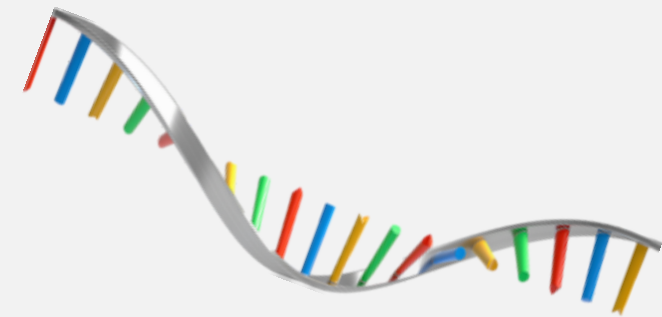


Multi-tissue transcriptome analysis of bovine herpesvirus-1 (BoHV-1) challenged dairy calves

Stephanie O'Donoghue BSc,
MSc

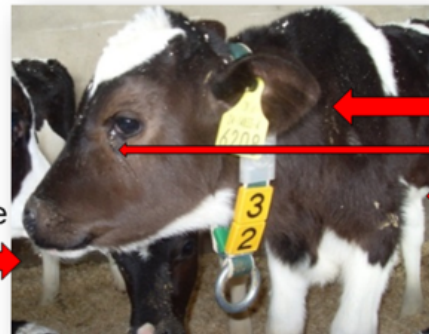


EAAP Annual Meeting, Lyon

29th August 2023

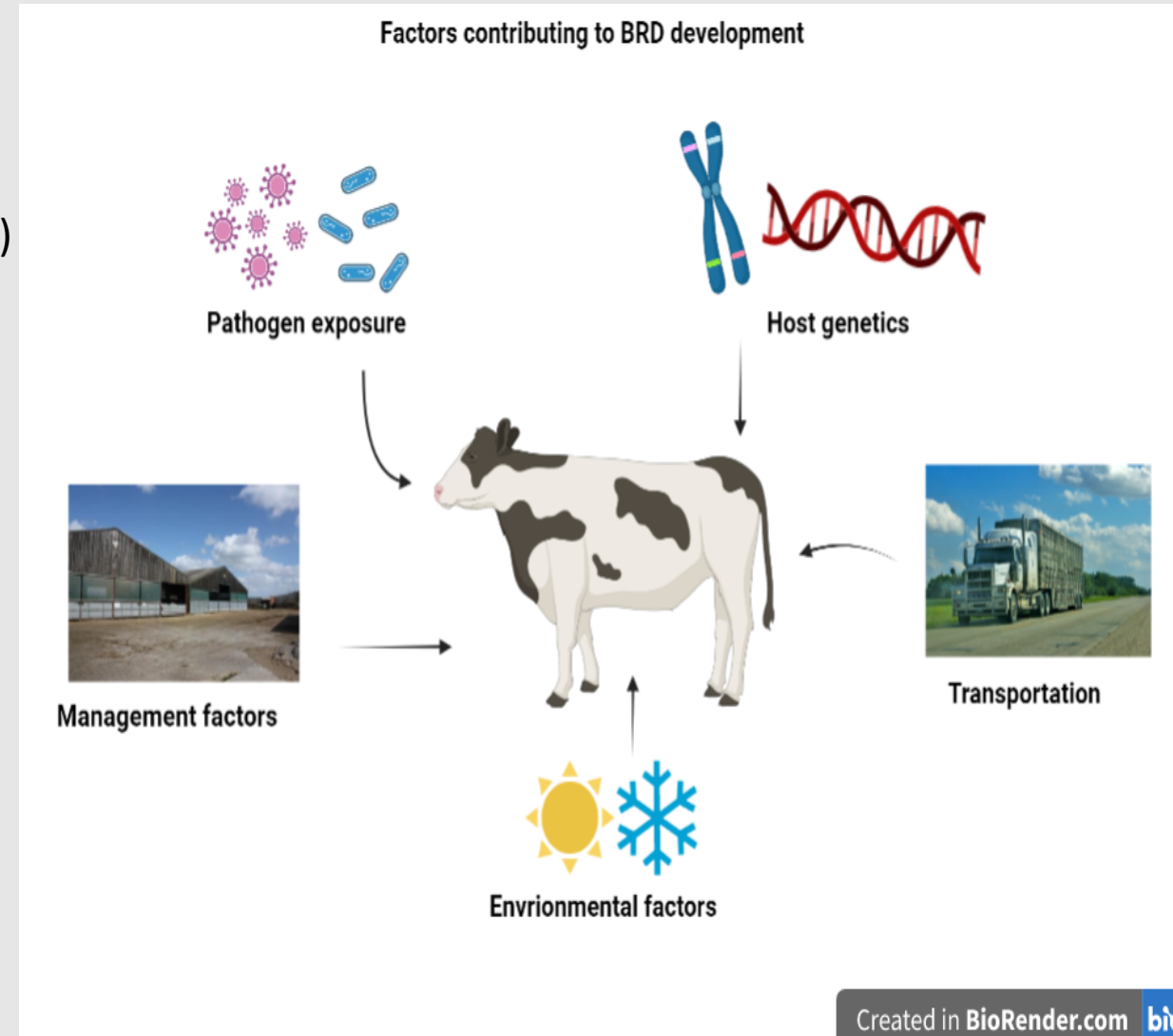
Background: Bovine Respiratory Disease (BRD)

- Major **health and welfare issue** in **Irish and international** cattle industries.
- **Multifactorial** disease
- 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**
- Both **bacterial and viral agents** capable of causing infection
- Diagnosis relies largely on **clinical signs** of infection



