treated animals.

- More abundant lactic acid bacteria such as *Bifidobacterium*, *Streptococcus* and *Sharpea*
- Archaeal community was dominated by *Methanobrevibacter* sp.
- Strong shift in the abundance of protozoa genera; *Polyplastron*, *Ostracodinium*, *Epidinium*, *Entodinium* sp more abundant in treated samples

GlasPort Bio

Ollscoil na Gaillimhe
University of Galway

Roskam, E, Mills S et al., 2025

Ollscoil na Gaillimhe
University of Galway

Application of the rumen microbiome to improve the accuracy of selection for low methane emitting cattle

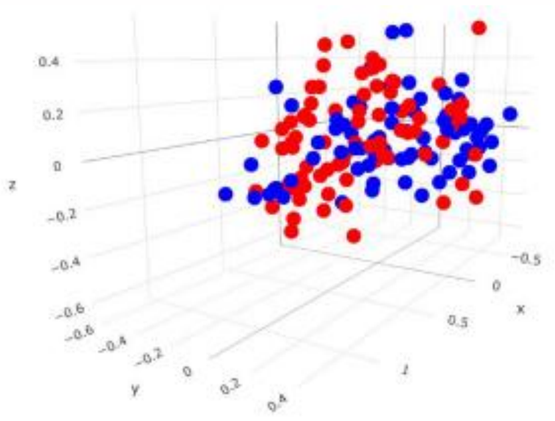
Animal breeding to mitigate methane

- Inclusion of CH_4 output in a breeding index
- Individual animal variation in methane emissions exists
- Methane output is heritable
- Good breeding decisions are cumulative and permanent
  - In sheep methane emissions reduced by 10% after three generations (Rowe *et al*. 2019).
- Ireland: Genomic selection breeding programme

Ollscoil na Gaillimhe
University of Galway

Relationship between the rumen microbiota and methane emissions in a population of finishing beef cattle

- 300 beef cattle
- 30% difference in methane emissions between high vs low
- Rumen metataxonomics n=300



Bacteria genus	RME Ranking[1] High n = 80	Medium n = 96	Low n = 74	SEM[2]	P-value
Anaeroplasma	0.71	0.77	0.76	0.11	0.59
Bifidobacterium	1.63	1.81	1.72	0.3	0.77
Butyrivibrio	1.35[a]	1.12[a]	0.80[b]	0.19	<0.001
Eubacterium	0.55	0.56	0.68	0.07	0.06
Fibrobacter	4.35	4.21	4.08	0.5	0.77
Intestinibaculum	0.37[a]	0.50[a]	0.76[b]	0.1	<0.0001
Mogibacterium	1.63[a]	1.16[b]	0.97[b]	0.16	<0.0001
Olsenella	0.53[a]	0.74[a]	1.02[b]	0.13	<0.0001
Prevotella	53.15	54.55	55.71	1.74	0.23
Pseudobutyrivibrio	0.69[a]	0.52[b]	0.43[b]	0.09	<0.001
Ruminobacter	1.77	1.61	1.08	0.43	0.08
Ruminococcus	9.92	9.3	8.65	0.67	0.16
Selenomonas	0.74[a]	0.85[a]	1.21[b]	0.15	<0.001
Sharpea	1.27[a]	1.75[ab]	1.86[b]	0.26	0.03
Succiniclasticum	11.48	11.26	10.51	0.69	0.23
Succinivibrio	2.78	2.41	1.84	0.46	0.41
Treponema	1.63	1.61	1.56	0.17	0.82

frontiers | Frontiers in Microbiology

ORIGINAL RESEARCH
published: 29 April 2022
doi: 10.3389/fmicb.2022.855565

Differences in the Composition of the Rumen Microbiota of Finishing Beef Cattle Divergently Ranked for Residual Methane Emissions

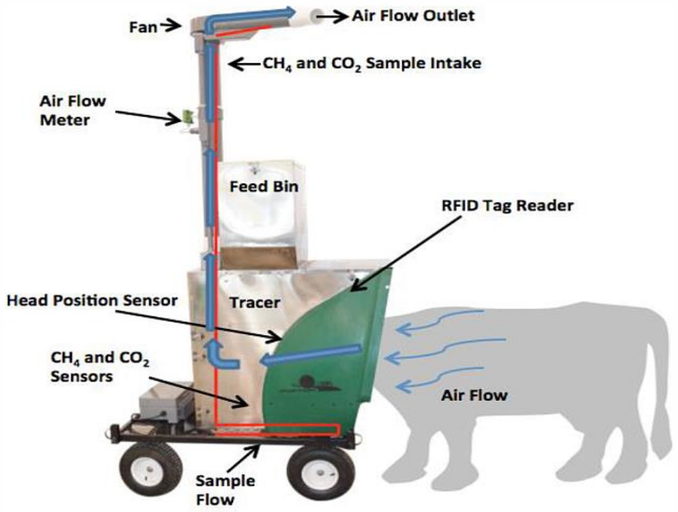
*Paul E. Smith[1,2], Alan K. Kelly[2], David A. Kenny[1] and Sinéad M. Waters[1]**

Rumen methanogens	RME ranking[1] High n = 80	Medium n = 96	Low n = 74	SEM[2]	P-value
Genus					
Methanobrevibacter	94.01	93.64	93.6	0.32	0.27
Methanosphaera	4.93[a]	5.22[a]	5.79[b]	0.39	<0.01
Methanobrevibacter clades					
RO	45.25[a]	48.07[ab]	53.92[b]	4.57	<0.01
SGMT	52.11	49.6	45.59	4.45	0.08

