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MicroRNAs modulate immunological and inflammatory responses in Holstein cattle naturally infected with *Mycobacterium avium* subsp. *paratuberculosis*

Gerard Badia-Bringué[1,2], María Canive[1], Cristina Blanco-Vázquez[3], Rosana Torremocha[4], Susana Ovalle[4], Ricardo Ramos-Ruiz[4], Rosa Casais[3] & Marta Alonso-Hearn[1]✉

MicroRNAs (miRNAs) regulate the post-transcriptional expression of genes by binding to their target mRNAs. In this study, whole miRNA sequencing was used to compare the expression of miRNAs in ileocecal valve (ICV) and peripheral blood (PB) samples of cows with focal or diffuse paratuberculosis (PTB)-associated lesions in gut tissues versus (vs) control cows without lesions. Among the eight miRNAs differentially expressed in the PB samples from cows with diffuse lesions vs controls, three (miR-19a, miR-144, miR32) were also down-regulated in cows with diffuse vs focal lesions. In the ICV samples, we identified a total of 4, 5, and 18 miRNAs differentially expressed in cows with focal lesions vs controls, diffuse lesions vs controls, and diffuse vs focal lesions, respectively. The differential expression of five microRNAs (miR-19a, miR-144, miR-2425-3p, miR-139, miR-101) was confirmed by RT-qPCR. Next, mRNA target prediction was performed for each differentially expressed miRNA. A functional analysis using the predicted gene targets revealed a significant enrichment of the RNA polymerase and MAPK signaling pathways in the comparison of cows with focal vs no lesions and with diffuse vs focal lesions, respectively. The identified miRNAs could be used for the development of novel diagnostic and therapeutical tools for PTB control.

Bovine paratuberculosis (PTB), often known as Johne's disease, is a chronic granulomatous enteritis that affects ruminants worldwide. This disease is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and is mainly characterized by a decrease in milk production and weight loss. Global economic losses from PTB cases are estimated to exceed US1.5 billion dollars per year[1], with US198.42 million dollars in the United States and US364.31 million dollars in Europe[2] primarily due to decreased milk production, increased management costs, and premature culling. Indeed, bovine PTB is considered endemic in the United States and Europe with more than 50% of herds being ELISA positive for anti-MAP antibodies[3]. In addition, scientific evidence links MAP to human inflammatory bowel disease (IBD), autoimmune diseases, as well as colorectal cancer and Alzheimer´s disease[4–6]. This potential threat to human health has stimulated interest in this disease and in the development of more sensitive diagnostic and control methods.

Transmission of MAP usually occurs early in the life of the animal by ingestion of MAP-contaminated feces or milk. MAP crosses the intestinal barrier via interaction with M and epithelial cells[7–9] and can survive within subepithelial macrophages by inhibiting apoptosis and phagosome acidification, as well as by preventing antigens´ presentation to the immune system[10]. There are different stages of MAP infection (silent, subclinical, clinical, and advanced clinical) each with distinct immunological and pathological characteristics[11,12]. Th1

[1]Department of Animal Health, NEIKER-Basque Research and Technology Alliance (BRTA), Derio, Spain. [2]Doctoral Program in Molecular Biology and Biomedicine, Universidad del País Vasco/Euskal Herriko Unibertsitatea (UPV/EHU), Leioa, Bizkaia, Spain. [3]Center of Animal Biotechnology, SERIDA-Regional Service of Agri-Food Research and Development, Deva, Spain. [4]Genomic Unit, Scientific Park of Madrid, Campus de Cantoblanco, Madrid, Spain. ✉email: malonso@neiker.eus