Application of next generation sequencing for the elucidation of genes and pathways involved in the host response to bovine respiratory syncytial virus

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Background

Bovine respiratory disease (BRD) is multifactorial, involving infectious agents (e.g. bovine respiratory syncytial virus (BRSV), host and environmental factors, and their interactions. Studies have shown that immunisation and antimicrobial therapies have not significantly reduced the prevalence or severity of BRD. This is largely due to the lack of comprehensive information concerning the biological mechanisms controlling the host response and the underlying genetic basis of host resistance to BRD.

In a controlled challenge study in dairy calves, the influence of the host response to BRSV was examined. The transcriptome of bronchial lymph nodes elucidated the molecular mechanisms comprising the host immune response to BRSV.

Objective: To identify genes and pathways involved in the host response to bovine respiratory syncytial virus.

Materials and Methods

- Holstein-Friesian calves were either challenged with BRSV (n=12) or mock challenged with PBS (n=6) (housed in a separate location) (Fig. 1).

Fig 1. Inoculation of a calf with $10^{4.7}$ $TCID_{50}$ BRSV virus

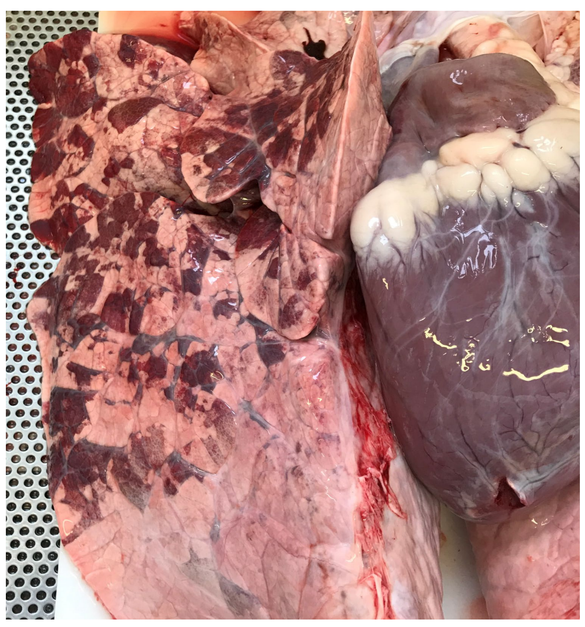

Fig 2. Lesioned lung

- Clinical assessments were performed and blood and nasal swabs were collected daily.
- Calves were euthanised on day 7 post challenge and lung pathology was assessed (Fig 2).
- Bronchial lymph nodes were collected and aliquots were frozen at -80^{o}C.
- RNA was extracted from frozen bronchial lymph nodes and sent for RNA-Seq library preparation and sequencing (University of Missouri).
- Sequenced reads were adapter trimmed, quality assessed using FastQC, aligned to the genome using STAR.
- Differential gene expression analysis was performed using EdgeR.
- Pathway and gene ontology analysis was carried out using Ingenuity pathway Analysis (IPA) and g:Profiler.

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Results

- Calves showed a mild clinical response to viral infection with BRSV.
- Lung lesions were present in one control calf and nine of the BRSV challenged calves.
- There was a clear separation between challenged and control calves based on log_2 fold gene expression changes (Fig. 3).
- There were 934 differentially expressed genes (DEG) ($p<0.05$, FDR<0.1, fold change >2) between the BRSV challenged and control calves.

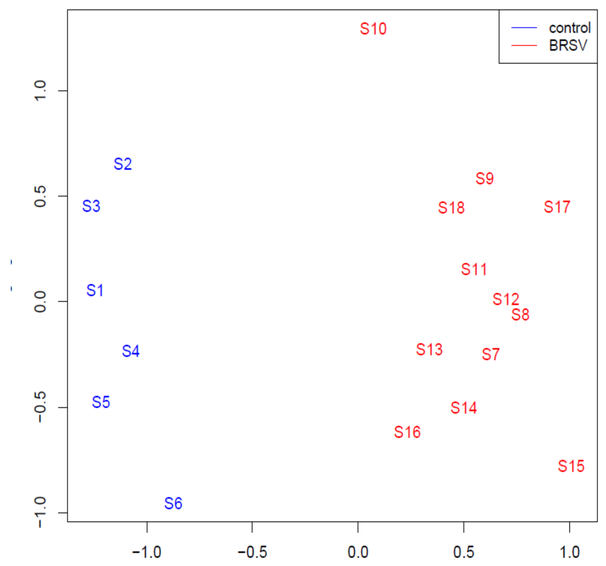

Fig. 3. MDS plot

- Influenza A was an over-represented KEGG pathway among the DEG (Fig. 4).