Smith et al. (2022)

Rumen Microbial Biomarkers Improved Prediction Equations for Enteric Methane Emissions

	Explanatory variables			R^2 adjusted
DME (g/day)	DMI; kg/d	BW; kg		0.45
DME (g/day)	CO2, kg/d	BW; kg	-	0.48
DME (g/day)	CO2; kg/d	BW; kg	Microbes; RA%	0.69
			Mogibacterium	0.10
			Intestinibaculum	0.05
			Selenomonas	0.02
			SGMT	0.01



- **Ruminal abundance of three bacterial genera (*Mogibacterium*, *Selenomonas* and *Intestinibaculum*) and the *Methanobrevibacter* SGMT clade accounted for a further 21% of the variation in methane emissions**

Ollscoil na Gaillimhe
University of Galway

Kelly et al., 2022 GGAA conference, Florida, USA.

Heritable Microbiomes

ScienceAdvances

A heritable subset of the core rumen microbiome dictates dairy cow productivity and emissions

R. John Wallace[1*†], Goor Sasson[2†], Philip C. Garnsworthy[3], Ilma Tapio[4], Emma Gregson[3], Paolo Bani[5], Pekka Huhtanen[6], Ali R. Bayat[4], Francesco Strozzi[7‡], Filippo Biscarini[7§], Timothy J. Snelling[1], Neil Saunders[3], Sarah L. Potterton[3], James Craigon[3], Andrea Minuti[5], Erminio Trevisi[5], Maria L. Callegari[8||], Fiorenzo Piccioli Cappelli[5], Edward H. Cabezas-Garcia[6¶], Johanna Vilkki[4], Cesar Pinares-Patino[4], Kateřina O. Fliegerová[9], Jakub Mrázek[9], Hana Sechovcová[9], Jan Kopečný[9], Aurélie Bonin[10], Frédéric Boyer[10], Pierre Taberlet[10], Fotini Kokou[2], Eran Halperin[11], John L. Williams[7#**], Kevin J. Shingfield[4**††], Itzhak Mizrahi[2***]

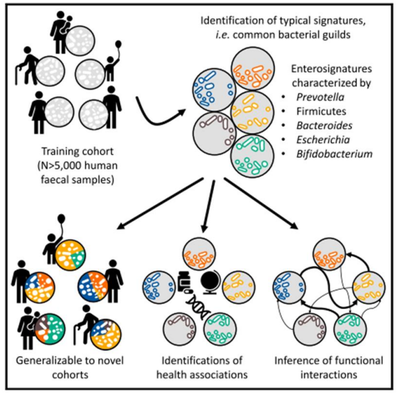
h^2= 0.2 - 0.6

Article

Cell Host & Microbe

Enterosignatures define common bacterial guilds in the human gut microbiome

Graphical abstract



Authors

Clémence Frioux, Rebecca Ansorge, Ezgi Özkurt, ..., Christopher Quince, Sebastian M. Waszak, Falk Hildebrand

Correspondence

clemence.frioux@inria.fr (C.F.), falk.hildebrand@quadram.ac.uk (F.H.)

In brief

Frioux et al. introduce enterosignatures as microbial guilds whose assemblies are accurate descriptors of the human gut microbiome composition. Enterosignature composition relates to changes and perturbations in the microbiome over a lifetime. The model generalizes to diverse human populations and can be used to detect anomalies in the gut microbiome.

Ecological guilds as units for microbiome data reduction

"**Guild**," "*a group of microbial species that exploit the same class of environmental resources in a similar way" – co-abundance patterns*

Guild-based analysis - ecologically relevant aggregation method for identifying bacterial functional groups

Reducing dimensionality and sparsity in data analysis

HoloRuminant
Understanding microbiomes of the ruminant holobiont

www.holoruminant.eu

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000213

Study populations and analytical workflow (n=2,492 animals)

Multi-Breed

Holstein
Italy (n=409)
UK (n=409)
France (n= 120)

Angus
Canada (n=203)

Charolais
Canada (n=114)

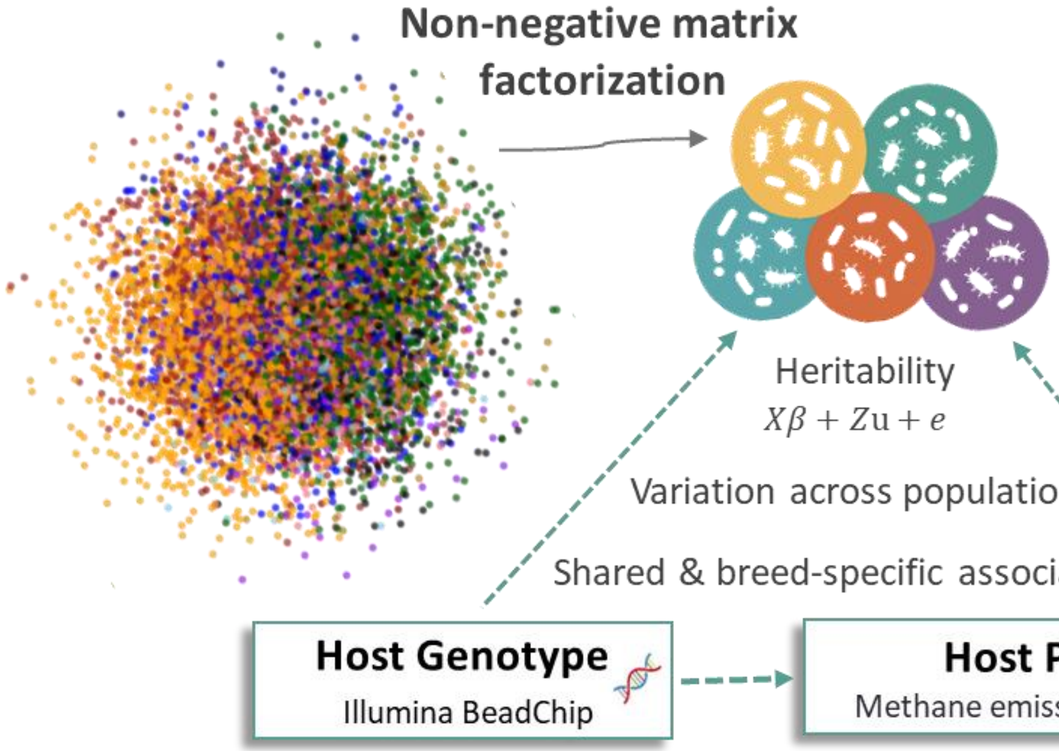
Kinsella composite
Canada (n=392)

