HoloRuminant
Understanding microbiomes of the ruminant holobiont

Identification of the core microbiota of bovine colostrum collected fresh from the dam

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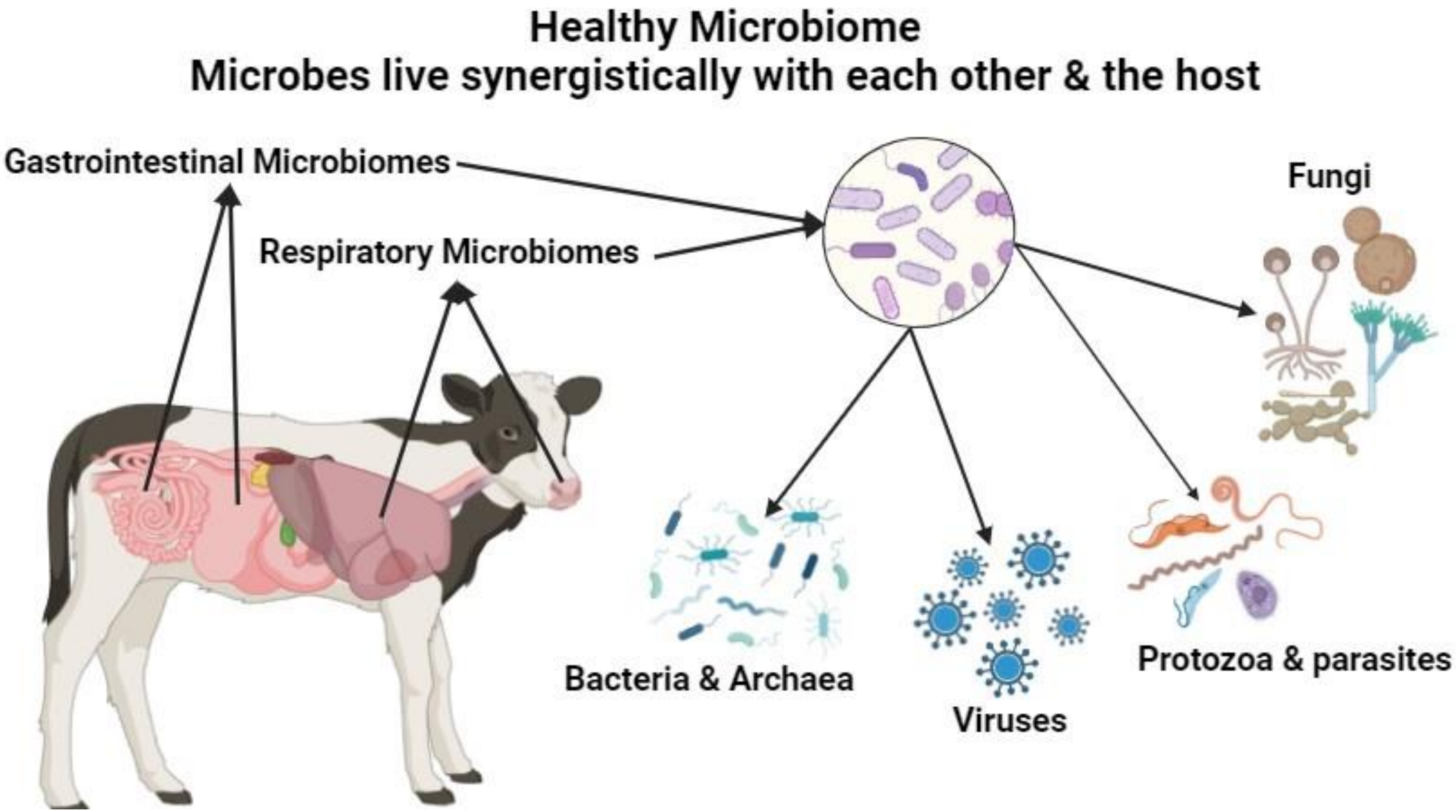
Microbiomes



