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# Introduction and aims

# Sovine mammary gland microbiota and immune response

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Bovine mastitis is an inflammation of the mammary gland generally due to an infection. It is responsible for considerable economic losses in dairy farms. Preventive and curative strategies that mainly rely on antibiotic therapies are not totally effective and contribute to antibiotic resistance dissemination, prompting the need for alternative or complementary strategies. We previously established a link between internal teat microbiota composition and bovine mastitis in quarters which had different histories regarding mastitis [1]. In this study, to further decipher the relationships between internal teat microbiota and immune and microbial responses, a switch from twice- to once-daily milking (ODM) was used to trigger an udder perturbation.

## Experimental design

Transition to once-daily milking used as a perturbation of mammary gland ecosystem

# Microbiota composition at D0 in relation to the response to the transition to ODM

Firmicutes

Actinobacteria





**Fig 1**. Experimental design. A switch to ODM was used in 31 initially healthy quarters of dairy cows. Immune and microbial responses including determination of teat microbiota were monitored just prior to the transition (day 0, D0), and 3 and 14 days following transition to ODM.

## Microbiota composition and response to transition to ODM



**Fig 4**. Discriminant taxa between C1 (G1-G2 quarters) and C2 (G4-G5 quarters) at day 0 for Firmicutes and Actinobacteria. Differences are represented by the color of the cluster where the taxon is more abundant.

## Temporal relationship between bovine internal teat microbiota composition and richness and the immune and microbial responses during transition to ODM



**Fig 2**. Hierarchical clustering of samples, based on the UniFrac distance using the ward linkage function, reveals separation in 2 main clusters, C1 and C2, corresponding mainly to G1-G2 and G4-G5 quarters respectively

# A higher richness at D0 in quarters that underwent transient inflammation (C2) without developing mastitis



#### Cluster C1 :

low initial richness at D0 -> low colonization resistance to pathogens. Microbiota not affected by ODM-induced perturbation (resistant microbiota) -> lack of immune system stimulation (green spot).

higher rate of pathogen entrance through the teat canal ->higher rate of mastitis cases **Cluster C2** :

higher initial richness at D0 -> higher colonization resistance. microbiota altered in response to the transition to ODM (sensitive microbiota) -> stimulation of the innate immune system (red spot) -> contributed to combatting pathogens

# **Conclusion and perspectives**

 Temporal relationship between initial internal teat microbiota composition and richness, the immune response to ODM, and mastitis development [2].
suggests a role of the bovine teat microbiota composition and richness

D0 D3 D14 D0 D3 D14

**Fig 3.** Alpha-diversity of teat cistern microbiota at day 0, day 3, and day 14 with regard to clusters, as illustrated by the distribution of several diversity indices:

in the immune response of the mammary gland during perturbations.
invites us to consider strategies that can preserve teat microbiota diversity and taxa associated to a healthy status for the design of next-generation probiotics.

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