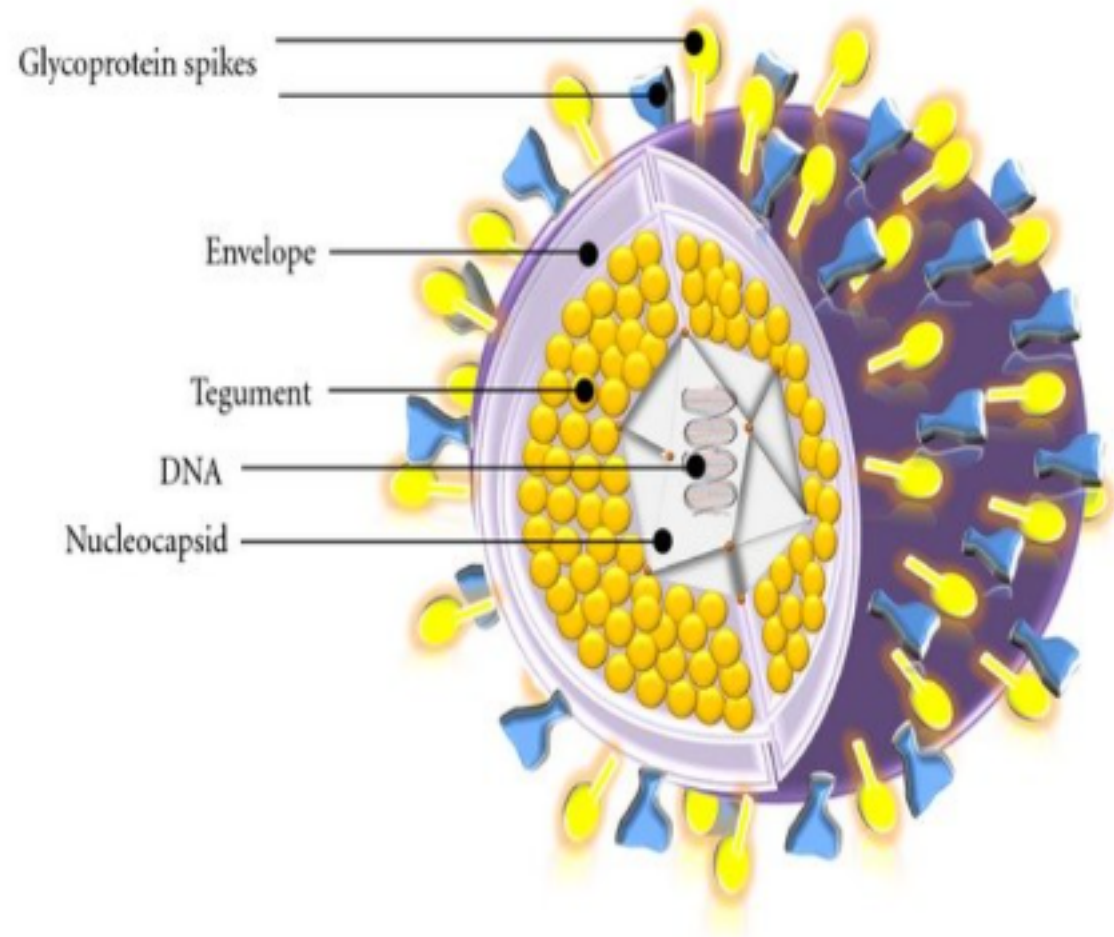
Nasal discharge
Coughing
Appetite loss

Elevated temperature
Depression
Ocular discharge
Elevated respiratory rate




Bovine herpesvirus 1 (BoHV-1)

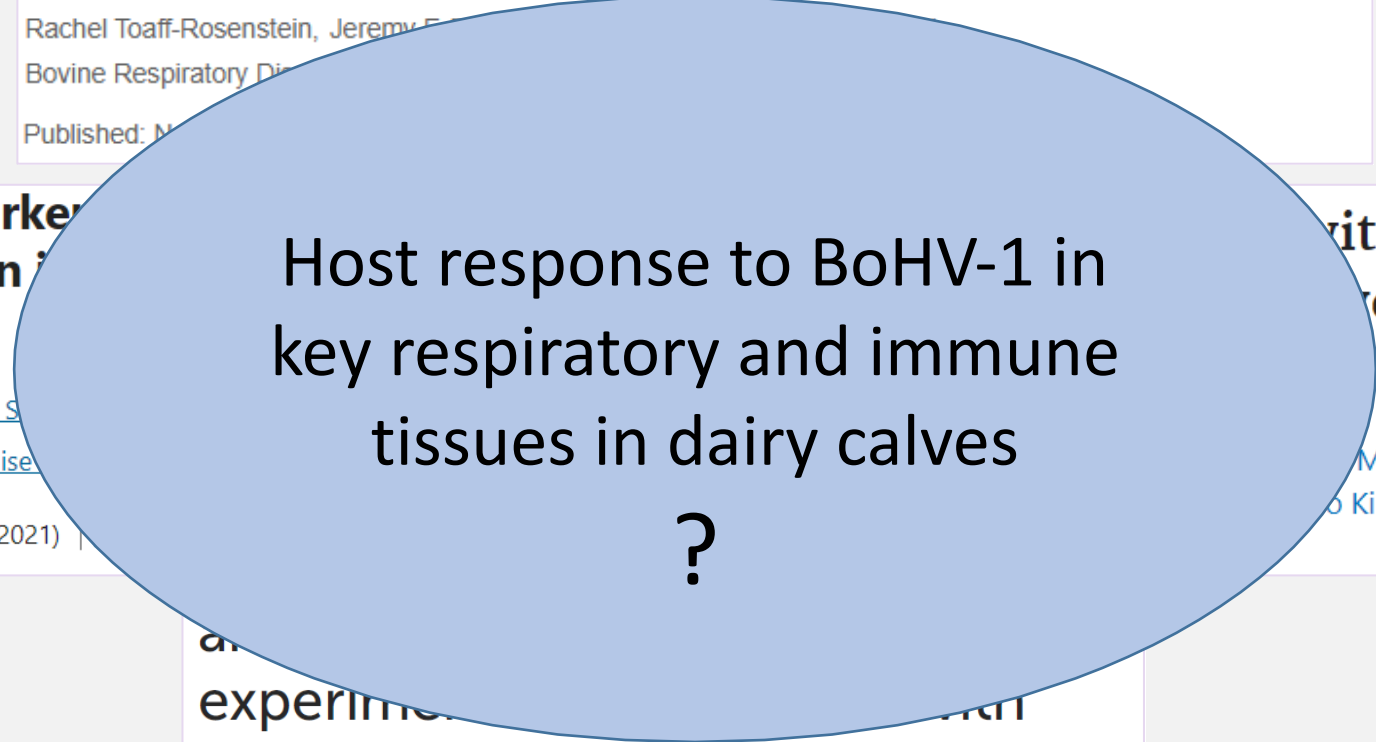
- *Varicellovirus* genus of the *alphaherpesvirus* subfamily
- Enveloped icosahedral capsid containing **double-stranded DNA**
- Causes Infectious Bovine Rhinotracheitis (**IBR**).
- Transmitted mainly through **nasal fluid 4-6 days** post infection.
- Can remain **latent** within the animal