Healthy Microbiome
Microbes live synergistically with each other & the host

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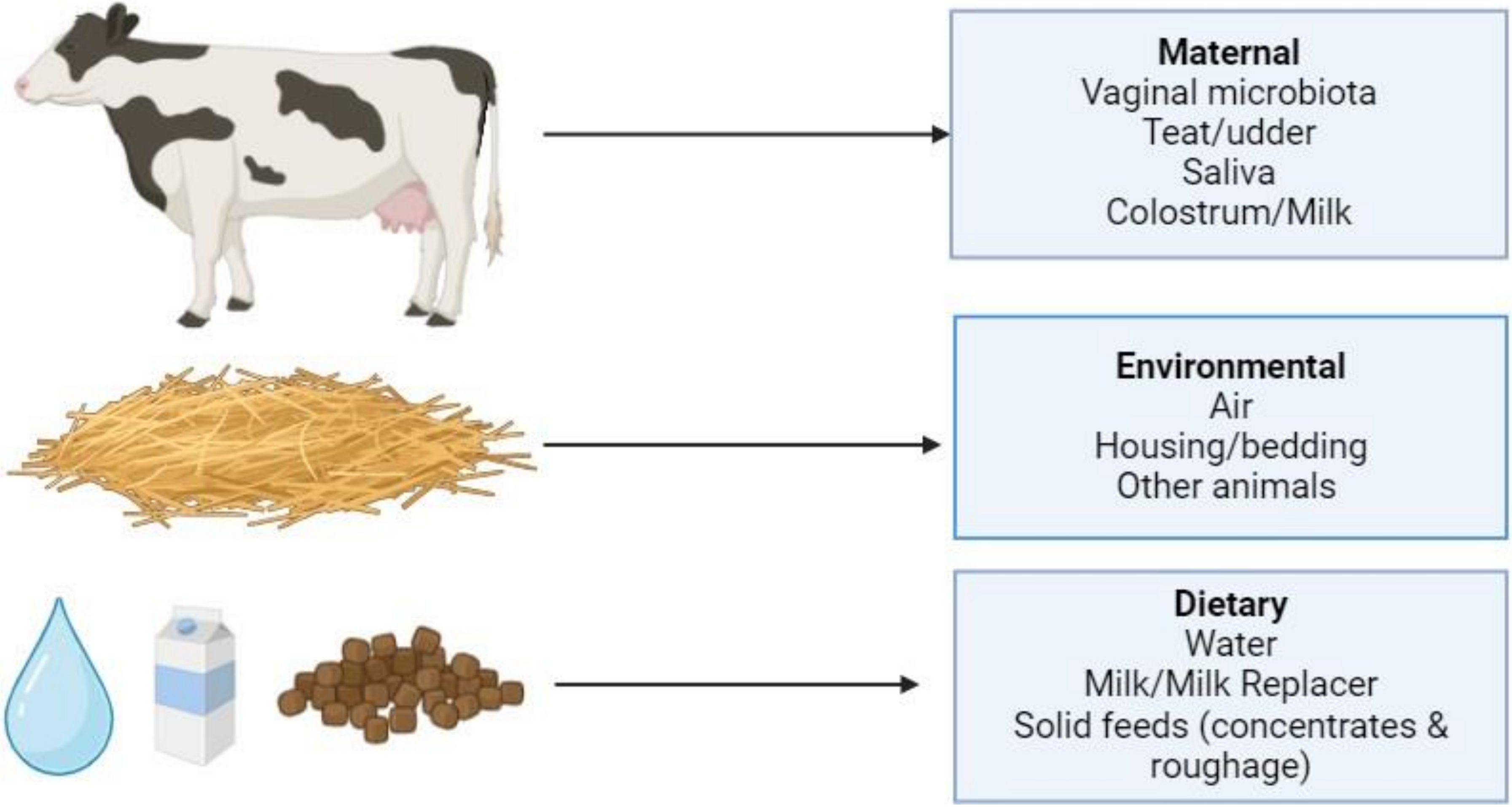
Sources of pioneering microbes

Primary Role:

- “seed” & colonize the various microbiome sites
- Transition calf gut environment

Secondary Role:

- Metabolites for host and other microbes
- Immune and physiological development



Maternal
Vaginal microbiota
Teat/udder
Saliva
Colostrum/Milk

Environmental
Air
Housing/bedding
Other animals

Dietary
Water
Milk/Milk Replacer
Solid feeds (concentrates & roughage)

