

Host response during experimental intramammary infection in dairy heifers with *S. chromogenes* and *S. fleurettii* - preliminary results

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Introduction

Coagulase-negative staphylococci (CNS) are the most commonly isolated bacteria in subclinical bovine mastitis cases. Conventionally, CNS are considered as one large bacterial group even though roughly a dozen different species are found in milk. Although CNS are generally regarded as minor pathogens, little information is available on the host-pathogen interaction of individual species. Previous research even demonstrates a positive association between CNS infection and milk production in heifers and multiparous cows. The aim of this study is to elucidate the immune response of dairy heifers after experimental infection with *Staphylococcus chromogenes*, a so-called udder-adapted species, and *Staphylococcus fleurettii*, a so-called environmental species.

Materials and Methods

Eight clinically healthy Holstein-Friesian heifers in mid-lactation are randomly selected for an experimental infection with 3 different bacterial isolates:

- (1) *S. chromogenes*: a strain isolated from a chronically infected cow
- (2) *S. chromogenes*: a strain that inhibits the growth of major pathogens *in vitro*
- (3) *S. fleurettii*: a strain isolated from sawdust bedding

A split-udder design is used in which three quarters of the uninfected heifers are inoculated with 1×10^6 colony-forming units (CFU) of the aforementioned bacteria and one quarter is infused with 5 ml sterile phosphate buffered saline, thereby serving as a control. During the study, the heifers are kept in a tie-stall barn and monitored for five days. The somatic cell count (SCC) and bacterial elimination rates are quantified repeatedly. Furthermore, the proportion of viable neutrophils in milk is determined using a double staining technique with annexine V-fluorescein isothiocyanate (FITC) and propidium iodide. Milk production is also measured at quarter level. Milk and blood samples are collected for further analysis of prolactin levels, several pro-inflammatory cytokines (TNF- α , IL-1 β , IL-8, GM-CSF) and serum amyloid A. The CNS isolates found in milk are stored at -80°C for later confirmation by means of tDNA-PCR and strain typing.

Preliminary results

Based on the descriptive results of 4 heifers, it appears that the chronic *S. chromogenes* strain remains in the udder for approximately 48 hours, causing the highest and longest increase of the quarter milk SCC. The other *S. chromogenes* strain and *S. fleurettii* are eliminated within 28 hours. None of the heifers showed any signs of clinical mastitis.