Study Rationale

Single Pathogen Challenge with Agents of the Bovine Respiratory Disease Complex

Laurel J. Gershwin , Alison L. Van Eenennaam, Mark L. Anderson, Heather A. McEligot, Matt X. Shao †, Rachel Toaff-Rosenstein, Jeremy F. Taylor
Bovine Respiratory Disease Complex
Published: March 2021



with bovine respiratory 2019 res: bronchial lymph node

Matthew S. McCabe ¹, Ken Lemon ², Catherine Duffy ²,
Jae Woo Kim ³, Gordon Blackshields ¹, Jeremy F Taylor ³,

Messenger RNA biomarker Syncytial Virus infection in calves

[Dayle Johnston](#), [Bernadette Earley](#), [Matthew S. McCabe](#),
[Catherine Duffy](#), [Michael McMenemy](#), [S. Louise Cosby](#)
Scientific Reports **11**, Article number: 9392 (2021) |

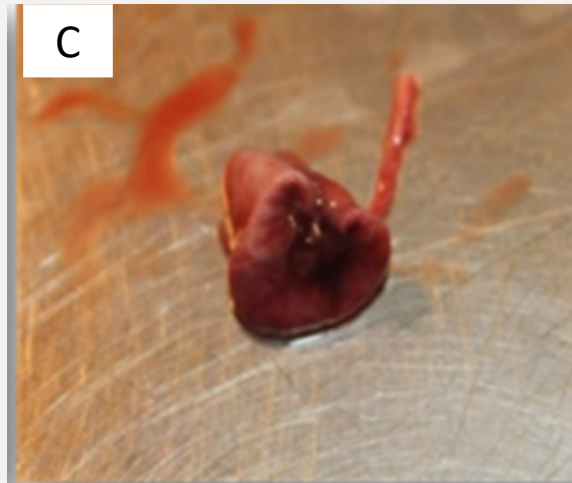
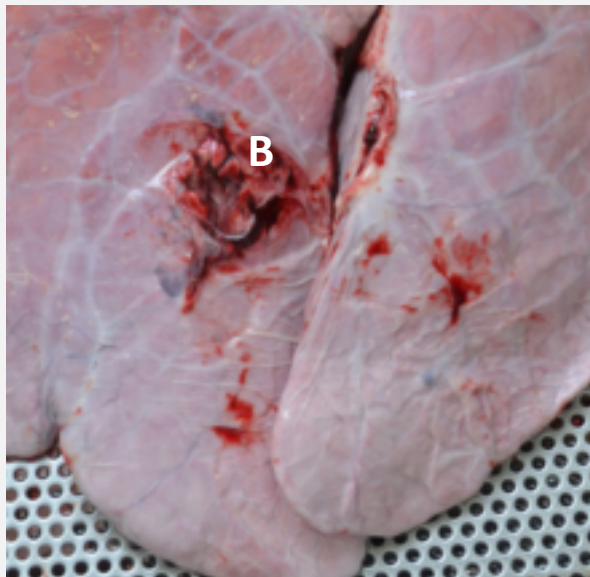
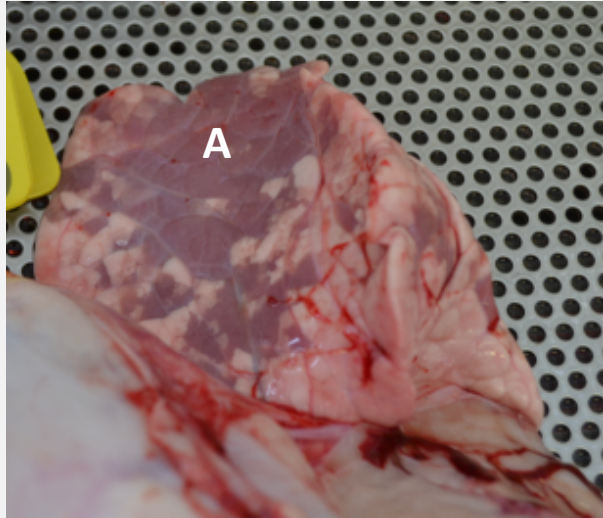
an experimen... with bovine herpesvirus 1 (BoHV-1) and comparison to a bovine respiratory syncytial virus (BRSV) challenge

Stephanie O'Donoghue^{1,2}, Bernadette Earley¹, Dayle Johnston¹,
Matthew S. McCabe¹, Jae Woo Kim³, Jeremy F. Taylor³,
Catherine Duffy⁴, Ken Lemon⁴, Michael McMenemy⁴,
S. Louise Cosby⁴, Derek W. Morris² and Sinéad M. Waters^{1*}

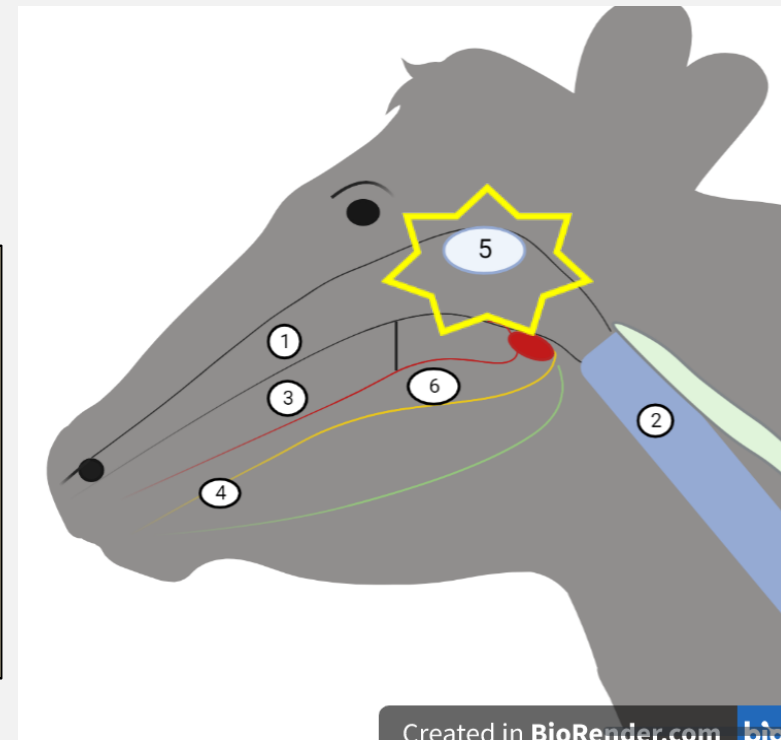
O'Donoghue et al., 2023

Tissue selection: Bronchial and mediastinal lymph node, pharyngeal tonsil and Lung (Right cranial lung lobe)

- **Right cranial lung lobe** is the most common location for lesion development
- **Bronchial lymph node** and **mediastinal lymph node** can become enlarged in cases of pneumonia – located in thoracic cavity
- **Pharyngeal tonsil** a key site of antigen processing and immune response initiation



