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154. Host variation in susceptibility to lumpy skin disease: gene expression analyses of experimentally infected cows

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Abstract

Lumpy Skin Disease (LSD) is becoming a global threat to cattle and related species. It requires annual vaccination to prevent further outbreaks. There is host variation in the response to infection both in field studies and under carefully controlled artificial infections. Here, we study the gene expression response in whole blood at four time points before and after experimental infection with LSD virus in five cows with divergent disease outcomes. Differentially expressed genes (FDR<0.05) were identified for comparisons between LSD symptomatic and asymptomatic animals at all time points as well as between time points for symptomatic animals. Gene set enrichment showed a role of oxidative phosphorylation as well as a range of disease pathways. While preliminary, these results are the first ever study of host transcriptomes related to LSD.

Introduction

Lumpy skin disease (LSD) is endemic in Africa and an immediate and serious threat to cattle in Europe and eastern and central Asia. The disease is caused by the capripoxvirus Lumpy Skin Disease Virus (LSDV). The disease has spread steadily north over the past 10 years from Africa through the Middle East and the eastern Mediterranean region before entering Europe, the Balkans and Caucasus in 2014-16 (EFSA *et al.*, 2020). Several Balkan countries currently vaccinate against LSD but this is costly (reviewed by Tuppurainen *et al.*, 2021)). There is clear host variation in response to LSD infection: in field studies about 50% of the animals develop no clinical signs when a herd is infected with LSDV (EFSA *et al.*, 2020). This is confirmed by experimental challenges where animals are infected by direct injection of LSDV into the vena jugularis and the neck (Haegeman *et al.*, 2021). Here we show a first attempt to explore mechanisms behind divergent disease outcomes by studying gene expression after experimental LSDV infection in whole blood.

Materials & methods

Five approximately 6-month-old male Holstein bulls which were tested free of BVD and IBR were experimentally infected at Sciensano with LSDV via injection in the vena jugularis and the neck with a LSDV strain derived from Israel (Haegeman *et al.*, 2021). Blood samples were taken five days prior to infection, at day 1 post infection and subsequently at least every other day until day 15. Three animals were symptomatic for LSD while the other two were asymptomatic. Here we used whole blood stored in Tempus™ Blood RNA Tubes (Applied Biosystems™) from days -5, 3, 7, and 15. RNA was isolated using the Tempus™ Spin RNA Isolation Reagent Kit according to the manufacturer's instructions. RNA integrity was validated using high sensitivity RNA Screentape reagents (Agilent) yielding integrity scores (RQN)≥9.6 for all samples.

RNA Sequencing. RNA sequencing was performed at the Neuromics Support Facility of VIB University of Antwerp, Center for Molecular Neurology. RNA sequencing followed a QuantSeq approach where sequencing libraries were constructed near the 3′ end of polyadenylated RNAs (Moll *et al.*, 2014). This results in only a single fragment per transcript thus simplifying the quantification of gene expression. Libraries were sequenced with Illumina NextSeq500 using 150 HO sequencing kit.

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RNA analysis. Bioinformatics analyses were performed by the SLU Bioinformatics Infrastructure (SLUBI). Quality control of sequencing data was performed using FastQC v0.11.8. Quality trimming and adapter removal was performed using bbduk.sh script available in BBMAP suite v38.94. After data pre-processing, the filtered data was input to STAR aligner v2.7.9a (Dobin *et al.*, 2013) for read mapping to *Bos taurus* reference genome. Top level assembly of the genome Bos_taurus.ARS-UCD1.2.105 and annotation in annotation v105 in GTF format was downloaded from ENSEMBL database. Differential Expression analysis was performed in R version 4.1.2 using DESeq2 v1.32.0 package (Love *et al.*, 2014). Comparisons were made between symptomatic and asymptomatic animals at each time points as well as within symptomatic animals between time points. Gene Set Enrichment Analyses were done for both gene ontology terms (GO) and KEGG pathways.

Results

All 20 libraries were sequenced successfully with an alignment rate of 90-93% of reads uniquely mapped to the reference genome. One sample of a symptomatic animal at day 3 was shown to be an outlier when doing a PCA plot of all the results. This sample also had the lowest RNA concentration and was hence removed from further analysis. When comparing the symptomatic animals to the asymptomatic animals at different time points the smallest number (17) of differentially expressed (false discovery rate (FDR)<0.05) genes was at 5 days prior to infection with the highest number (376) at 7 days post infection. At 3 days and 15 days post infection, we detected 36 and 40 differentially expressed genes, respectively. When comparing gene expression patterns over time within the symptomatic animals, the largest number of differentially expressed genes was between day 3 and day 7 post infection (81). The Venn diagrams in Figure 1 show limited overlap between different analyses, in particular when comparing symptomatic and asymptomatic animals. Table 1 shows the results for the functional annotation of the differentially expressed genes. While day 7 after infection showed the largest number of differentially expressed genes, day 3 after infection gives the clearest gene set enrichment related to disease response, both for GO terms and KEGG pathways. The GO terms point towards several metabolic processes while the KEGG pathways highlight a broad spectrum of disease-related pathways. While only nominally significant, the GO terms for day 7 post infection included terms like coagulation, platelet activation, wound healing and hemostasis. Both GO and KEGG point towards a role of oxidative (de-)phosphorylation.