Crossbred
Ireland (n=849)

Microbial communities

Metataxonomic
Bacteria, Archaea, and Protist

Ruminosignatures
Ruminal microbial guilds



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000213

Co-occurring Bacterial Guilds: Diversity, Composition and Influence on Host Performance

- **RS-UBA2** was consistently negatively associated with CH_4 emission and positively associated with feed efficiency across all breeds and countries.
  - Promising candidate for integration into breeding programmes

Key findings:

- Breed-specific and common RS capturing between 96% and 99% of the variance in the bacterial ecosystem.

- RS abundances linked with methane emissions and feed efficiency, and were under host-genetic control, with heritability estimates ranging from 0.09 to 0.51.

Heritability for each Ruminosigantures for the Irish dataset

RS	$\widehat{h}_a^2$
RS_Prev	0.19
RS_UBA2	0.48
RS_SMFI	0.59
RS_Rumi	0.49
RS_Succ	0.51
RS_Shar	0.38

Ollscoil na Gaillimhe
University of Galway

IRTA

HoloRuminant
Understanding microbiomes of the ruminant holobiont

Ramayo et al., 2025 EAAP; Vourlaki et al., 2025 In Preparation

www.holoruminant.eu

Ollscoil na Gaillimhe
University of Galway

International Rumen Gateway Project

- **Global Research Alliance Flagship**
- Understanding rumen microbiomes hindered by its complexity and poor culture collections.

- ***Expansion, analysis and exploitation of the Hungate rumen microbial culture collection Flagship Goal: To generate new knowledge on the rumen microbiome which will enable novel interventions to reduce methane emissions from livestock***

- Anaerobic culturing technologies and ‘rumen simulated’ culture media, which mimic the unique rumen environment.

- Whole genome sequencing and MALDI MS

- Diversity, ontogeny, and functionality of the rumen microbiome - combined with sequencing technology ‘culturomics’

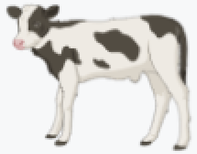
GLOBAL RESEARCH ALLIANCE
ON AGRICULTURAL GREENHOUSE GASES

Ollscoil na Gaillimhe
University of Galway

Role of ruminant microbiomes in animal health

Bovine Respiratory Disease (BRD)