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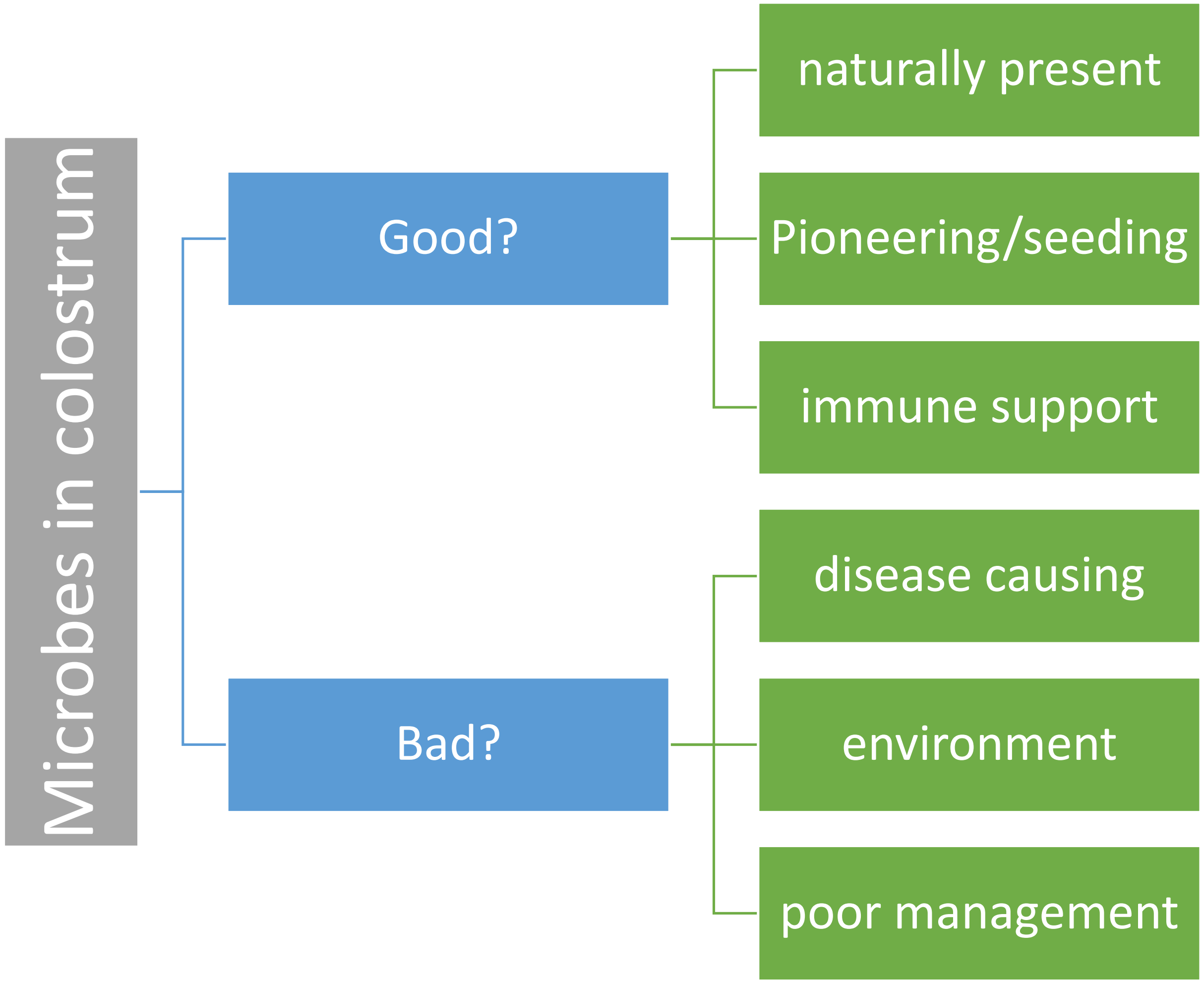
Colostrum and microbes



Microbes in colostrum

- Good?
  - naturally present
  - Pioneering/seeding
  - immune support
- Bad?
  - disease causing
  - environment
  - poor management

Entero-mammary pathway

- Proven in humans, mice and pigs
- Growing evidence in cattle

Colostral microbes

- Essential in monogastric GIT Microbiome & immunological development
- Growing evidence in ruminants

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Objective

to examine the core bacteria identified in fresh colostrum collected from a single, spring-calving dairy herd.*

**Core bacteria is defined as bacteria observed as present in all samples*

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Animal model and sampling protocols

Hygienic conditions

- Boiling water
- Anti-microbial soap
- Sterile collection tubes
- Gloves + 70%ETOH/MolH2O
- 70% ETOH+MolH2O

1 cow = 1 sample

- Milked within 2hr parturition
- 3 x 10ml per sample

Snap Frozen immediately

- Stored at -80degC

SAMPLE POPULATION BY BREED AND PARITY

	Multiparous **(n=17)**	Primiparous **(n=10)**	**Total (n=27)**
Holstein-Friesian **(n=17)**	12	5	17
Jersey **(n=10)**	5	5	10
Total (n=27)	17	10	27

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Microbial DNA extraction and sequencing

27 Colostrum Samples

10 mL Colostrum thawed → 600µL EDTA per 10mL colostrum → 4500 × g, 20 min, 4°C → Cream discarded → Supernatant (fat-free colostrum) → Colostrum Pellet