- 1 = Nasal cavity
- 2 = Trachea
- 3 = Hard palette
- 4 = Oral cavity
- 5 = Pharyngeal tonsil**
- 6 = Oropharynx
- 7 = Epiglottis
- 8 = Oesophagus



A = Lung consolidation **B** = lesioned lung **C** = mediastinal lymph node from BRD infected calf

Experimental model

- **18 Holstein-Friesian bull calves** selected from a pool of 43 (BoHV-1) based on low maternally derived antibodies (MDA) and negative BoHV-1 PCR status.
- Two treatment groups:
 1. **Mock challenged** with sterile PBS (n=6)
 2. **Challenged** (n=12) with **BoHV-1** (6.3×10^7 /m x 1.35mL)
- **Clinical signs** recorded daily (day (d) 0 to 6)
- Euthanised on **d 6 (BoHV-1)** post challenge for tissue collection
 - **mediastinal lymph node**
 - **bronchial lymph node**
 - **pharyngeal tonsil**
 - **right cranial lung lobe (healthy/lesioned)**



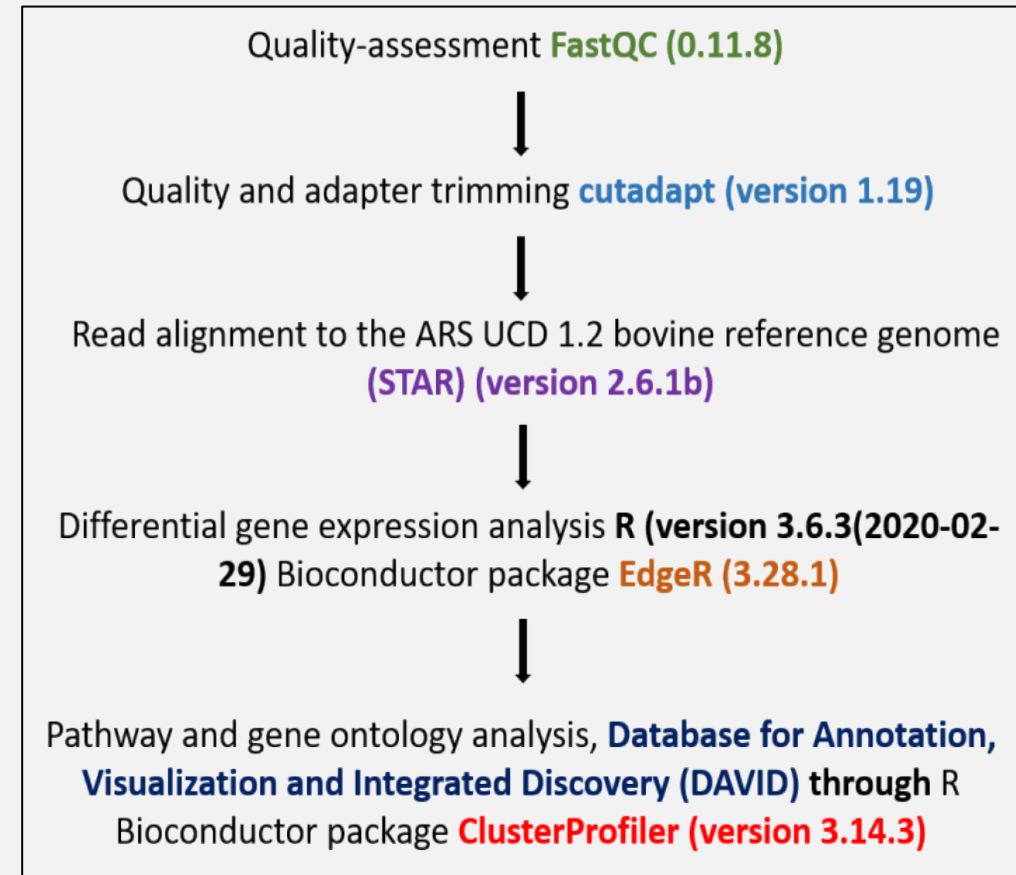
Methods : RNA extraction and bioinformatics

- RNA extracted (right cranial lung lobe, bronchial and mediastinal lymph node and pharyngeal tonsil) using the **Qiagen Rneasy Plus Universal mini kit**

Tissue type	Average RIN (SD)
Pharyngeal tonsil	9.5 (0.17)
Bronchial lymph node	9.07 (0.11)
Mediastinal lymph node	9.32 (0.21)
Healthy lung	8.83 (0.16)
Lesioned lung	9.14 (0.3)

- Library preparation and RNA-sequencing (**100-150bp, NovaSeq 6000**) were performed at the University of Missouri's DNA core facility, Missouri, US.

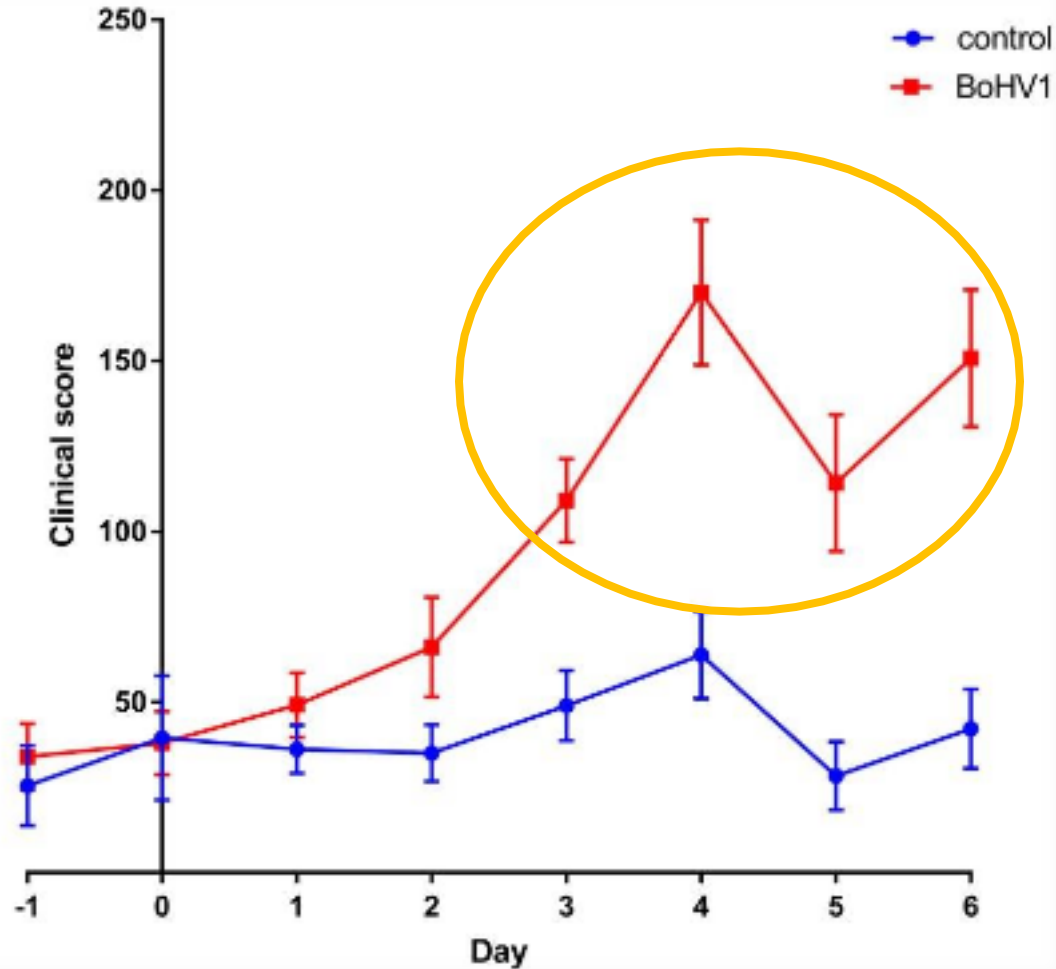
- **Pathway and gene ontologies examined using DAVID**