Multifactorial syndrome caused by complex interactions



Younger animals most vulnerable

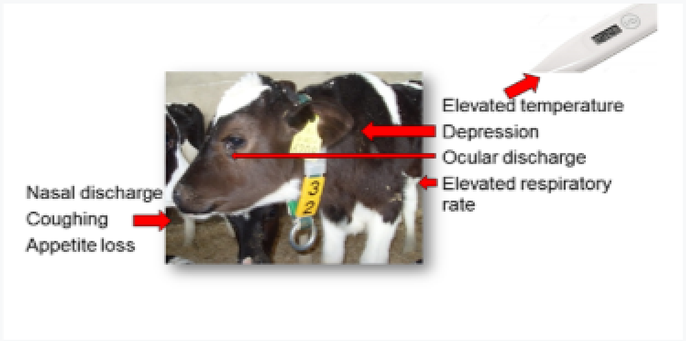
Environment

Pathogens

Host immunity

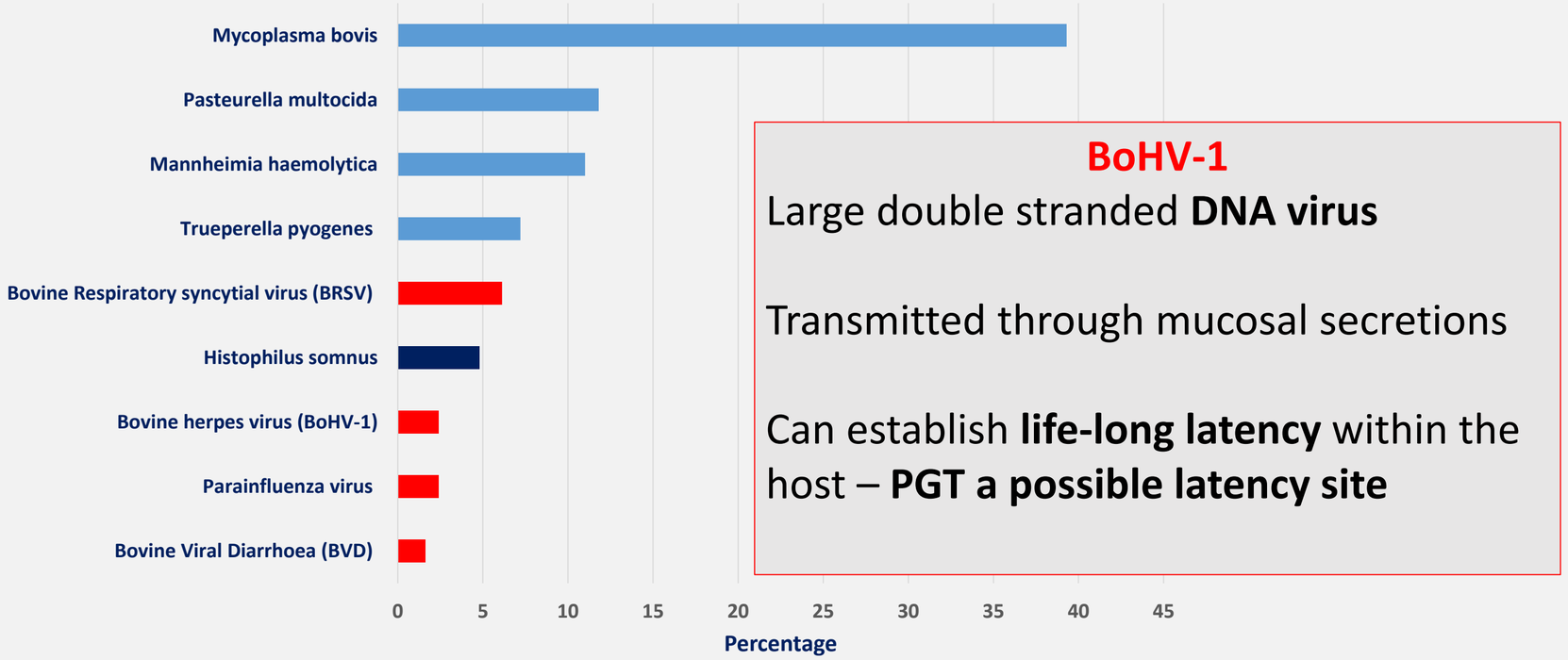
Impact the host immune defence mechanisms

Development of clinical signs and secondary infections



BRD pathogens

- Both bacterial and viral pathogens can be responsible for disease onset
- Initial viral infection leading to secondary bacterial infection
- Respiratory disease was the most common (38.8%) amongst calves 1-6 months old (DAFM, 2023)
- This trend continued in 6-12 month old calves, with 41.1% identified to have respiratory infection



BoHV-1

Large double stranded **DNA virus**

Transmitted through mucosal secretions

Can establish **life-long latency** within the host – **PGT a possible latency site**

Bacterial microbiota of the URT and LRT of dairy calves in response to BoHV-1

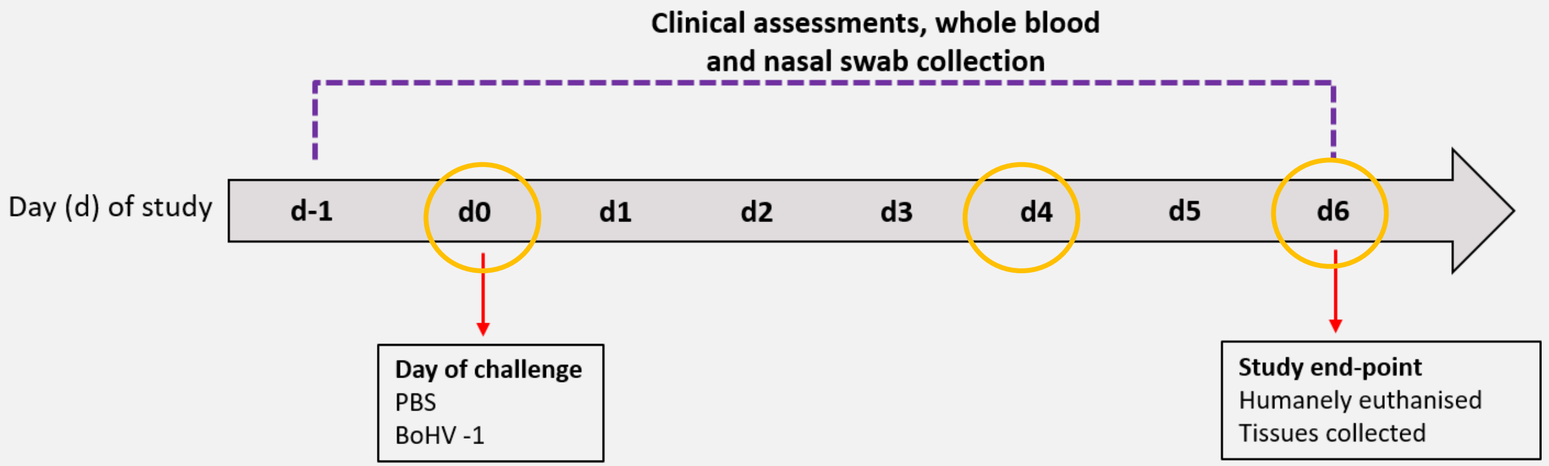
Characterisation of the bacterial microbiota of the upper and lower respiratory tracts of dairy calves after an experimental challenge with bovine herpesvirus 1 (BoHV-1)