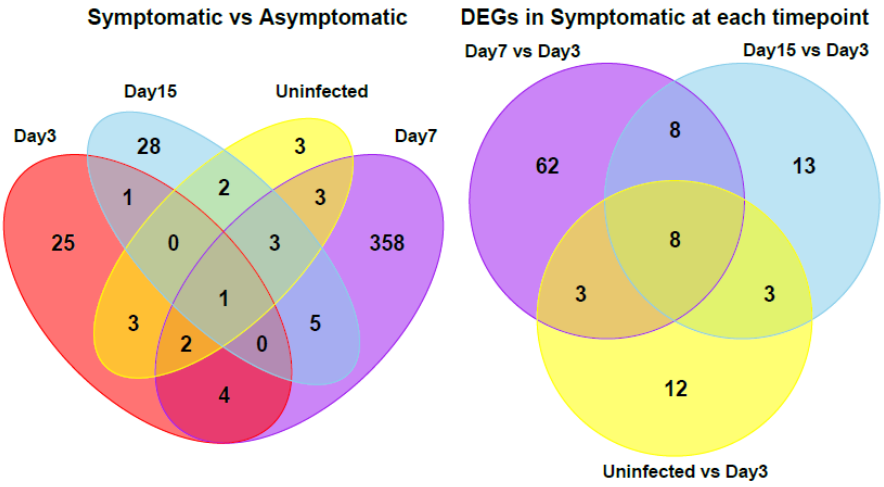

Figure 1. Differentially expressed genes between experimentally infected cows with different clinical outcomes (left), and within symptomatic animals over time (right).

Table 1. Functional annotation of differentially expressed genes.

Comparison	GO_Terms	FDR	KEGG Pathways	FDR
Symptomatic versus asymptomatic, day 3	ATP metabolic process	0.021	Oxidative phosphorylation	<0.001
	Oxidative phosphorylation	0.038	Chemical carcinogenesis – reactive oxygen species	<0.001
	Generation of precursor metabolites and energy	0.054	Prion disease	0.001
			Parkinson disease	0.001
			Diabetic cardiomyopathy	0.002
			Thermogenesis	0.004
			Amyotrophic lateral sclerosis	0.004
			Huntington disease	0.01
			Pathways of neurodegeneration – multiple diseases	0.011
			Alzheimer disease	0.021
Symptomatic versus asymptomatic, day 7	-	-	Vitamin digestion and absorption	0.08
Symptomatic versus asymptomatic, day 15	-	-	Complement and coagulation cascades	0.03
			Hematopoietic cell lineage	0.087
Symptomatic, before infection versus day 3	-	-	Systemic lupus erythematosus	0.002
			Transcriptional misregulation in cancer	0.007
			Alcoholism	0.033
			Neutrophil extracellular trap formation	0.038
Symptomatic, day 7 versus day 3	Nervous system process	0.042	-	-
	Sensory perception	0.08		
	Positive regulation of dephosphorylation	0.08		
	Regulation of organ growth	0.08		
	Behavior	0.08		
	Bone morphogenesis	0.08		

Discussion

To the best of our knowledge this is the first study of host transcriptome response to infection with LSDV. While modest in terms of sample size, we could show clear differences between animals with different disease outcomes for all time points. The finding of differentially expressed genes, while only a handful, between symptomatic and asymptomatic animals at five days before infection is intriguing. While it may simply be an artifact, it could also reflect the prior immune status of animals that has a subsequent effect on the outcome of the infection. The most significant gene prior to infection, Interleukin 1 Receptor Accessory Protein (IL1RAP) is also significant at other time points, together with other genes related to interleukin. In further analyses we also look at genet works and pathways over time using the whole data. In order to make efficient use of limited blood volumes and other resources, we opted for a QuantSeq approach in this study. While this is an efficient way for gene counting, we did not capture any splice variants or get a complete whole blood transcriptome of LSDV response. In ongoing studies, we will perform whole transcriptome sequencing in a cohort of 15 animals which will facilitate a comparison with more statistical power and a more detailed analysis of the transcriptome itself.

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