Upper respiratory tract microbiota of dairy calves experimentally challenged with BRSV

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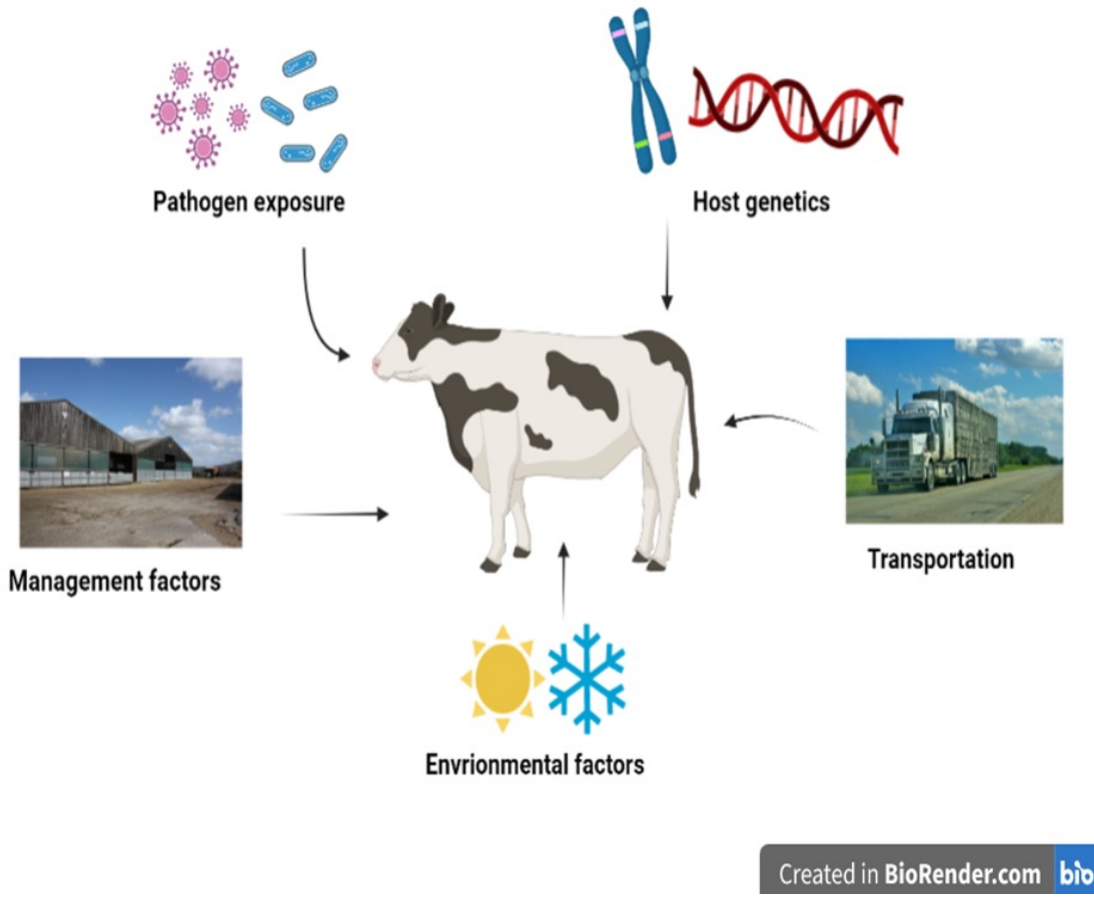
**EAAP Annual Meeting, Lyon
30th August 2023**

Factors contributing to BRD development

Pathogen exposure

Host genetics

Management factors

Transportation

Envrionmental factors

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- Major **health and welfare issue** in **Irish and international** cattle industries.

- Responsible for **33.4% mortality** in calves (both dairy and beef) aged between **1 and 5 months** (DAFM, 2021)

- Costs range from **approx. €49 – €95 per infected animal**

- **Multifactorial** disease

- Impacts cattle of all ages – **younger** calves **most vulnerable**

- Diagnosis largely centred on **clinical signs**

Elevated temperature
Depression
Ocular discharge
Elevated respiratory rate
Nasal discharge
Coughing
Appetite loss
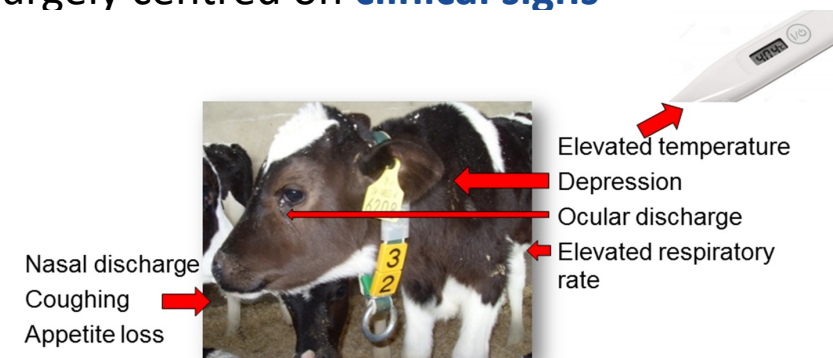
- Both **viral** and **bacterial** pathogens responsible for BRD onset

- Initial viral infections can lead to secondary bacterial infections

- BRD bacteria can be **commensals in URT**

- **Stress/illness can** compromise immune system and cause an **imbalance in natural communities** present