Stephanie O'Donoghue[1,2], Bernadette Earley[1], Matthew S. Finnie[1], Dayle O Johnston[1], S. Louise Cosby[3], Ken Lemon[3], Jeremy F. Taylor[4], Derek W. Morris[2], and Sinead M. Waters[2]

Objective: Characterise the bacterial microbiota of the upper and lower respiratory tract following an experimental challenge with BoHV-1 in dairy calves

Bacterial microbiota of the URT and LRT of dairy calves in response to BoHV-1

Nasal swabs, pharyngeal tonsil (PGT) and lung tissue



- DNA extracted from **nasal, lung and PGT tissue -** DNeasy PowerSoil kit
- Libraries prepared – Oxford Nanopore Technologies(ONT)**16S Barcoding kit**
- **Pooled libraries** were sequenced on a ONT MinION MK1C device on a MinION flow cell
- **Analysis performed with EPI2ME platform** with the **Fastq 16S pipeline**
- Statistical analysis conducted in R using phyloseq and vegan packages

Key findings

No difference in abundance of any genera between the control and challenged calves for any tissue type.

BoHV-1 versus control

- *Mycoplasma spp*. numerically higher in the PGT and lung of BoHV-1 calves
- *Pasteurella* numerically higher in nasal cavities of BoHV-1 calves
- BoHV-1 significantly altered the bacterial community structure in the PGT and lung

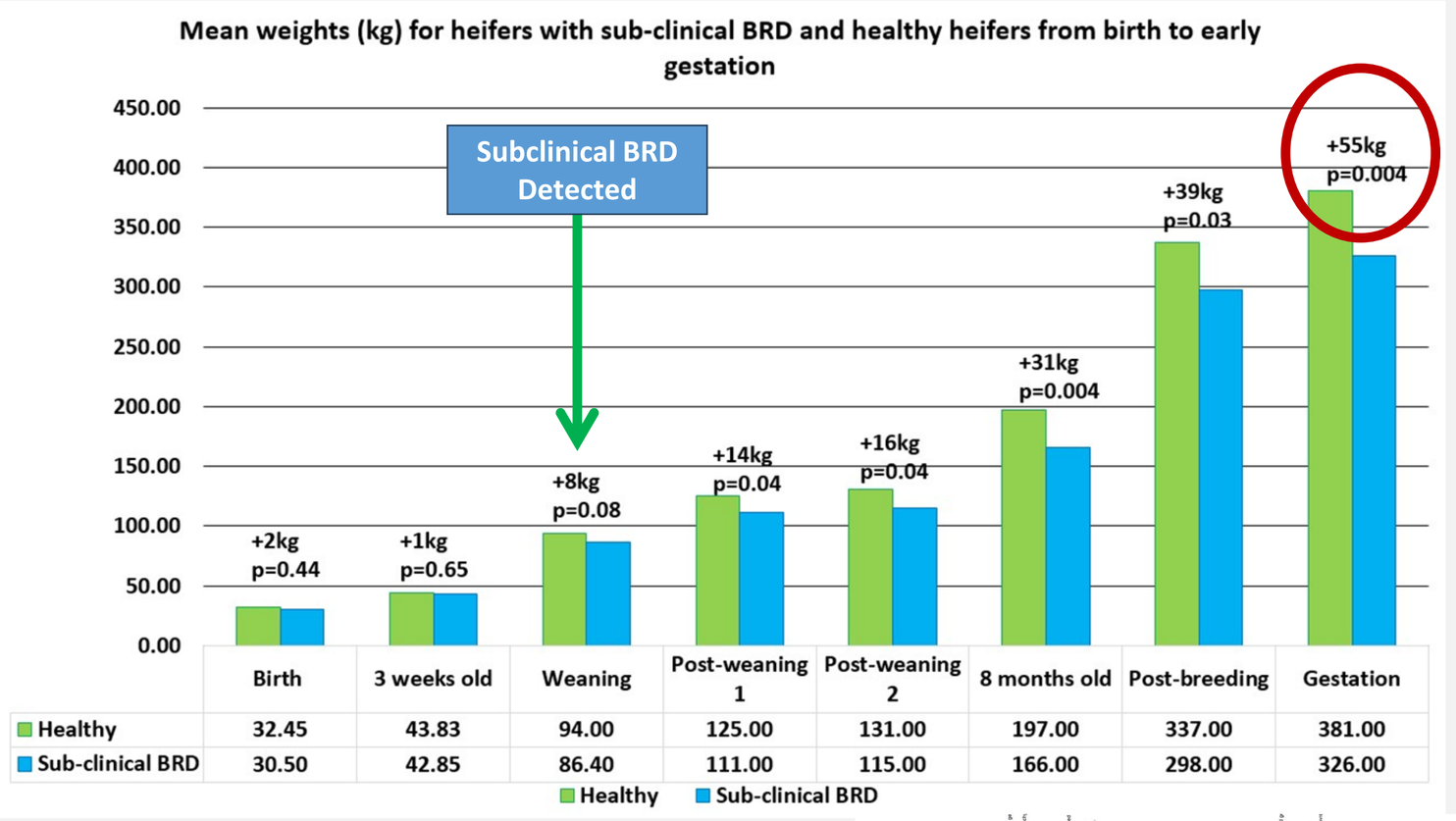
- **Under favourable conditions a symbiosis exists within respiratory microbiome**
- **Times of stress, disease etc. a dysbiosis occurs**
- **Causes commensal bacteria to become opportunistic pathogens**