10 mL Colostrum thawed ↓

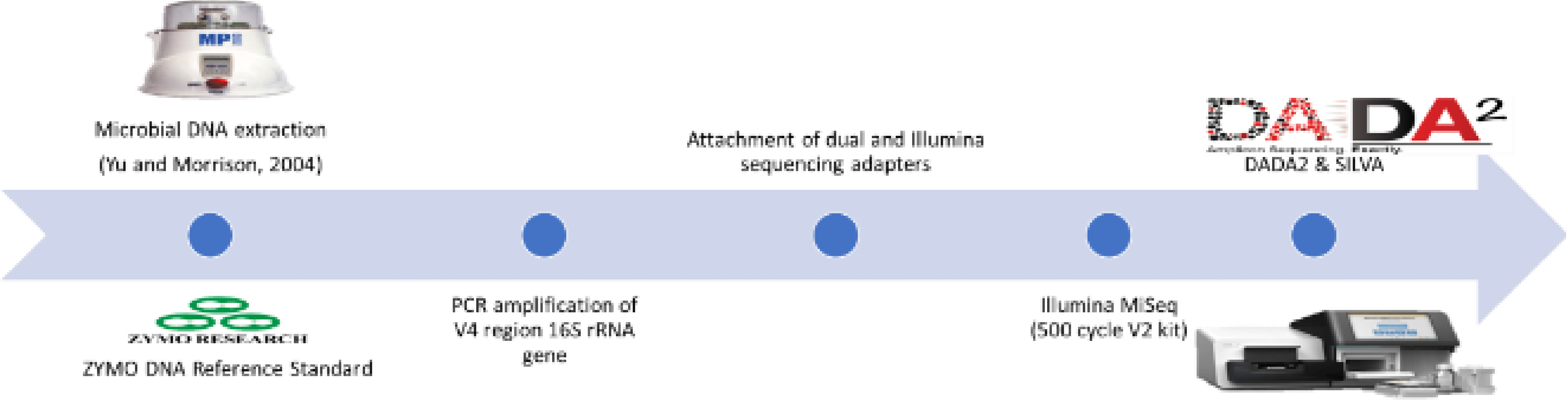
-80 to -20, 24hrs
-20 to +4, 24hrs

qPCR prior to sequencing

Supernatant (fat-free colostrum) ↓

Used for sRID IgG

Colostrum Pellet



R packages: Phyloseq, Vegan, Microbiome

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Colostrum quality

Statistics: SAS

- Normality and Homogeneity (UNIVARIATE procedure)
- ANOVA (MIXED procedure), fixed effects: Breed, Parity & Breed*Parity

Mean Colostrum Quality									
	Breed (B)			Parity (P)			P Value		
	HO	JE	SE	Primi	Multi	SE	B	P	P*B
BRIX (%)	27.2	25.5	0.76	25.84	26.99	0.75	0.23	0.54	0.68
IgG (mg/mL)	152.09	148.04	8.14	141.47	155.95	7.95	0.73	0.24	0.19

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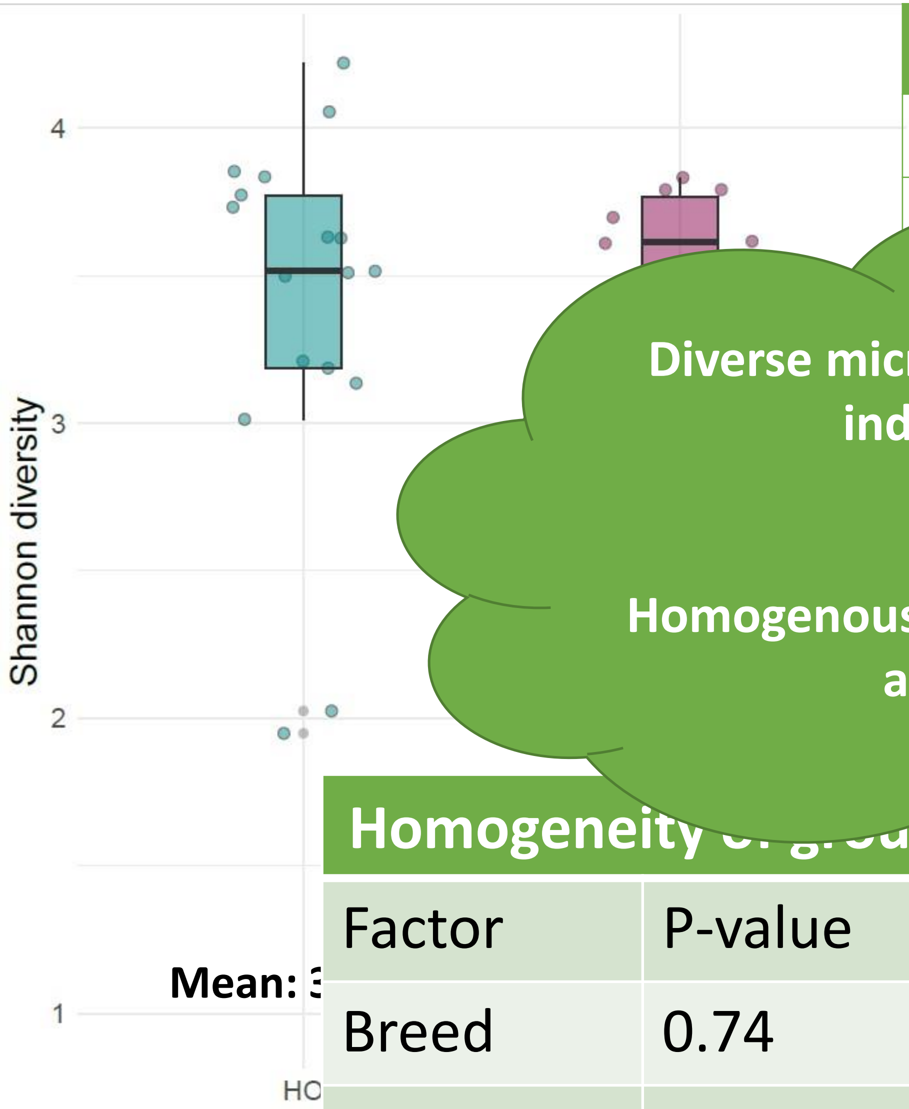
Colostrum microbial diversity and community composition