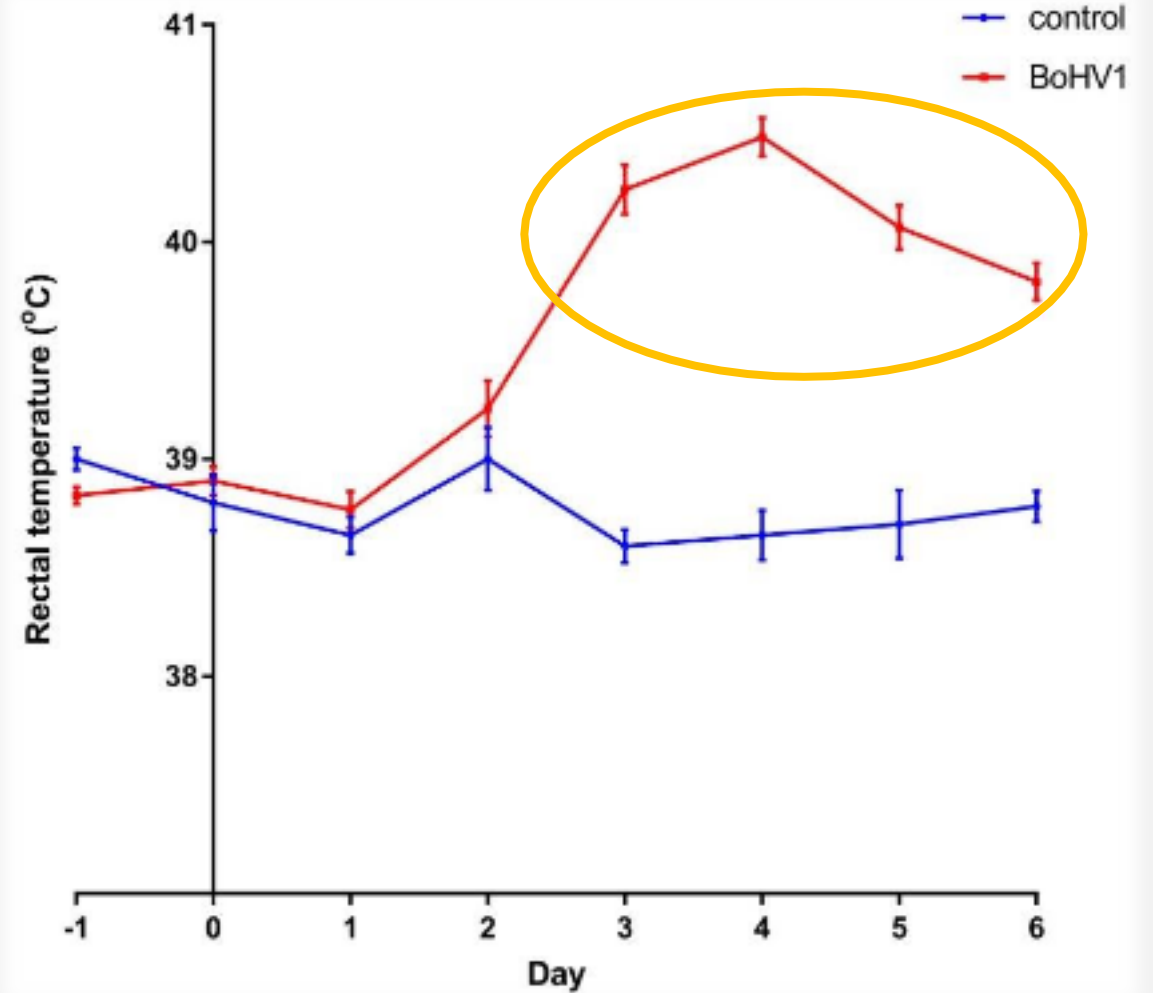
Bioinformatics pipeline

Results: Clinical scores

Clinical scores differed between BoHV-1 challenged and control calves with a **treatment x day** interaction ($P < 0.0001$)



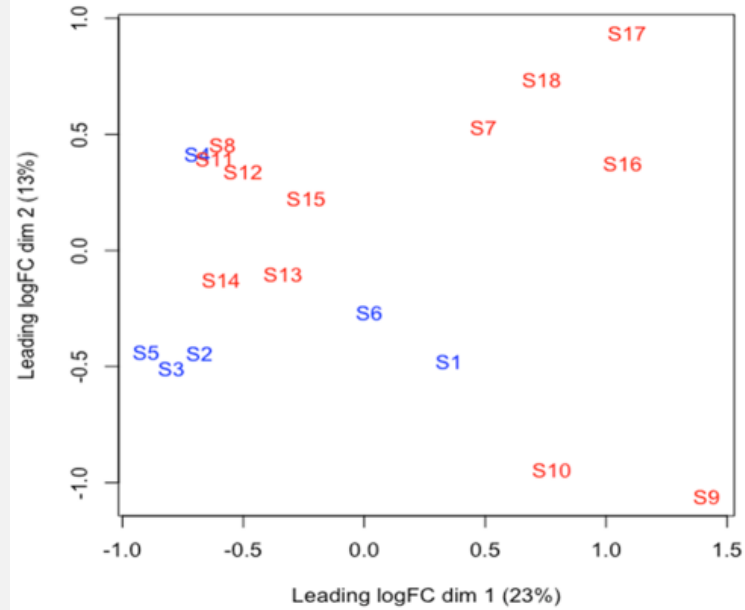
Higher rectal temperatures on days 3, 4, 5 & 6, in challenge calves relative to control calves ($P < 0.01$)



