

Next-generation sequencing of bovine milk-derived exosomal microRNA to determine transcriptome expression for efficient recovery from mastitis infection

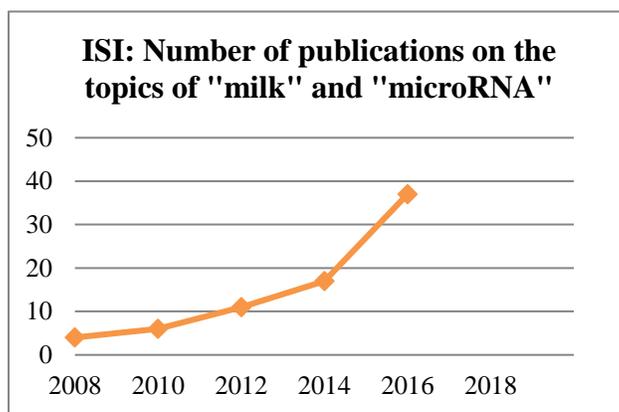
Andrea Zukowski, Jamie Kraft, Emanuela Ferretti¹, Illimar Altosaar

Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ontario, Canada; ¹Dept of Pediatrics, CHEO/The Ottawa Hospital, Division of Neonatology, Ottawa, Ontario.

Exosomes are extracellular microvesicles (30-100 nm diam.) released by various cell types. Milk-derived exosomes contain degradation-resistant microRNAs (miRNAs), long non-coding RNA, mRNA, and proteins. The miRNAs are thought to play an important role in intercellular communication, especially during an immune system response (Floris et al 2016). Human, bovine, porcine, and murine milk miRNA transcriptomes have been sequenced, and organism-specific miRNA target sites are being postulated. Currently, interest in miRNAs is growing in the dairy industry, as miRNAs may be useful biomarkers for the onset of mastitis (Altosaar 2015).

Mastitis, an inflammatory disease of the mammary gland, presents one of the greatest economic challenges to the dairy industry, due to decreased milk production, contamination of bulk tank milk, and culled animals. Most mastitis infections are the result of the intramammary presence of environmental pathogens, including prominent infectious agents like *Staphylococcus aureus* and *Escherichia coli*. Depending on the bacterial species, the resulting symptoms of infection in dairy cows can vary from acute severe clinical infection to chronic subclinical disease. Milk somatic cell count is the standard for detection of subclinical infection, but is not diagnostic, pre-emptive, and is prone to false-positives. Biomarkers using miRNA for susceptibility and response to treatment may provide the herdsman with a more significant means of managing mastitis (Taga 2012).

Prior work suggests that milk exosomal miRNAs differ between healthy cows and those that have been inoculated with pathogenic bacteria. In order to compare the exosomal miRNA profiles of healthy and mastitis-infected cows, Sun et al. (2015) collected milk from four Holstein cows prior to and post-infection with *Staphylococcus aureus*. However, post-mastitis bovine milk exosomal miRNA expression has yet to be elucidated. With next-generation sequencing, within-host miRNA expression can be monitored over the course of antibiotic treatment for the discovery of novel recovery-mediating miRNAs or time-dependent changes in the levels of these miRNAs in exosomes. The altered miRNA expression in response to infection may not be unique to the infected quarter, resulting in inter-quarter communication and affecting all quarters of the cow. Thus, mastitis diagnosis can additionally be verified by the cow's non-infected quarters and milk. Inter-herd miRNA expression variability can hold important differences between cows with fast responses to treatment that can be taken advantage of to treat those with sub-optimal recovery. We modelled flux of miRNA profiles in milk collected from mastitis-infected cows at various time-points during/after antibiotic treatment. MiRNA expression was analyzed for time-dependent, inter-quarter, and intra-herd differences to identify biomarkers of effective and efficient mastitis recovery.



Floris I, Kraft JD, Altosaar I. Roles of microRNA across prenatal and postnatal periods. *Int. J. Mol. Sci.* 2016; 17:1994; doi:10.3390/ijms17121994

Altosaar I, Siggers J. Micromolecules to Nanoparticles – Human Milk: More Than Nutrition. Proceedings from the 3rd Annual International Conference on Human Milk Science and Innovation, September 9-11, 2015, Pasadena, California, USA. J. Bruce German, William Rhine (Eds). pp 6-8.

Taga I, Lan CQ, Altosaar I. Plant essential oils and mastitis disease: Their potential inhibitory effects on pro-inflammatory cytokine production in response to bacteria related inflammation. *Natural Product Communications.* 2012, 7(5): 675-682.

Sun J, Aswath K, Schroeder SG, Lippolis JD, Reinhardt TA, Sonstegard TS. MicroRNA expression profiles of bovine milk exosomes in response to *Staphylococcus aureus* infection. *BMC Genomics.* 2015;16(1):806.