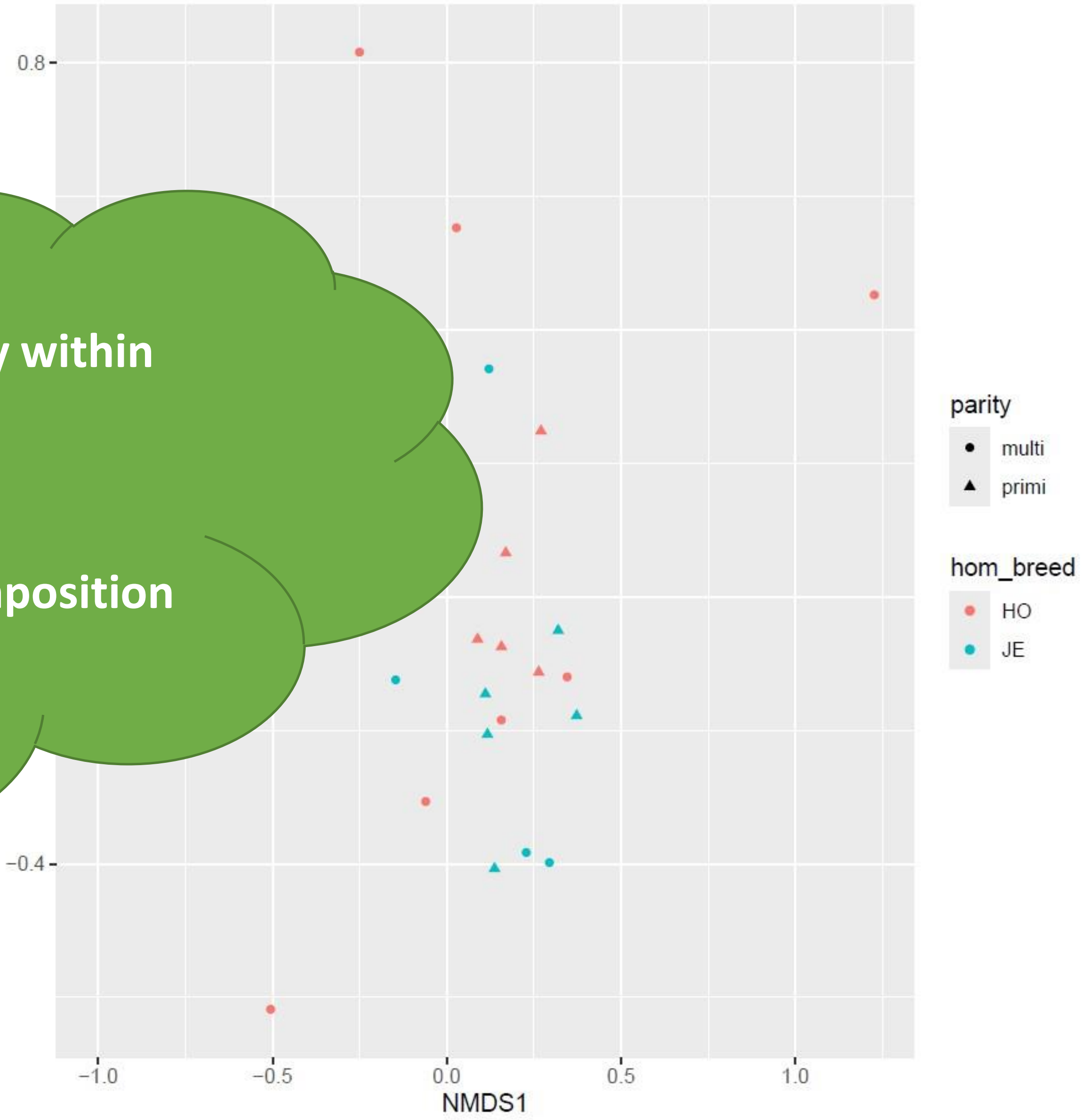
Mean: 3

Effects on al
Factor

Diverse microbial community within individual samples

Homogenous community composition across samples

Homogeneity of group	
Factor	P-value
Breed	0.74
Parity	0.28



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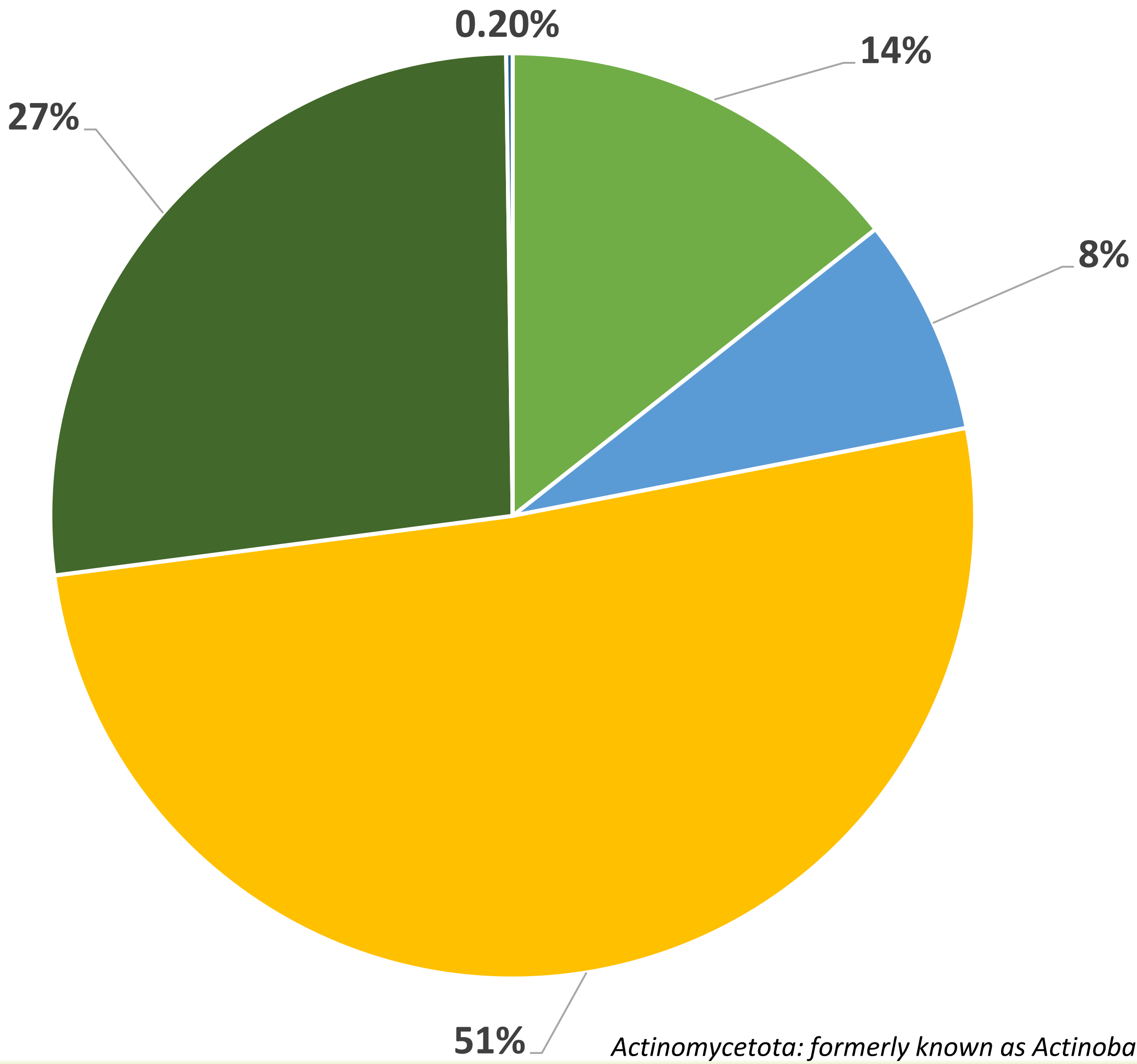
Predominant bacterial phyla

Relative Abundance of Bacterial Phyla in fresh colostrum



709 different genera

Actinomycetota: formerly known as Actinobacteria; Bacillota: formerly known as Firmicutes; Pseudomonadota: formerly known as Proteobacteria

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Core bacteria in fresh colostrum