Results: Gene expression analysis

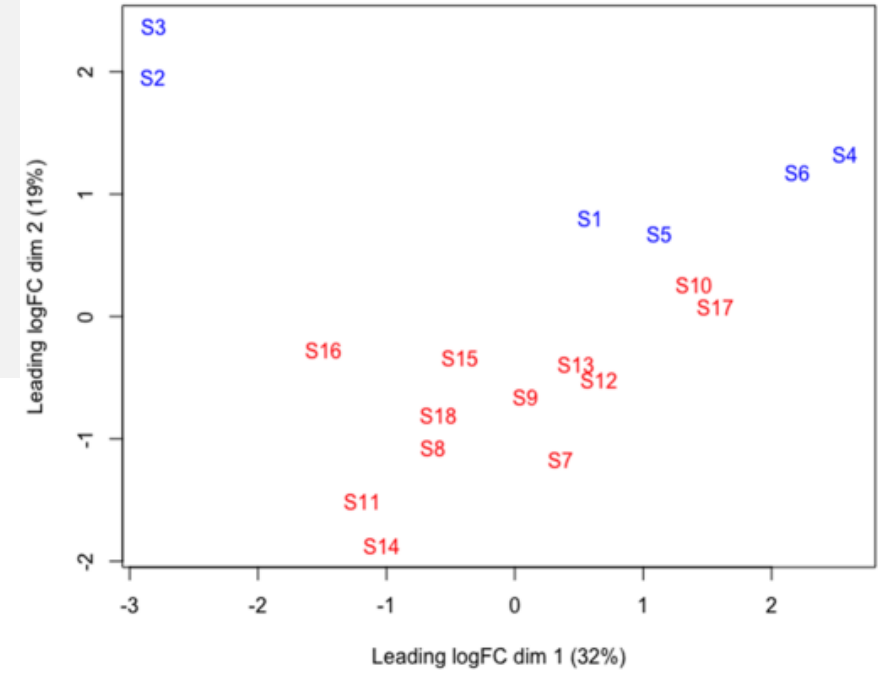
Mediastinal lymph node

81 DEGs (P < 0.05, FDR < 0.1, FC >2)



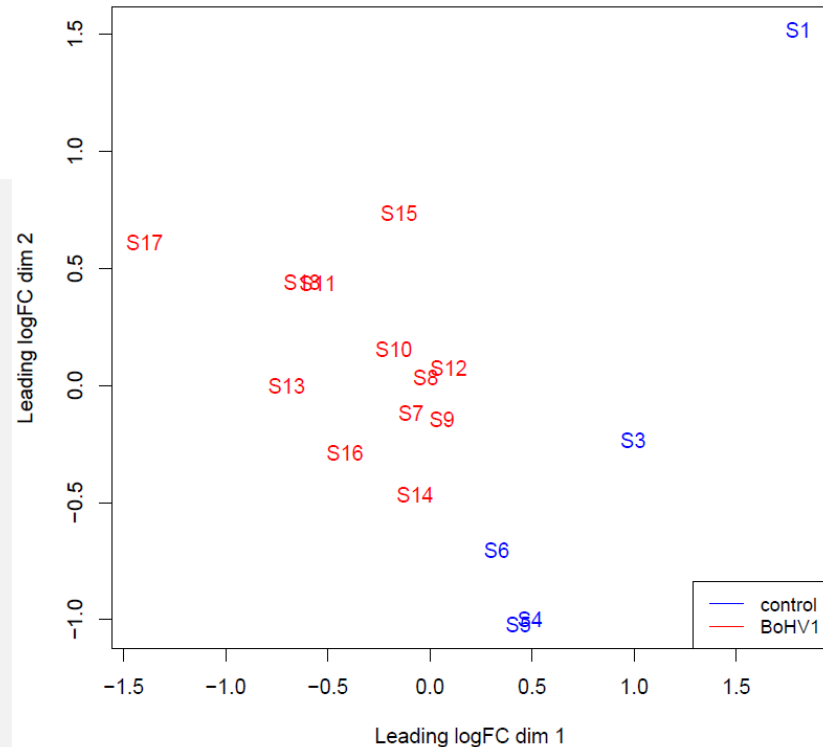
Pharyngeal tonsil

1833 DEGs (P < 0.05, FDR < 0.1, FC >2)



Bronchial lymph node

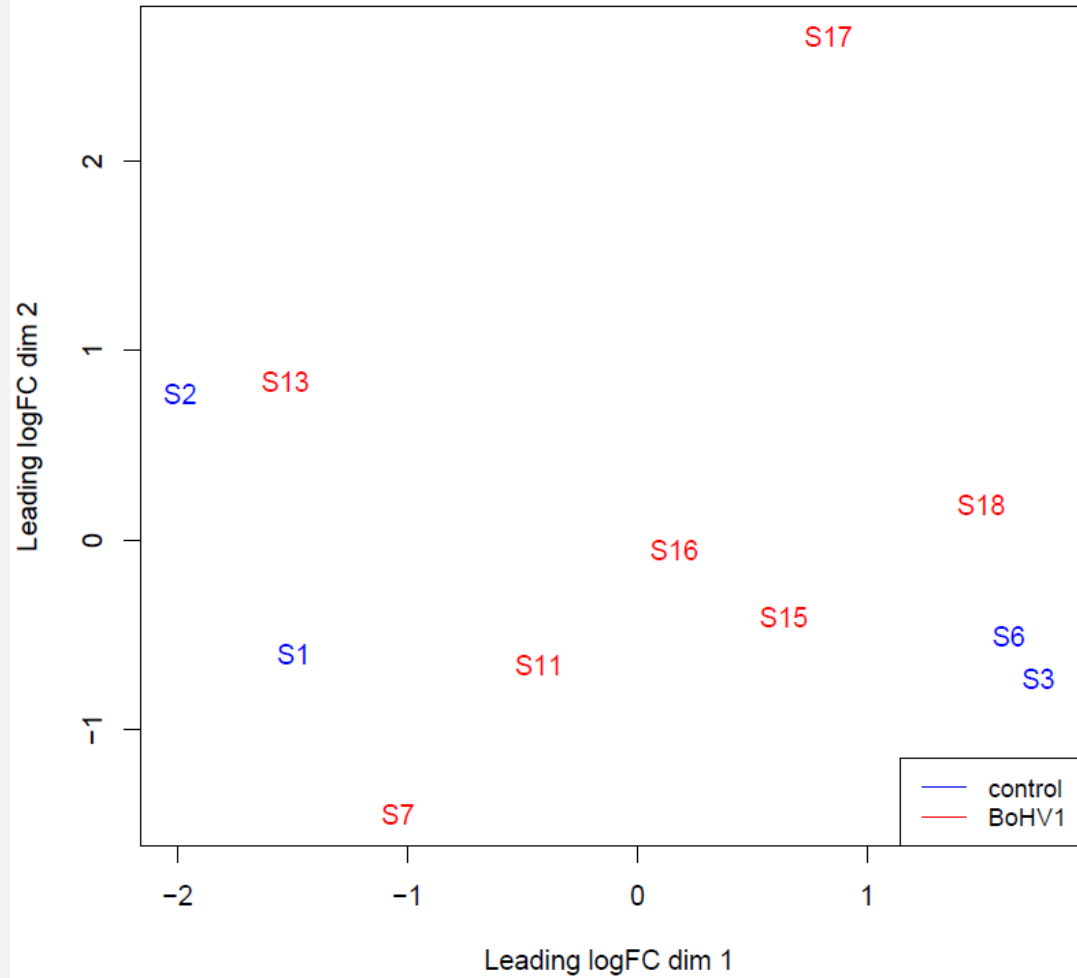
337 DEGs (P < 0.05, FDR < 0.1, FC >2)



Results: Gene expression analysis

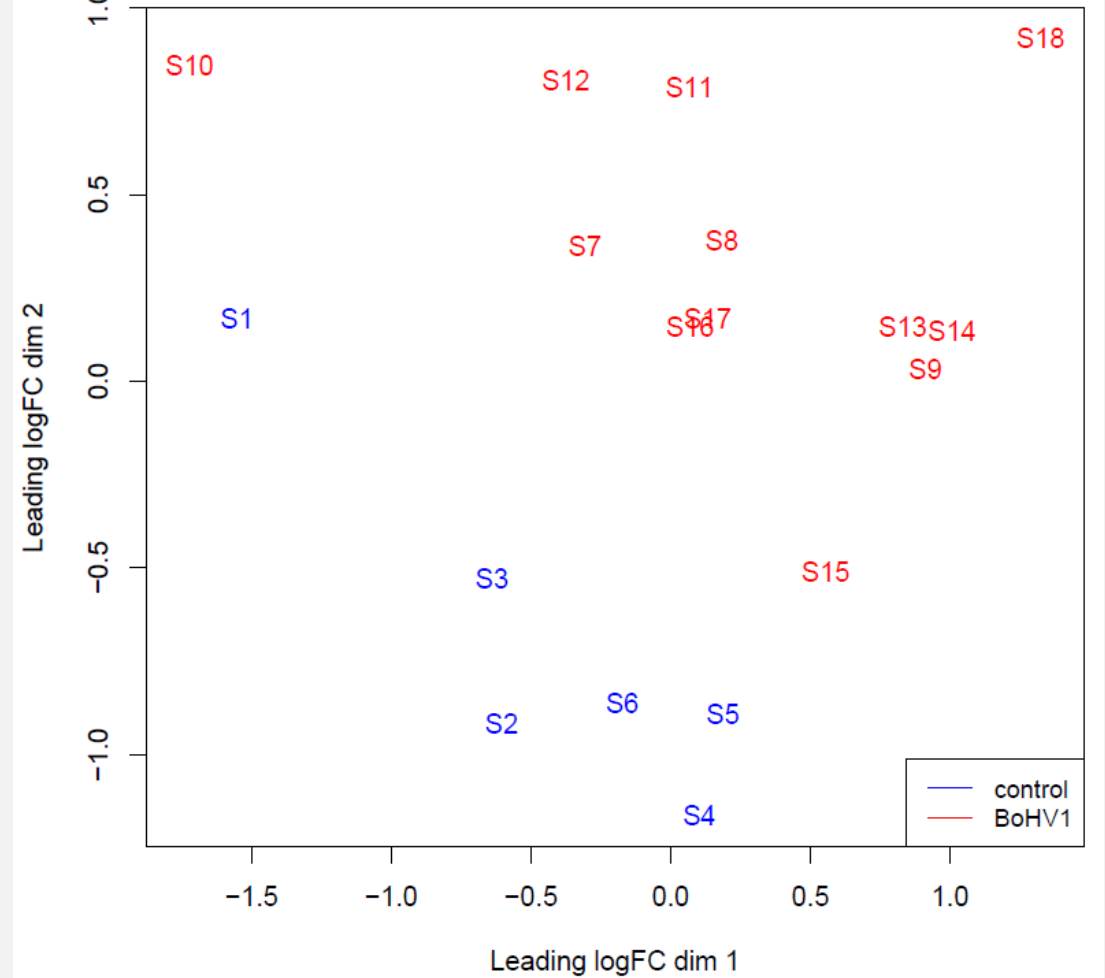
Lesioned right cranial lung lobe

67 DEGs (P < 0.05, FDR < 0.1, FC > 2)



Healthy right cranial lung lobe

334 DEGs (P < 0.05, FDR < 0.1, FC > 2)



Results: Gene expression analysis

<u>MLN</u>	Fold change	Function
<i>PRSS2</i>	2354.306822	Inflammation
<i>CATHL2</i>	23.80360266	Anti-microbial activity
<i>CATHL3</i>	21.42144889	Anti-microbial activity
<i>FREMI</i>	-2.687261732	Extracellular matrix protein
<i>PTPRQ</i>	-2.691068212	Cardiac muscle development
<i>EPHA1</i>	-5.845166497	Extracellular matrix protein

<u>BLN</u>	Fold change	Function
<i>PRSS2</i>	1841.67522	Inflammation
<i>ENSBTAG00000050398</i>	176.238858	Extracellular matrix
<i>ENSBTAG00000019018</i>	39.667145	Immune system process
<i>ACTG2</i>	-7.4266621	Cytoskeleton regulation
<i>EPHA1</i>	-8.7739654	Extracellular matrix
<i>SFRP4</i>	-11.944142	Cell signalling