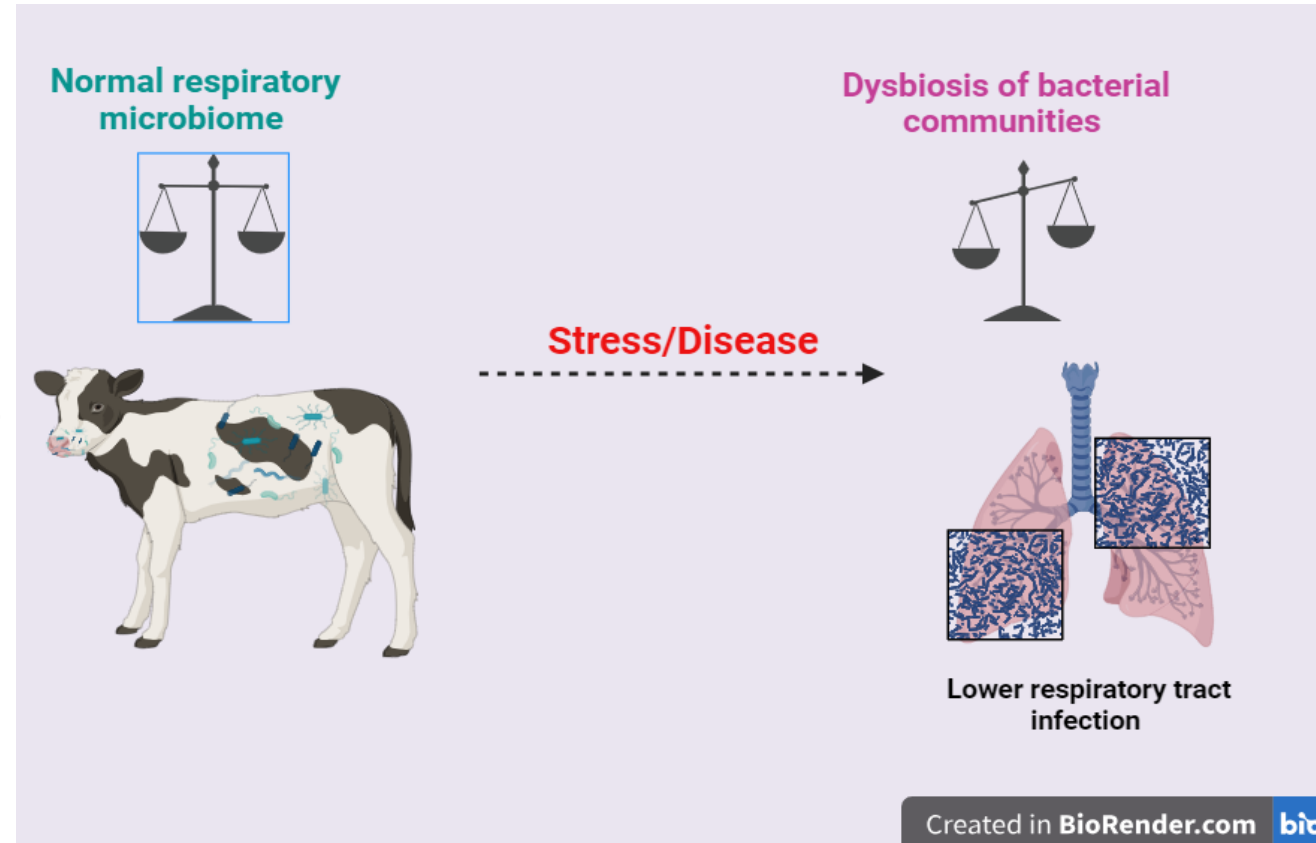| BRD-associated viruses	BRD-associated bacteria
Bovine Respiratory Syncytial Virus (BRSV)	Pasteurella multocida
Bovine herpesvirus 1 (BoHV-1)	Mannheimia haemolytica
Bovine Coronavirus (Bcov)	Histophilus somni
Bovine parainfluenza 3 virus (PI3)	Mycoplasma bovis

Studies to date:

2016

Microbiome of the upper nasal cavity of calves prior to weaning[12] 🔓

Tara G McDaneld ✉, Larry A Kuehn, John W K...

2017

...mplicon sequence analysis of ...ease associated bacteria in lung ...node tissue

...an, Gerard Murray, David Anthony Kenny, Sinead Mary ...ew Sean McCabe ✉

2019

Lower Respiratory Tract ... Bovine Respiratory Disea...

Cassidy L. Klima, Devin B. Holman, Brenda J. Ralston, ... Tim A. McAllister ✉

Bacterial microbiota
over the course of a
known viral infection
?