Phylum	**Family**	**Genus**	**Relative Abundance (%)**
Pseudomonadota	Moraxellaceae	**Acinetobacter**	10.68
Bacillota	Peptostreptococcaceae	**Romboutsia**	8.19
Pseudomonadota	Pseudomonadaceae	**Pseudomonas**	7.18
Bacillota	Staphylococcaceae	**Staphylococcus**	5.75
Actinomycetota	Corynebacteriaceae	Corynebacterium	5.25
Bacillota	Lachnospiraceae	**NK3A20 group**	3.52
Bacillota	Oscillospiraceae	**UCG-005**	2.41
Bacillota	Peptostreptococcaceae	**Clostridioides**	2.19
Bacillota	Christensenellaceae	**R-7 group**	2.07
Pseudomonadota	Moraxellaceae	Psychrobacter	2.01
Actinomycetota	Micrococcaceae	Glutamicibacter	1.63
Bacillota	Erysipelotrichaceae	Turicibacter	1.31
Bacteroidota	Flavobacteriaceae	**Flavobacterium**	1.26
Bacteroidota	Bacteroidaceae	**Bacteroides**	1.26
Bacillota	Aerococcaceae	**Facklamia**	1.22
Bacillota	Clostridiaceae	**Clostridium sensu stricto 1**	1.20

26 core bacteria identified

Opportunistic pathogen/bovine commensal from other body site

Primary pathogenic agent

Known bovine gut commensal

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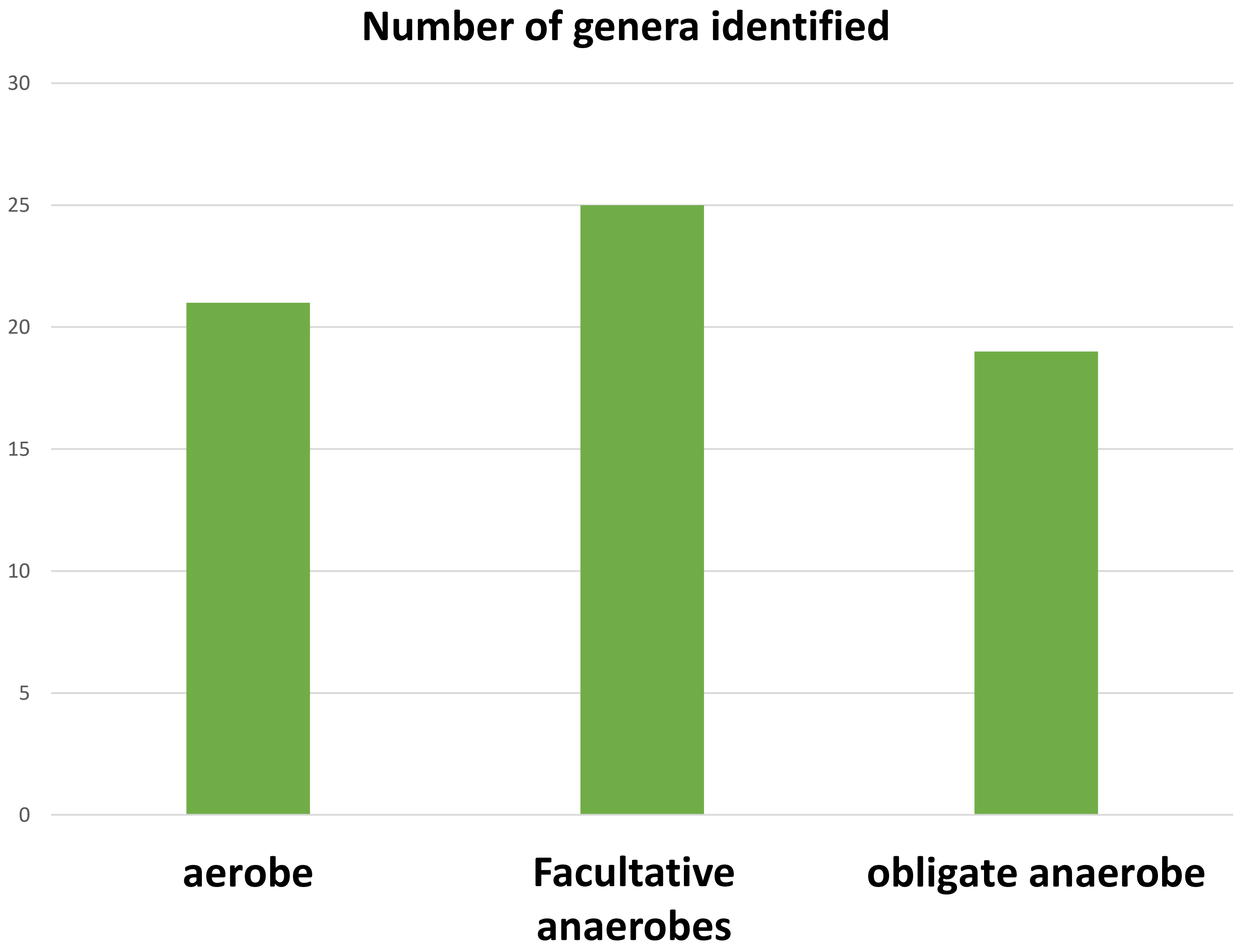
Metabolic classification of genera identified