O'Donoghue et al., 2025

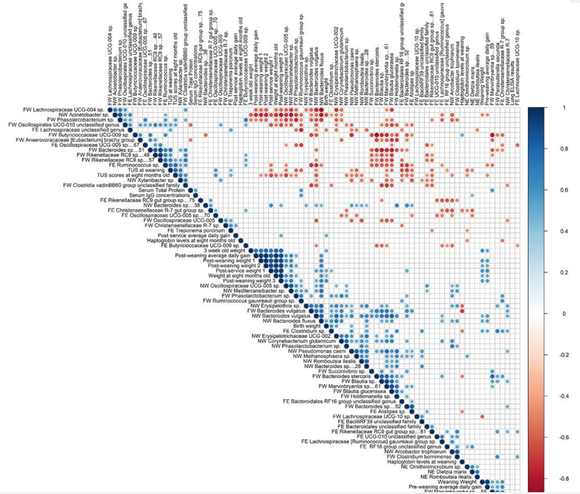
Subclinical bovine respiratory disease

- Early detection is key to management
  - Detection tools rely on observation of clinical signs
- Subclinical BRD affected both the nasal and faecal microbiota at time of detection
- Microbes significantly associated with subclinical BRD were not common BRD pathogens
- Normal nasal community members that behave opportunistically
- Lowly abundant
  - Nasal: *Romboutsia ilealis*
  - *Fecal: Butyricioccaceae UCG*-009

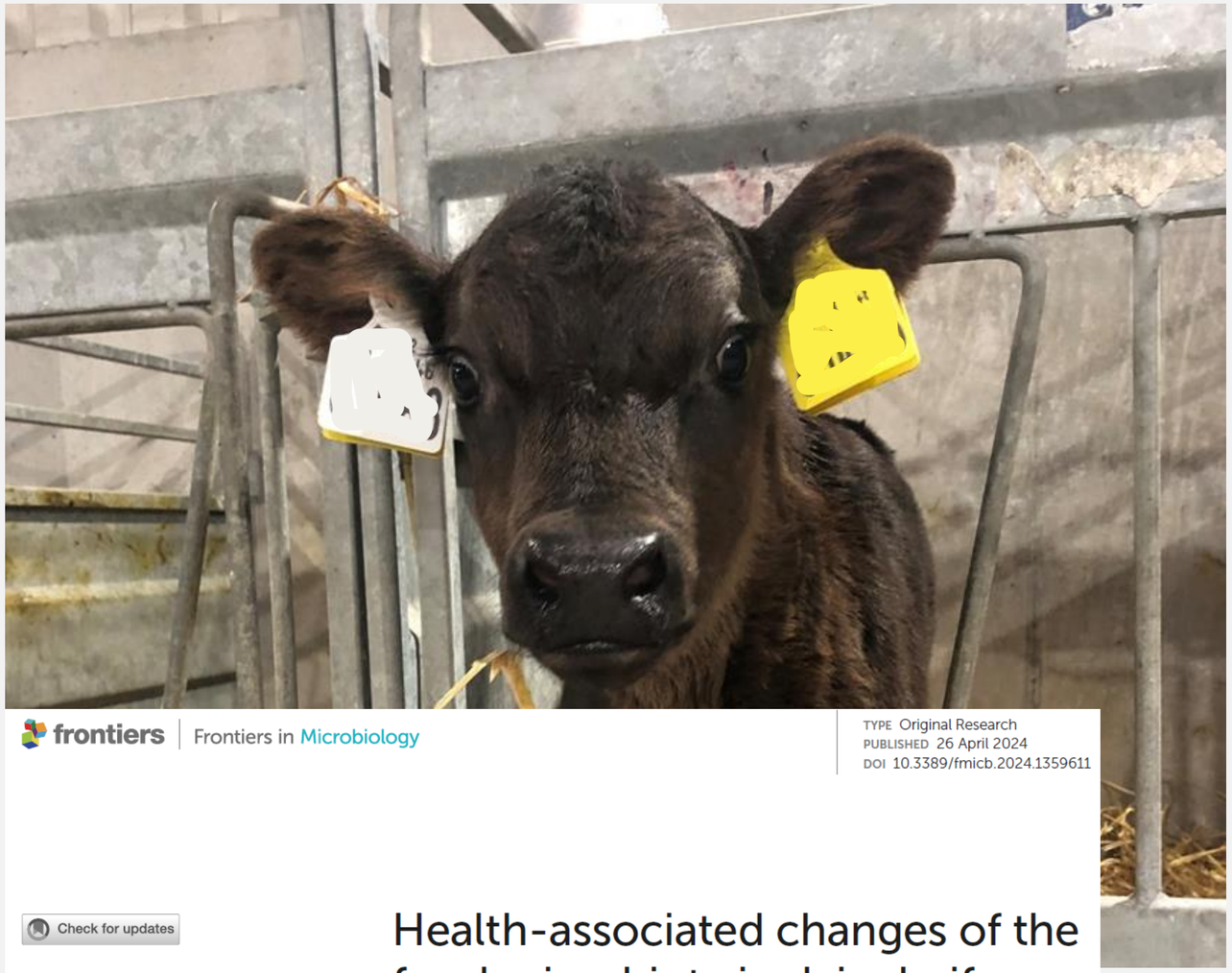
Scully et al., 2025 In Prep.