2022

...bovine respiratory disease through the nasal microbiome

Ruth Eunice Centeno-Martinez, Natalie Glidden, Suraj Mohan, Josiah Levi Davidson, Esteban Fernández-Juricic, Jacquelyn P. Boerman, Jon Schoonmaker, Deepti Pillai, Jennifer Koziol, Aaron Ault, Mohit S. Verma & Timothy A. Johnson ✉
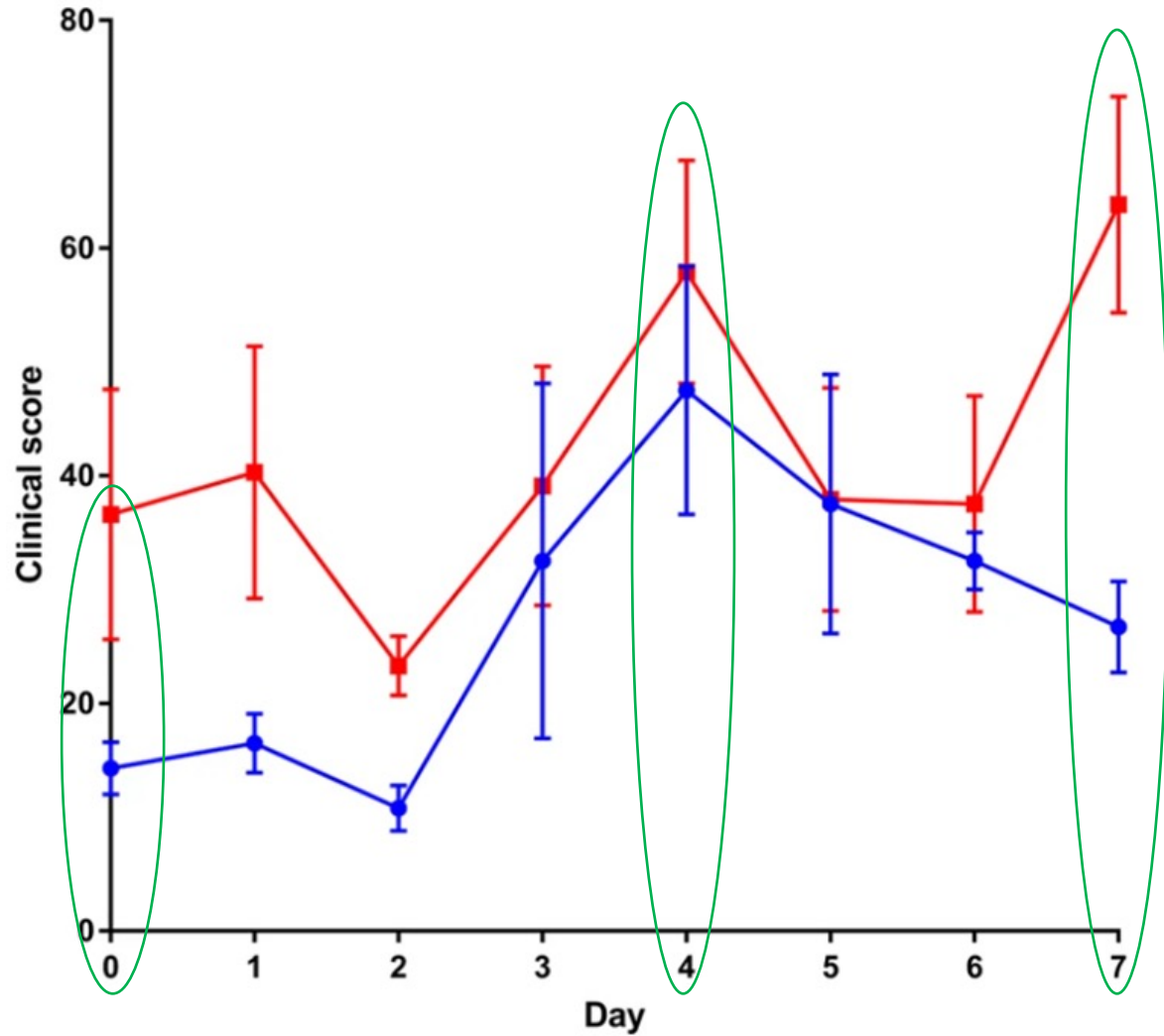
- **18 Holstein-Friesian bull calves** selected from a pool of 30 animals based on low maternally derived antibodies (MDA) and negative BRSV PCR status.

- Two treatment groups:
    1. **Mock challenged** with sterile PBS (n=6)

    2. **Challenged** (n=12) with BRSV($10^{3.5}$ TCID$_{50}$/ml × 15 ml)

- **Clinical signs** recorded daily (day (d) 0 to 7)

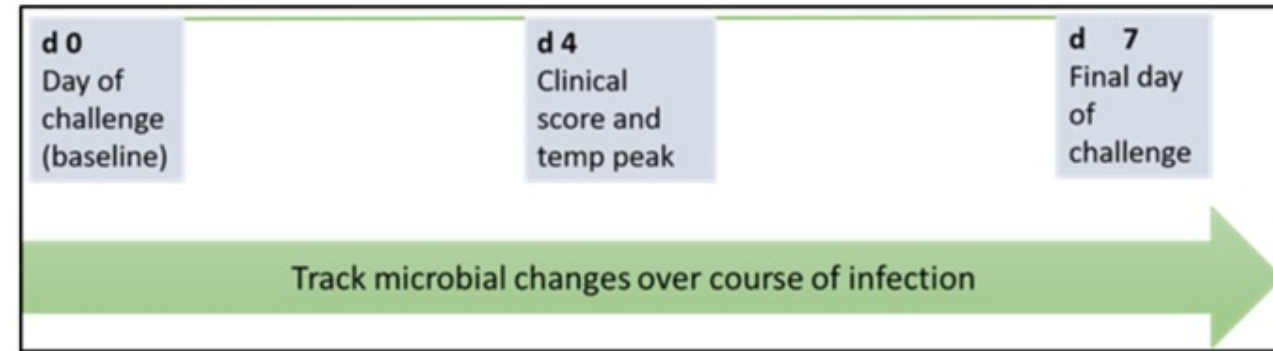- **Nasal swabs** collected each day

- Euthanised on **d 7** post-challenge

(Johnston et al., 2019)

d 0 Day of challenge (baseline)	**d 4** Clinical score and temp peak	**d 7** Final day of challenge

