1. Introduction

The milk somatic cell count (SCC) is a routine parameter used for monitoring udder health and milk quality in cows, and it is currently implemented also in ewes and goats. Nevertheless, the question of which thresholds to use in small ruminants is still strongly debated. In fact, adding to intramammary infections, a number of other factors impact on small ruminant SCCs more significantly than in cows. Therefore, for an earlier and sensitive monitoring of udder health, as well as for a better definition of thresholds, SCCs would benefit from the integration with additional markers. Several biomarkers were suggested as potential indicators of IMI, such as haptoglobin, serum amyloid A, lactoferrin, and others. We propose the presence of cathelicidins as a reliable and pathogen-independent signal of an inflammatory process of the mammary gland.

2. Biomarker Discovery and validation

Milk from healthy sheep, from sheep naturally infected by M. agalactiae, and from sheep experimentally infected with S. uberis was subjected to 2D-DIGE-MS followed by ESI-Q-TOF/MS/MS, and in parallel by SDSPAGE followed by band cutting, digestion, and LTQ-Orbitrap Velos, and then by label-free quantitation and Ingenuity Pathway Analysis for characterization of differential proteins. The cellular origin of the markers of interest was investigated and validated by immunohistochemistry (IHC) on infected mammary tissues. Among the proteins with the highest fold change, cathelicidin shows the lowest variation between intramammary infections by the two infectious agents. Thus, the antimicrobial peptide was chosen for validation in large cohorts of milk samples from different farmed animals. Cathelicidins expression was determined by means of a specific and sensitive sandwich ELISA developed in house.

3a. Cathelicidins in cow’s milk

Expression of cathelicidins was also tested on 70 single udder halves milk samples of Sarda sheep, from 3 flocks, in Sardinia, Italy. As shown in Fig.3g, the SCC in ewes has a larger overlap of ELISA negatives and positives, compared to cow’s milk samples. Specifically, the negative group in ewes has a higher median value (144,000 cells/mL) than SCC in cattle (3,000 cells/mL in quarters and 8,000 cells/mL in composites).

3b. Cathelicidins in ewe’s milk

Expression of cathelicidins was also tested on 70 single udder halves milk samples of Sarda sheep, from 3 flocks, in Sardinia, Italy. As shown in Fig.3g, the SCC in ewes has a larger overlap of ELISA negatives and positives, compared to cow’s milk samples. Specifically, the negative group in ewes has a higher median value (144,000 cells/mL) than SCC in cattle (3,000 cells/mL in quarters and 8,000 cells/mL in composites).

3c. Cathelicidins in goat milk

The presence of the biomarker in goat milk is currently under investigation. 436 milk samples from a single flock were studied for the expression of cathelicidins in udder halves. Preliminary results suggest a translational clustering of the distribution of goat milk samples between cattle and sheep. As highlighted in Fig.5, the distribution of ELISA negative goat samples is closely related to the group from ewes. Positive samples distribution, instead, has a similar trend to the cattle milk samples distribution. Nevertheless, only post-partum samples have been examined to date.

4. Conclusions

The molecules identified in the proteomic discovery study hold promise as novel mastitis markers, and open valuable perspectives for the development of diagnostic tools enabling a better monitoring of udder health and milk quality. In addition, these novel markers can support the cross-validation of SCCs for monitoring small ruminant mastitis, as well as a better evaluation of SCC levels, dynamics, and reliability in these dairy animals. Furthermore, the expanded parameters to be monitored should improve the early detection of both clinical and, especially, sub-clinical mastitis with significant economical and animal health implications.