	Birth	3 weeks old	Weaning	Post-weaning 1	Post-weaning 2	8 months old	Post-breeding	Gestation
Healthy	32.45	43.83	94.00	125.00	131.00	197.00	337.00	381.00
Sub-clinical BRD	30.50	42.85	86.40	111.00	115.00	166.00	298.00	326.00



Pre-weaning diarrhoea



frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 26 April 2024
DOI 10.3389/fmicb.2024.1359611

Check for updates

OPEN ACCESS

EDITED BY
Kun Li,
Nanjing Agricultural University, China

REVIEWED BY
Jana Koscova,
University of Veterinary Medicine and

Health-associated changes of the fecal microbiota in dairy heifer calves during the pre-weaning period

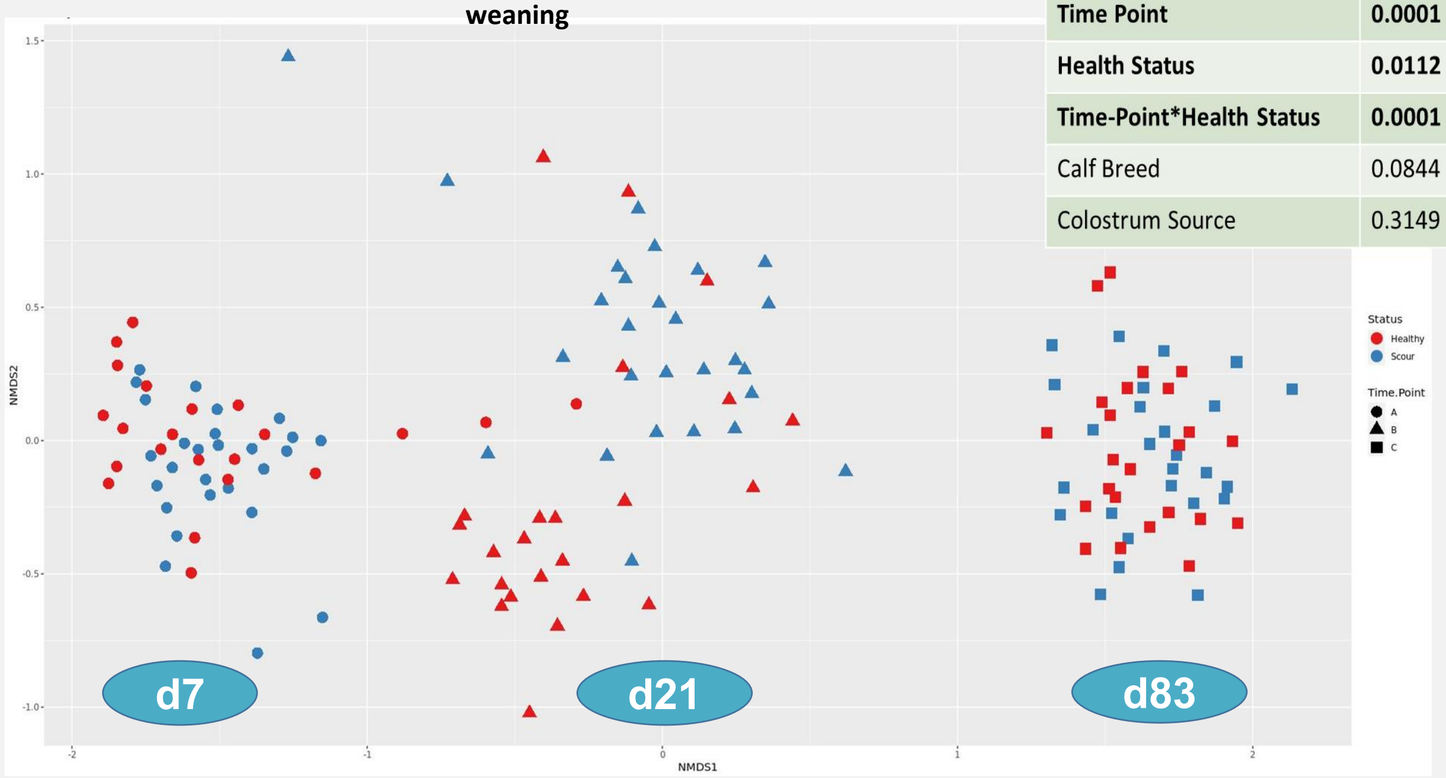
- Diarrhoea is the N°1 cause of death in calves 1 month old or less
- Complex, usually co-infection, e.g. rotavirus and cryptosporidiosis
- Changes in relative abundances from before diarrhoea to disease manifestation confirms gut dysbiosis before diarrhoeal disease
- Diarrheic calves had reduced alpha diversity and different microbial composition
  - Diarrhoea significantly altered the faecal microbiota
- The gut microbiota able to recover after diarrhoea if they are properly supported during disease

Scully et al., 2024

Pre-weaning Diarrhoea

- No effect of health status on ADG → calves recovered well
- Diversity and composition of faecal microbiota
  - α-diversity increased with time
  - Interaction between **time*health** was significant
    - No difference in composition pre- and post-disease
    - Reduced bacterial diversity at disease manifestation

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



Effect	P-value
Time Point	0.0001
Health Status	0.0112
Time-Point*Health Status	0.0001
Calf Breed	0.0844
Colostrum Source	0.3149

