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**ABSTRACTS**



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groups revealed significant enrichment of KEGG pathway associated with adipocytokine signaling pathway associated with metabolic homeostasis and metabolic disorders. The results of this study show that perinatal exposure to PFOA can result in several transcriptomic alterations, including those associated with metabolic disorder, in the hypothalamus of mice. It remains to be determined whether these genes mediate PFOA-induced metabolic disorder disruptions.

**Key Words:** rodents, RNA-seq, gene expression, PFOA, endocrine system

**P214 Polymorphisms associated with bovine paratuberculosis: Investigation of their role in DNA-protein interactions and transcriptional regulation.** C. Beltramo, A. Dondo, K. Varello, M. Gorla, A. Di Blasio, S. Nodari, S. Colussi, P. Modesto, P. L. Acutis\*, and S. Peletto, *Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta*.

Genetic variants associated with disease resistance/susceptibility and located in gene regulatory regions may affect the binding sites for DNA-binding protein and gene expression patterns, thus influencing the host response following pathogen exposition. Previous studies led to identify 3 SNPs in putative regulatory regions of the *SLC11A1* and *CARD15* genes with association to paratuberculosis (paraTBC) in cattle. Aim of the study was to investigate the role of these mutations at the regulatory level by DNA-protein interaction analyses and transcriptome comparison between wild-type and mutated animals. Gene regions carrying the SNPs of interest were analyzed by bioinformatic tools to predict allele-dependent binding sites for transcription factors (TFBS). Putative TFBS were in vitro explored by Electrophoretic Mobility Shift Assays (EMSA). Three putative binding sites for GATA3, Sp1, and MYOD were identified in intron 10 of *CARD15* and in the promoter and intron 11 of *SLC11A1*. EMSA did not show specific gel shifts for any allele, indicating that these SNPs may eventually influence gene transcription without altering TFBS. Whole transcriptome expression analysis was performed on intestinal tissues of wild-type and mutated cattle by RNA-Seq to identify differentially expressed genes. Total RNA was sequenced on HiSeq Illumina system and data were compared by PCA and Cluster analysis. Differential regulation of 5 genes involved in innate immune system was detected. Specifically, *ULBP3* was downregulated, while *S100A8*, *S100A12*, *LOC510860*, and *IFI27* were upregulated. These significant modulations were linked to the SNP in intron 11 of *SLC11A1*, with the exception of *ULBP3*, related to the mutation in the *SLC11A1* promoter. In previous studies, *ULBP3*, *S100A8*, and *S100A12* resulted differentially expressed in cattle affected by paraTBC, suggesting a possible implication in the pathogen response. Further investigations are necessary to elucidate the functional role of these SNPs and to understand the gene network involved in the interactions between non-coding SNPs and other genome regions.

**Key Words:** paratuberculosis, SNP, EMSA, RNA-Seq

**P215 Immune-related microRNA absorption in newborn calves.** H. T. Do\*<sup>1,2</sup>, J. L. Williams<sup>1</sup>, T. Chen<sup>1</sup>, K. Petrovski<sup>1</sup>, and C. D. K. Bottema<sup>1</sup>, <sup>1</sup>School of Animal & Veterinary Sciences, Davies Research Centre, University of Adelaide, Roseworthy, Australia, <sup>2</sup>Vietnam National University of Agriculture, Hanoi, Vietnam.

In addition to immunoglobulin G (IgG), bovine colostrum contains many immune-related factors that are absorbed by the neonate, including microRNAs (miRNAs) which may stimulate immune development. If essential colostrum immune-related miRNAs can be identified, the information could be useful in breeding programs to improve calf immunity. Herein, the levels of colostrum immune-related miRNAs were compared between calves that received colostrum from different sources to determine if these miRNAs are absorbed by the calves. Thirty-eight bull calves were randomly divided into groups and fed equal amounts of colostrum from 2 sources (dam colostrum or colostrum pooled from 1 to 7 d postpartum cows). Dam colostrum was collected after birth (d 0), d 1 and d 2 postpartum and calf blood was collected

