

Comparison of healthy and diseased free-stall barn cow milk microbiota, cytokines and antimicrobial proteins in seasonal aspect

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Objectives. Bovine mastitis is still challenging in the dairy industry. The invasion of udder by pathogens induces mastitis. Despite the experimentally induced mastitis the research on free-stall barn mastitis is limited and practically absent is the seasonal character of the mastitis inducers.

Materials and methods. Milk from 15 Holstein Friesian cows was used. Cows were divided into 3 groups: 5 healthy, 5 subclinical and 5 clinical animals. Samples were tested using immunohistochemistry for IL-2,-4,-10,-17A, TGF- β 1 and β -Def 3. The milk microbiota in the spring and autumn seasons was detected. Statistics were performed to compare protein expression over time and status.

Results. High expression of IL-2, IL-4, IL17A, TGF- β 1 was detected in healthy, subclinical and clinical mastitis cows. Expression of IL-10 and β -Def 3 in the milk of healthy cows was high with a significant decrease in infected cows. *S.uberis* and *S.aureus* prevailed in the milk without seasonal preference. Seasonal lack were rarely detected *E. coli* and *S. agalactiae*, while *Klebsiella*, *Enterobacter*, *Citrobacter spp.* were seen mainly in the spring milk. All the detected cytokines varied in spring but returned to high expression in the autumn. In milk from cattle with clinical mastitis, the most cells were IL-2-positive, followed by IL-17A cells, but IL-4 and TGF- β 1 increased more in autumn.

Conclusions. The stable expression of IL-2,-4,-17A and TGF β -1 from day 4 to day 14 in the milk of subclinical and clinical mastitis affected and in the healthy cows' milk indicates the possible insignificant role of these cytokines in mastitis. Decreased expression of IL-10 and β -Def 3 in the milk of subclinical and clinical mastitis indicates the significance of these factors for the diagnosis. The number of ILs positive cells in the milk of cows with subclinical mastitis has the greatest variation and decreases in spring, indicating the beginning of summer as an exhaustion of the immune system and autumn as the local immunity restoration time.