Results: Gene expression analysis

<u>LL</u>	Fold change	Function
<i>PRSS2</i>	1297.941724	Inflammation
<i>ENSBTAG00000019018</i>	72.94089826	Immune system process
<i>ENSBTAG00000025258</i>	26.69654039	Extracellular region/enzyme activity

<i>NOX5</i>	-5.207426667	Calcium ion binding
<i>ENSBTAG00000047764</i>	-7.103813693	Transmembrane transport
<i>GSTA3</i>	-14.29748272	Xenobiotic metabolic process

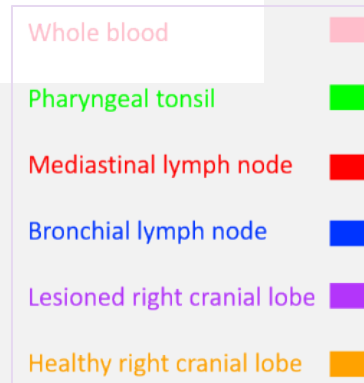
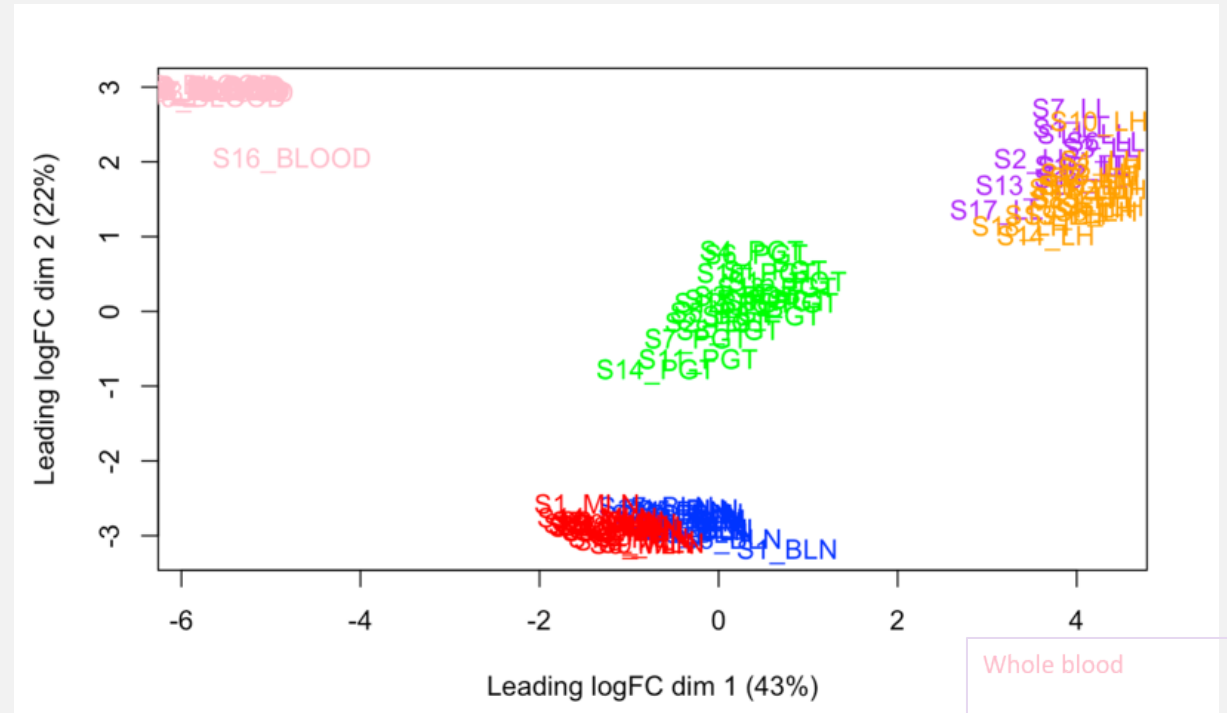
<u>LH</u>	Fold change	Function
<i>PRSS2</i>	895.7028391	Inflammation
<i>ENSBTAG00000019018</i>	46.6078165	Immune system process
<i>ENSBTAG00000025258</i>	42.03871851	Extracellular region/enzyme activity

<i>SSUH2</i>	-6.739052127	Heat shock response
<i>HBB</i>	-6.932044664	Oxygen transport from the lung
<i>PRG4</i>	-8.488772336	Immune response

Results: Gene expression analysis

<i>PGT</i>	Fold change	Function
<i>PRSS2</i>	2842.714714	Inflammation
<i>CLDN2</i>	417.9448511	Tight junction formation – mucosal immunity
<i>ENSBTAG00000047816</i>	261.8928369	Innate immunity
<i>MYL1</i>	-1419.60888	Muscle function
<i>XIRP2</i>	-2181.409584	Cell-cell junction organization
<i>MYH2</i>	-3577.581173	Skeletal muscle contraction

- Genes both uniquely DE and commonly DE in all tissues
- Tissue expression profiles cluster based on tissue type



Results: Pathway and gene ontology analysis

Pathway	PGT	MLN	BLN	HL	LL	BLOOD
Influenza A	Y	Y	Y	Y	Y	Y
Measles	Y	Y	Y	Y	Y	Y
Hepatitis C	Y	Y	Y	Y	Y	Y
Coronavirus Disease	Y	Y	Y	Y	Y	Y
NOD-like receptor signalling	Y	Y	-	Y	Y	Y
Herpes simplex infection	-	Y	-	Y	Y	-
Staphylococcus aureus infection	Y	Y	-	-	-	Y
Cytokine-cytokine receptor interaction	Y	-	-	-	-	Y
Cytosolic DNA sensing pathway	Y	-	-	Y	Y	-
RIG-I-like receptor signalling pathway	Y	-	-	-	Y	-
ECM-receptor interaction	Y	-	Y	-	-	-
Viral life-cycle HIV-1	-	-	Y	Y	Y	-
Salivary secretion	Y	Y	-	-	-	-

Y = enriched

*PGT = Pharyngeal tonsil
 MLN = mediastinal lymph node
 BLN = Bronchial lymph node
 HL = Healthy lung
 LL = Lesioned lung*

Conclusions

- First study to generate RNA-Seq data from key tissues in **BoHV-1 infected dairy calves**
- Key genes and pathways identified provide further insight into the **host molecular immune response to BoHV-1**
- Some DEGs may harbour variants associated with **BRD resistance – targets for future work**



Acknowledgements



Prof Sinéad Waters



Dr Bernadette Earley



Dr Derek Morris



OLLSCOIL NA GAILLIMHÉ
UNIVERSITY OF GALWAY



University of Missouri



Dr Dayle Johnston



Dr Matthew McCabe