at d 0 (before feeding), d 1 and d 7 postpartum. IgG concentration was measured by refractometry and ELISA. Five immune-related miRNAs were quantified by RT-qPCR with the addition of Cel-miR-39 as the internal standard. The differences between groups were analyzed using one-way ANOVA. Only miR-150 was moderately correlated with the IgG concentration in the dam colostrum at d 0. The concentration of miR-142-5p, miR-150 and miR-181a in the dam colostrum was highest at d 0 and decreased dramatically by d 1. The concentration of miR-155 increased over time though, while the level of miR-223 did not change. In the pooled colostrum, miR-223 was the only miRNA found at high levels and was the only miRNA at high levels in the calf blood at d 0. The concentration of all the miRNAs increased by d 1 in the calf blood, but returned to the d 0 levels by d 7. Interestingly, there was no significant difference between the calves fed the dam colostrum or pooled colostrum for any of the miRNAs at d 1 or 7 despite the absence of miRNA in the pooled colostrum. This suggests that the calves do not just absorb the miRNA from the colostrum but synthesize some or all of the miRNA themselves. Therefore, these miRNAs may not be good biomarkers of colostrum quality for breeding programs. However, the importance of the miRNAs for calf immune development and health is being further investigated.

**Key Words:** miRNA, bovine, colostrum

**P216 Combined transcriptomic analysis of ileocecal valve and peripheral blood in Holstein dairy cattle at different stages of *Mycobacterium avium* ssp. *paratuberculosis* (Map) infection revealed CXCL8/IL8 as a common effector molecule.** M. Alonso-Hernández\*, M. Canive<sup>1</sup>, C. Blanco-Vázquez<sup>2</sup>, R. Torremocha<sup>3</sup>, B. Soriano<sup>4</sup>, A. Balseiro<sup>5</sup>, J. Amado<sup>5</sup>, R. Ramos<sup>3</sup>, C. Llorens<sup>4</sup>, and R. Casais<sup>2</sup>, <sup>1</sup>NEIKER-Instituto Vasco de Investigación y Desarrollo Agrario, Derio, Bizkaia, Spain, <sup>2</sup>SERIDA, Servicio Regional de Investigación y Desarrollo Agroalimentario, Deva, Asturias, Spain, <sup>3</sup>Science Park of Madrid, Genomic Unit, Madrid, Spain, <sup>4</sup>Biotechvana, Paterna, Valencia, Spain, <sup>5</sup>LSAPA, Animal Health Laboratory of the Principality of Asturias, Gijón, Asturias, Spain.

Paratuberculosis (PTB) caused by infection with *Mycobacterium avium* ssp. *paratuberculosis* (MAP) is a major endemic disease affecting global cattle production. Since the blood transcriptome is widely used as a source of biomarkers, we analyzed whether it recapitulates at least in part the transcriptome of the ileocecal valve (ICV), the primary site of MAP colonization. Total RNA was prepared from peripheral blood (PB) and ICV, and RNA-Seq was used to compare gene expression between animals with focal or diffuse histopathological lesions versus control animals. As expected, the number of differentially expressed (DE) genes was larger in ICV than in PB samples and in animals with diffuse versus focal lesions. Our results demonstrated both shared, and PB or ICV-specific regulation of gene expression in response to MAP infection. Among the identified DE transcripts in PB and ICV, there were 5 common transcripts irrespective of the type of lesion including the C-X-C motif chemokine ligand 8 (CXCL8), apolipoprotein L domain containing 1 (APOLD1), interferon  $\alpha$ -inducible protein 27 (IFI27), KIAA1324-like and ArfGAP with RhoGAP domain ankyrin repeat (ARAP2). Two putative biomarkers were DE exclusively in PB and ICV of animals with focal lesions, the major histocompatibility complex class II (BOLA-DOB) and ENSBTAG0000038080. Thirty 2 genes were DE only in animals with diffuse lesions; 17 appeared up-regulated and 11 downregulated in both blood and ICV of cows with diffuse lesions. Five biological processes (BP) were enriched in ICV of cows with focal lesions; killing of other organisms (GO0031640), defense response (GO0006952, GO0050832), immune response (GO006955) and regulation of neutrophil chemotaxis (GO0090023). Two BP, GO0006952 and GO006955, were also enriched in PB and ICV of cows with diffuse lesions. Some of the identified DE genes GO and metabolic pathways will be studied further to aid in better diagnostic tools, vaccines and/or immunotherapeutics.

**Key Words:** cattle and related species, immunology, RNA-Seq, infectious disease, animal health