Track microbial changes over course of infection

Time-Point A = **d 0**
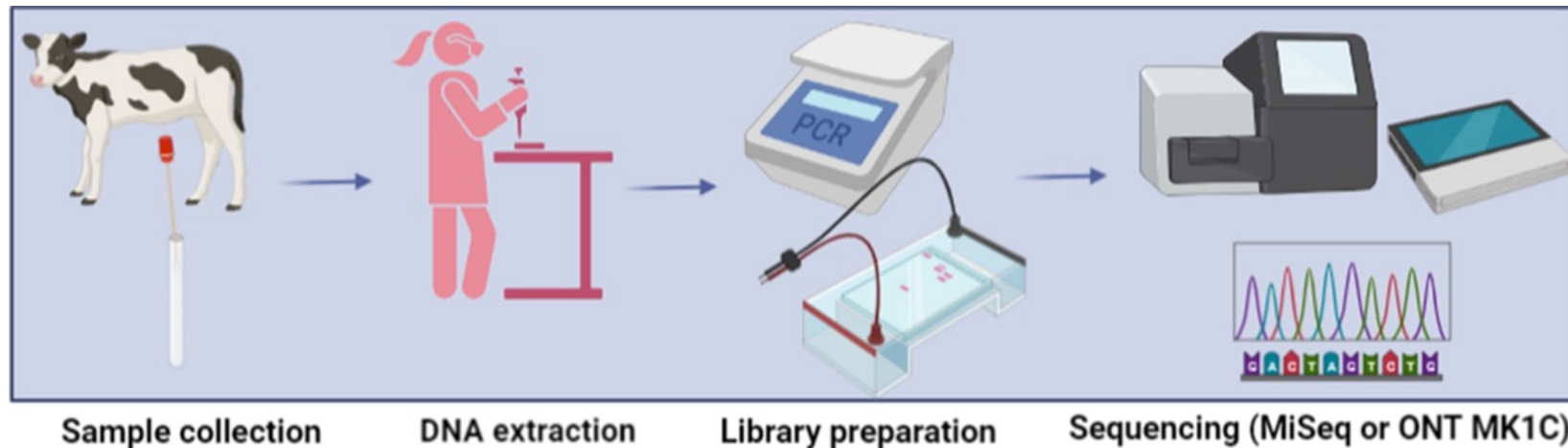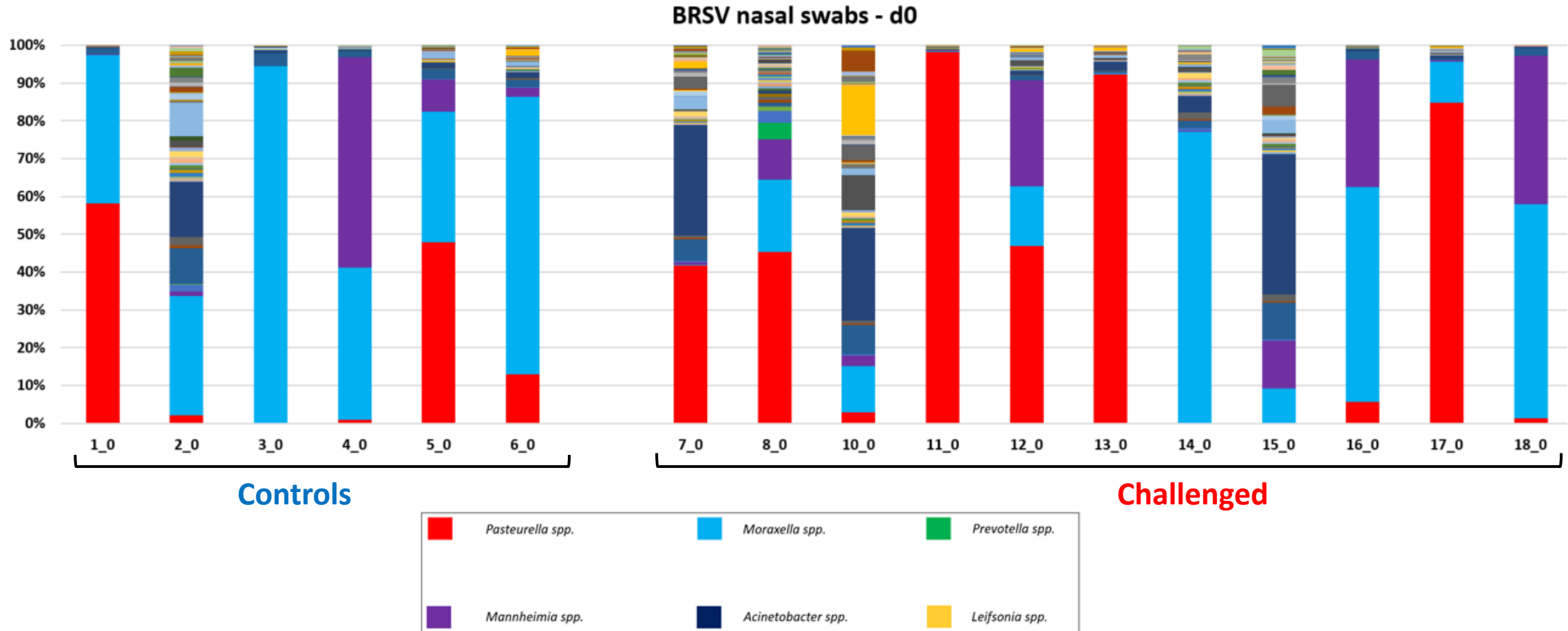
Time-Point B = **d 4**