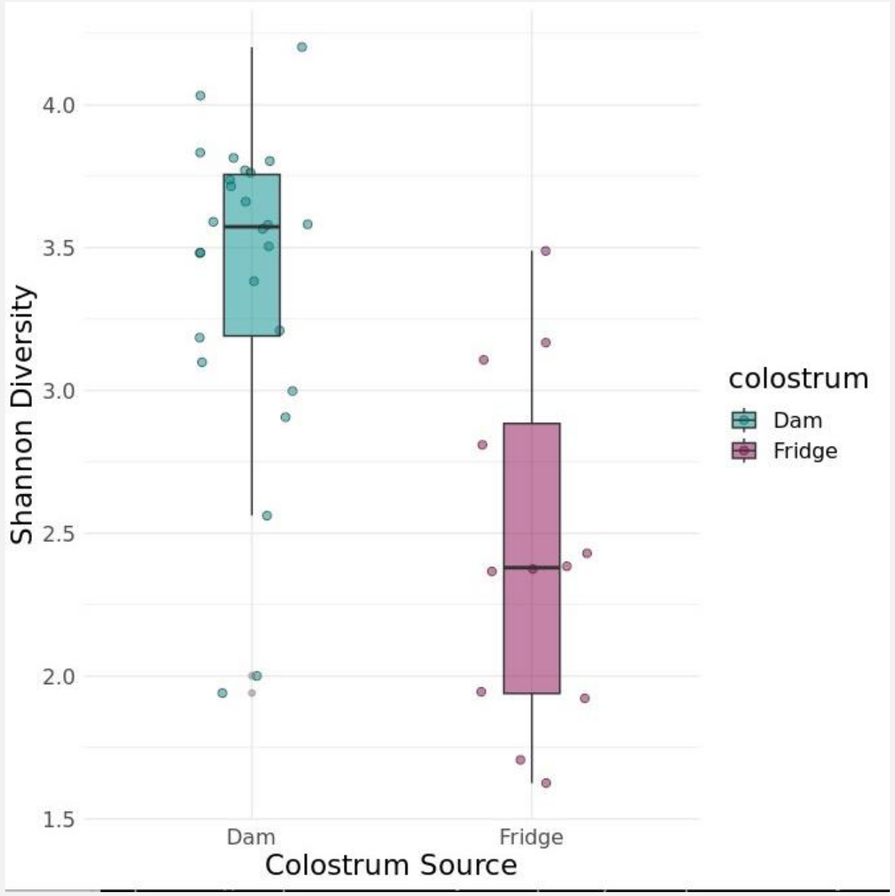
- **Changes in relative abundance** of microbes during **diarrhoeal incidence** confirms **bacterial dysbiosis**
  - ***Alloprevotella*** dominated the faecal microbiota of diarrheic calves at disease manifestation
  - ***Bifidobacterium*** dominated in healthy calves

Scully et al., 2024

The colostrum microbiota

- Colostrum is critical to calf health and development
  - Antibodies, nutrients, growth factors and hormones
- Increasing interest in the colostrum microbiome:
  - Possible entero-mammary pathway: transfers microbes from the gut to the mammary
  - Role in seeding, colonization and immune development
- **Fresh colostrum:**
  - Diverse and homogenous community
    - Metabolically versatile, many microbes known to be bovine gut commensals
  - Presence of *Lachnospiraceae* is linked to quality and passive transfer of immunity
- Colostrum management practices, like storage and refrigeration, may drastically alter community composition

Alpha Diversity of Colostrum collected fresh and colostrum stored and refrigerated



PLOS One

RESEARCH ARTICLE

Characterisation of the bacterial and archaeal microbiota in fresh colostrum collected from a single, spring-calving dairy herd

Sabine Scully[1,2], Bernadette Earley[1], Paul E. Smith[1], Matthew S. J. Finnie[1], Catherine McAloon[2], Frank Buckley[3,4], David A. Kenny[1], Sinéad M. Waters[1]*

Scully et al., 2025

Conclusions

Understanding the **rumen microbiome** contributes to reducing methane emissions from agriculture via:

- Development of methane mitigation strategies
- Mode of action of feed additives
- Breeding strategies

Breed-specific, and common heritable microbial guilds associated with CH_4 emissions and feed efficiency

Progress in culturing ruminant microbiomes e.g Rumen Gateway project

Knowledge of ruminant microbiomes (respiratory, GIT, colostrum) is critical to improve animal health (e.g., BRD) via diagnostics and development of therapeutics.

Thank you for your attention!

Acknowledgement

Teagasc:
Sabine Scully
Dr Paul Smith
Prof David Kenny
Dr Emily Roskam
Dr Stuart Kirwan (UC Davis)
Dr Bernadette Early
Dr Stephanie O'Donougue

University of Galway
Prof Vincent O'Flaherty
Dr Simon Mills
Gizem Karakaya

ICBF
Dr David Kelly
Dr Ross Evans
Dr Stephen Conroy

IRTA:
Dr Yuliaxis Ramayo and HoloRuminant team

Funders:

- **Irish Department of Agriculture, Food and the Marine**
  - Projects: 'Meth-Abate; MAGS', 'Rumen-Gateway' 'BRD-SEQ'
- **ERA-NET & ERA-GAS (*RumenPredict); ERA-GAS (INTEGRITY)***
  - 16/RD/ERAGAS/1RUMENPREDICT-ROI2017
- **EU Horizon:** ***MASTER*** Grant agreement No 818368
  HoloRuminant Grant agreement No 101000213

HoloRuminant
Understanding microbiomes of the ruminant holobiont

www.holoruminant.eu

University *of* Galway.ie