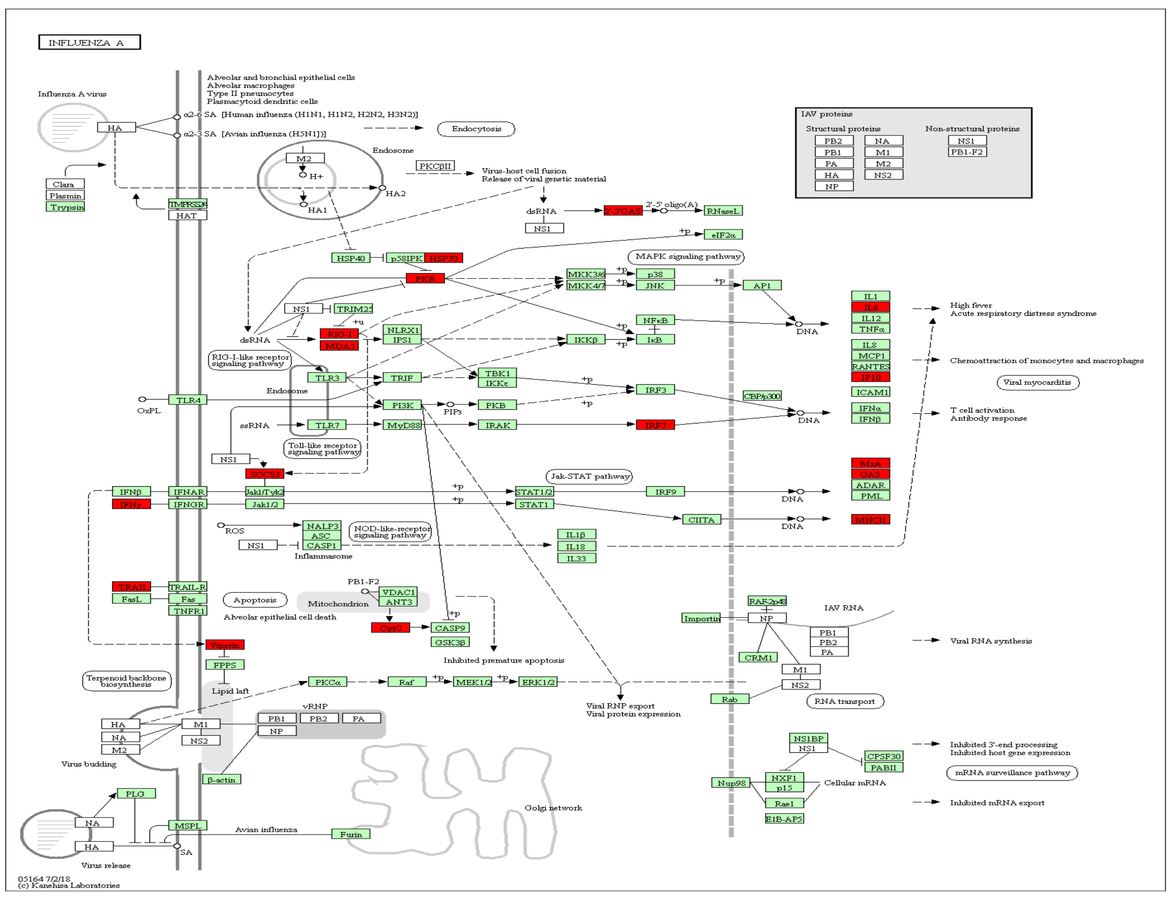

Fig. 4. Genes upregulated (red) in BRSV challenged calves in KEGG: Influenza A pathway (https://www.genome.jp/kegg/tool/map_pathway2.html)

- Over-represented “biological process” gene ontology terms among DEGs between the BRSV challenged and control calves were associated with immune responses.
- Interferon-signaling was the most over-represented “IPA canonical pathway”.

Conclusions

- The majority of the observed alterations in gene expression due to the BRSV challenge were associated with the immune response, particularly the Th1-mediated response.
- As BRSV is closely related to human respiratory syncytial virus (HRSV), and induces similar pathologies, the gene expression changes induced by BRSV infection observed in the present study may also provide an insight into the human transcriptional response to HRSV.