Time-Point C = **d 7**

- DNA from **nasal swabs** extracted using DNeasy Powerlyzer PowerSoil kit

- Sequencing libraries prepared using the Oxford Nanopore Technologies (ONT) **16S Barcoding kit (SQK16S024)**

- Libraries quantified and quality assessed on the **Nanodrop Spectrophotometer**

- **Pooled libraries** were sequenced on a ONT MinION MK1C device on a MinION flow cell (9.4 chemistry)

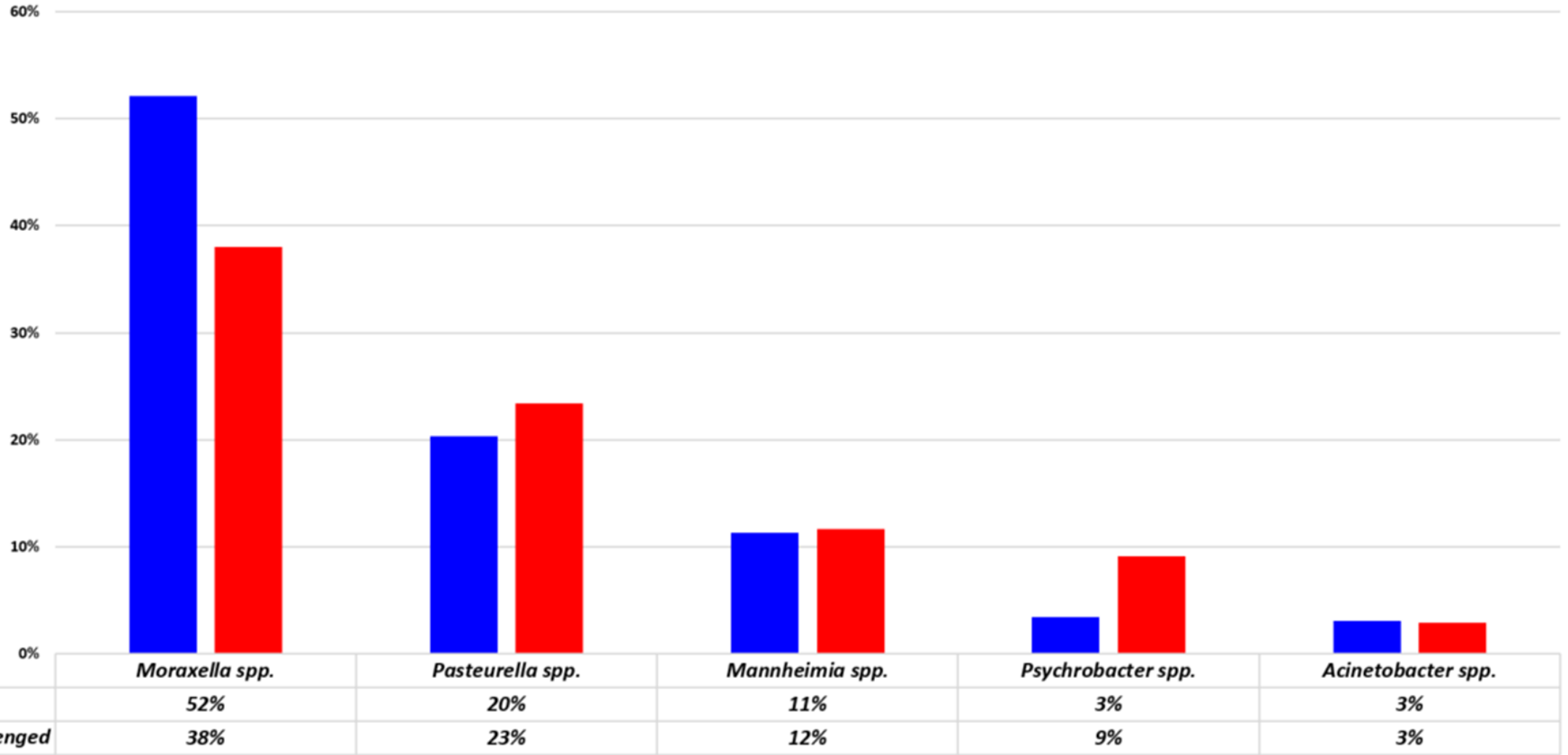- **Raw FASTQ reads** uploaded to the **EPI2ME platform** and analysed with the **Fastq 16S pipeline** (v2022.01.07)

Sample collection   DNA extraction   Library preparation   Sequencing (MiSeq or ONT MK1C)

BRSV nasal swabs - d0

Controls

Challenged

Legend:
- Pasteurella spp. (red)
- Moraxella spp. (light blue)
- Prevotella spp. (green)
- Mannheimia spp. (purple)
- Acinetobacter spp. (dark blue)
- Leifsonia spp. (yellow)

No significance difference in any genus between treatments at TP A (P < 0.05, FDR < 0.05)