Number of genera identified

Short Chain Fatty Acids

- Butyrate
- Acetate
- Propionate

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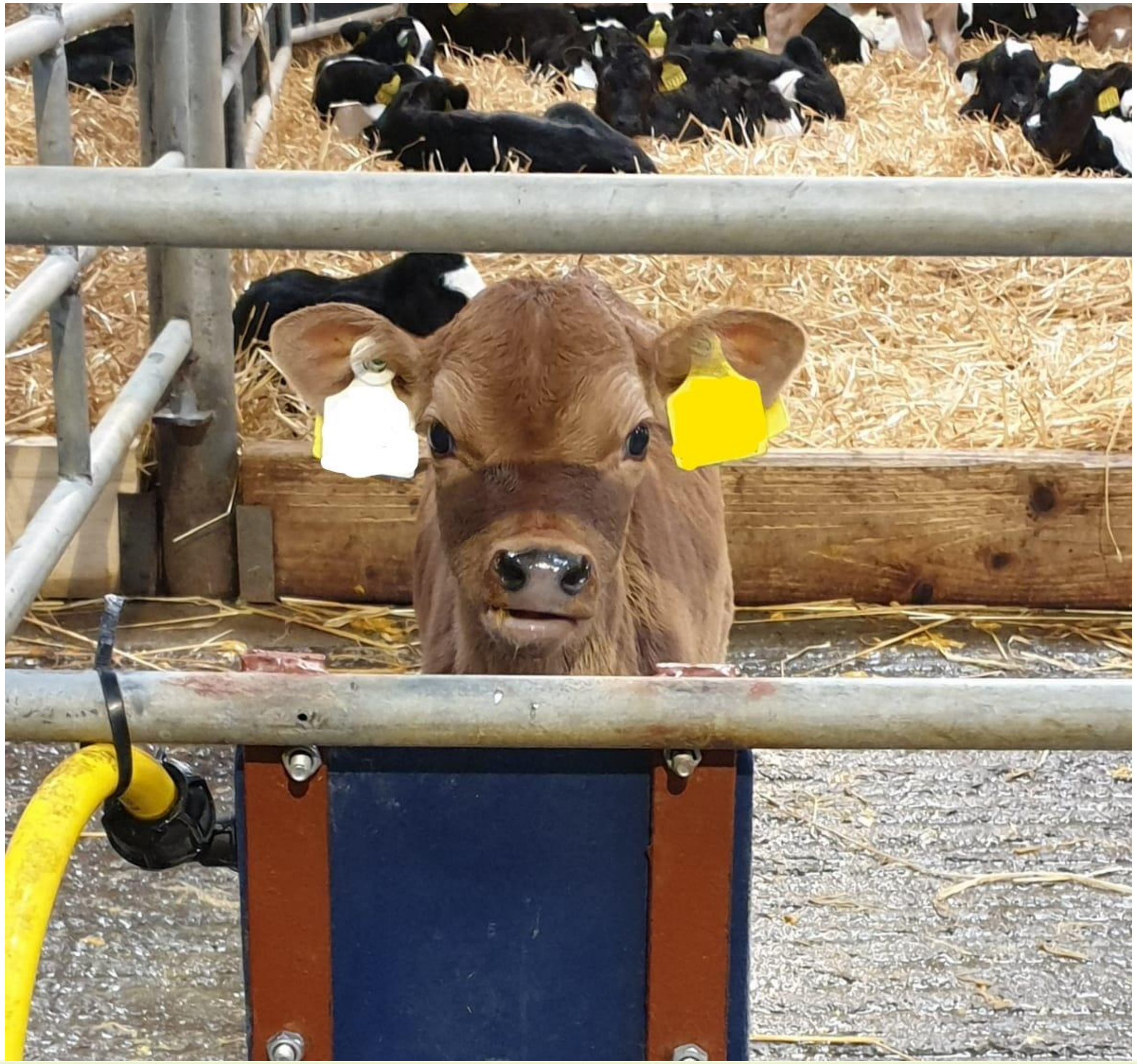
Future work



- Implications to calf health?
  - Viability of the microbes
    - Are these microbes alive when the calf consumes colostrum
  - If viable: Function of microbes
    - interactions with the host & other microbes
  - Contribution to microbiome development
    - Seeding and pioneering the gut in early life
- Implications of colostrum storage & management practices…

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Conclusions

- This characterization may be indicative of naturally occurring colostral microbiome
- Fresh colostrum was observed to be:
  - diverse in community membership
  - homogenous composition within sample population
- Work is ongoing to understand impact on calf health and development
  - Viability and function
  - Seeding
  - Colostrum management practices

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Thank you for your time

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