Average % abundances BRSV vs Controls - Day 0

	Moraxella spp.	Pasteurella spp.	Mannheimia spp.	Psychrobacter spp.	Acinetobacter spp.
Controls	52%	20%	11%	3%	3%
BRSV challenged	38%	23%	12%	9%	3%

BRSV nasal swabs - d4

Controls

Challenged

Legend:
- Pasteurella spp.
- Moraxella spp.
- Psychrobacter spp.
- Mannheimia spp.
- Acinetobacter spp.
- Jeotgalicoccus spp.
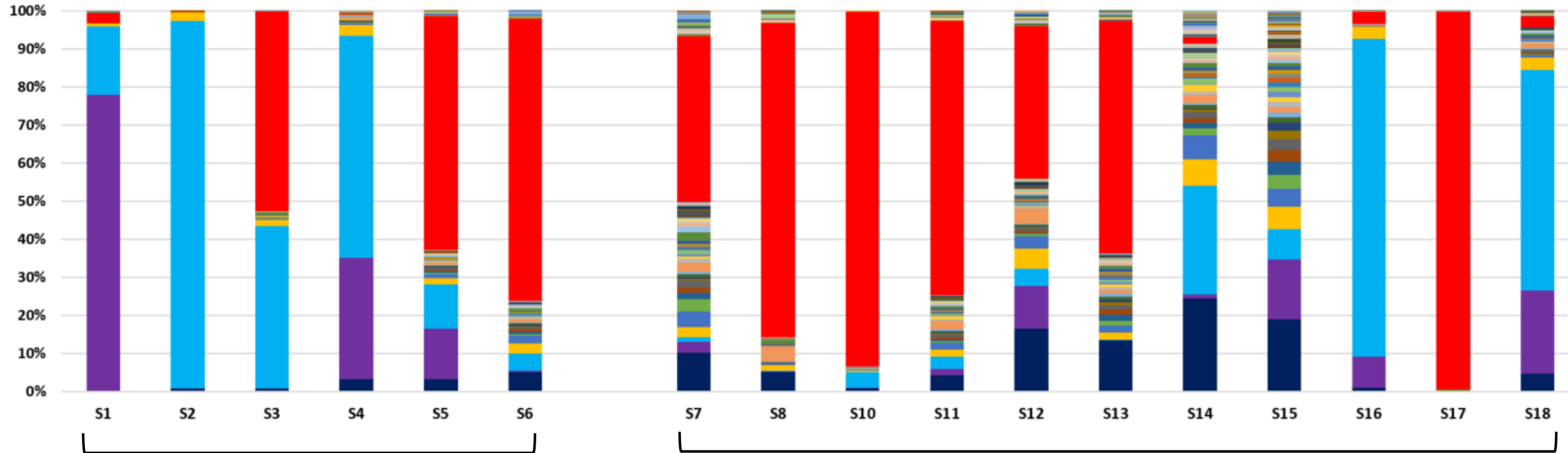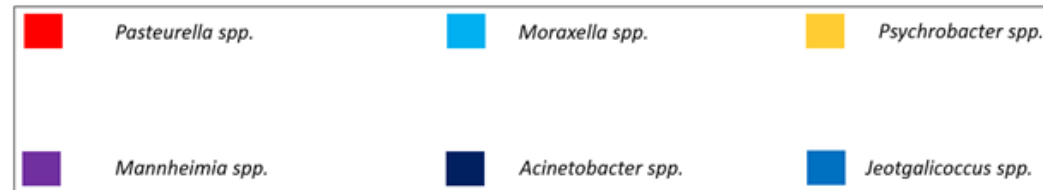
No significance difference in any genus between treatments at TP B (P < 0.05, FDR < 0.05)

Average % abundances BRSV vs Controls - Day 4

	Moraxella	Pasteurella	Mannheimia	Acinetobacter	Psychrobacter
Controls	38%	31%	21%	2%	2%
BRSV challenged	17%	45%	6%	9%	3%
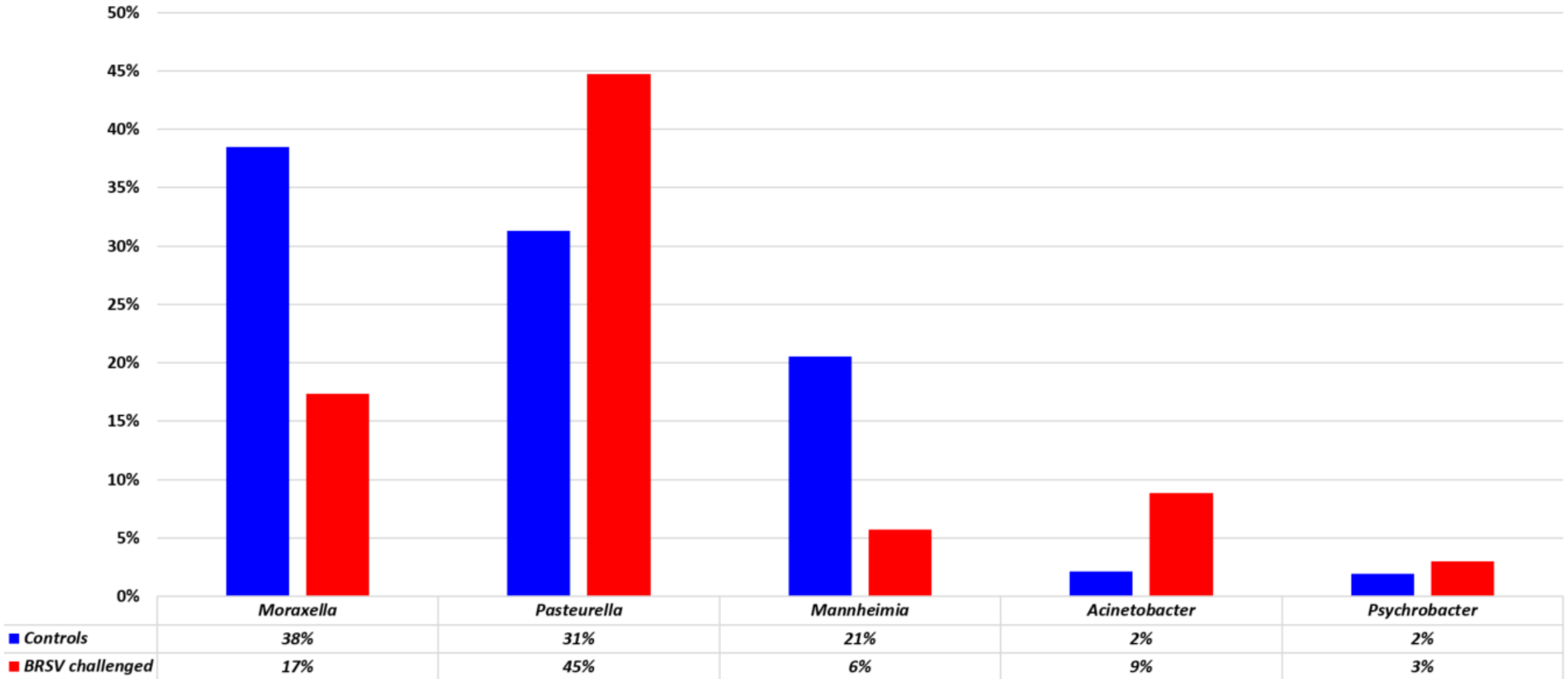
BRSV nasal swabs - d7

Controls

Challenged

Legend:
- Pasteurella spp. (red)
- Moraxella spp. (light blue)
- Mycoplasma spp. (yellow)
- Mannheimia spp. (purple)
- Acinetobacter spp. (dark blue)
- Psychrobacter spp. (orange)
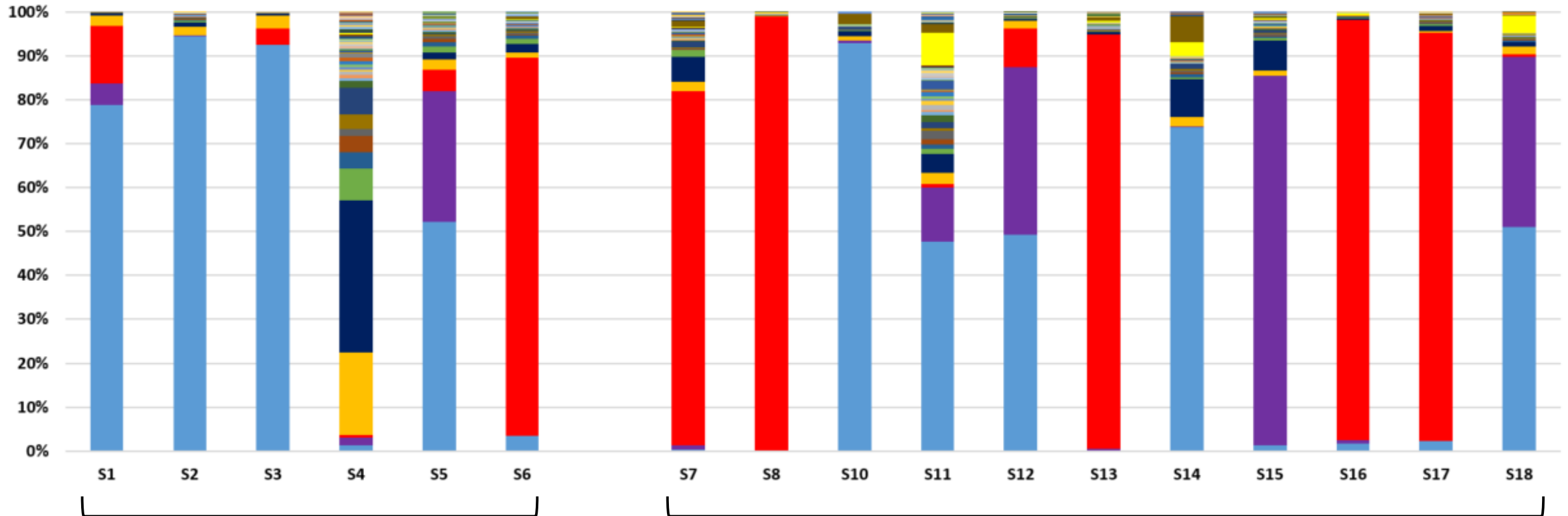
No significance difference in any genus between treatments at TP C (P < 0.05, FDR < 0.05)

Average % abundances BRSV vs Controls - Day 7
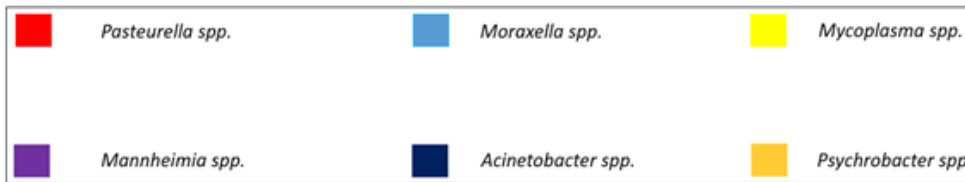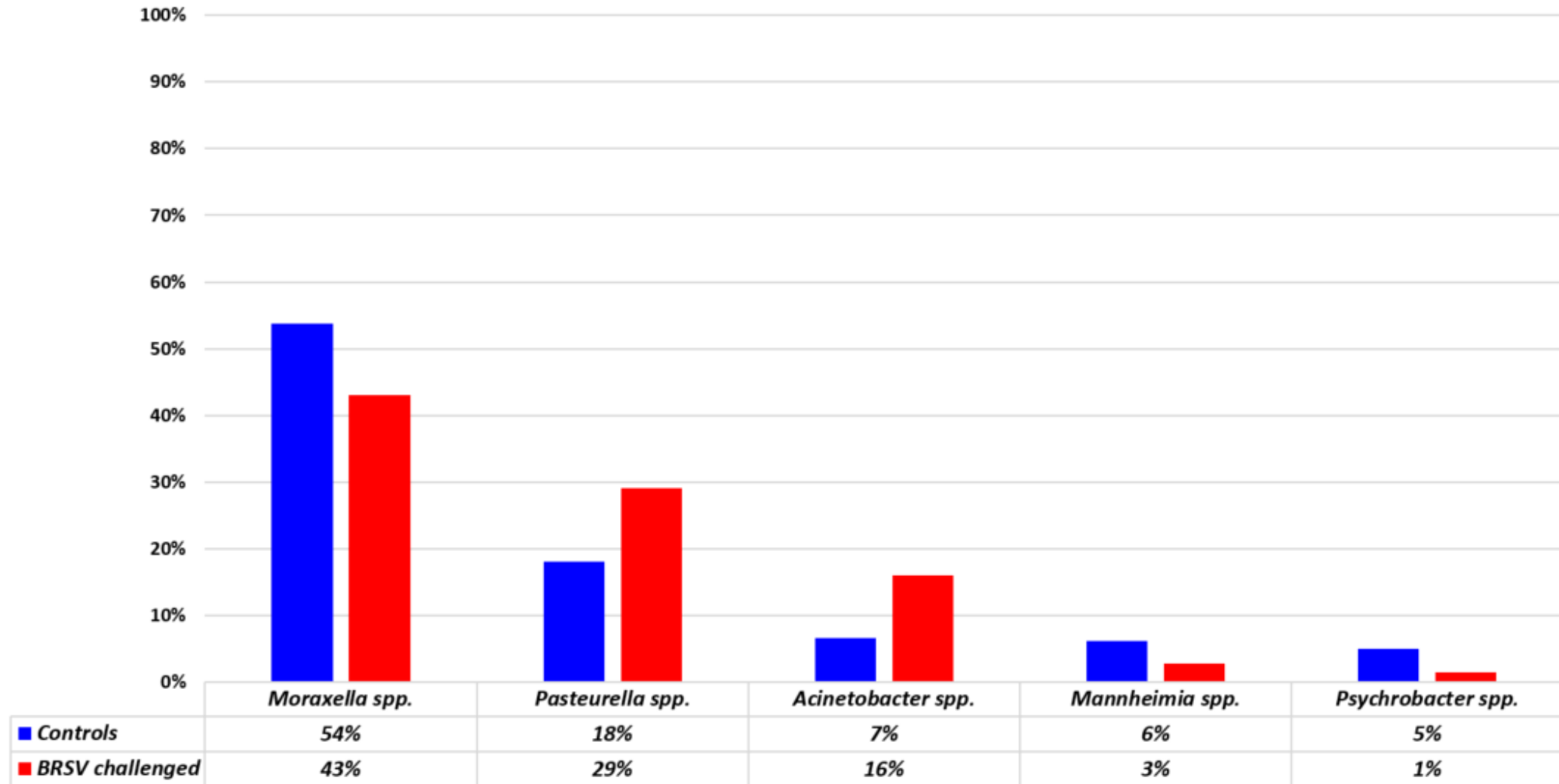
	Moraxella spp.	Pasteurella spp.	Acinetobacter spp.	Mannheimia spp.	Psychrobacter spp.
Controls	54%	18%	7%	6%	5%
BRSV challenged	43%	29%	16%	3%	1%

- Characterised the **bacterial nasal microbiome of BRSV infected calves**

- Top genera identified across all animals include *Pasteurella, Moraxella* and *Mannheimia spp*.

- BRSV infection seems to **alter the bacterial microbiome of the URT in infected calves**

- Potential for the use of the MinION device as an 'pen-side' laboratory testing tool

ONT MK1C

Prof Sinéad Waters   Dr Bernadette Earley   Dr Derek Morris   Dr Matthew McCabe

Project 16/RD/US-ROI/11

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**Agriculture,
Food and the Marine**
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Department of
**Agriculture, Environment
and Rural Affairs**

USDA

United States Department of Agriculture
National Institute of Food and Agriculture

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USDA

United States Department of Agriculture
National Institute of Food and Agriculture

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Thank you for your attention

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