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Cross-sectional pilot field study on *Streptococcus uberis*'s mastitis in the Tessin region, Switzerland

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Introduction

Bovine mastitis is a significant disease affecting dairy cattle worldwide, characterized by inflammation of the mammary gland due, in most cases, to bacterial infections. It leads to reduced milk production, altered milk composition, and substantial economic losses for farmers and the dairy industry (Ruegg 2017). In Switzerland alone, 129.4 million Swiss francs are lost each year due to issues related to mastitis (Heiniger et al., 2014). *Streptococcus uberis* (*S. uberis*) is one of the main mastitis's pathogens, classified as "environmental" pathogen associated with single quarter infection, but based on previous research, a contagious nature was also described (Zadoks et al., 2003).

To investigate the epidemiology and circulation of different subtypes of *S. uberis* in dairy herds, milk and body samples from cows, as well as environmental samples, were collected from 10 voluntary farms in the Canton of Tessin. The samples were analyzed for the presence of *S. uberis*, along with its antimicrobial resistance (AMR) profile and subtypes. Furthermore, the associated inflammatory response in the mammary gland was assessed by evaluating Somatic Cell Count (SCC) and Differential Somatic Cell Count (DSCC).

Material & methods

The study was conducted between April and May 2025, prior to the cows being sent to common alpine pasturing. A total of 260 cows were involved. From these, 1,048 aseptic quarter milk samples were collected. Furthermore, random bedding samples (at least

one sample per every ten cow beds) were collected from all farms. Additionally, swabs from the teats and perineal region of cows were collected in five of the herds.

Milk samples were cultured on blood agar and on a selective chromogenic medium (CHROMagar™ Streptococcus) designed for streptococci identification. For bedding samples, 10 g of material was enriched in 90 ml of Brain Heart Infusion (BHI) broth, incubated for 12 hours, and then streaked onto blood agar and CHROMagar. After 24 hours of incubation, colonies with distinct morphologies were selected for bacterial identification using MALDI-TOF analysis.

From 146 strains isolated from both milk and environmental samples, genotyping was performed using Random Amplification of Polymorphic DNA (RAPD) to compare strains from different sources. A minimum of four colonies per sample type (when available) were analyzed (Schmitt-Van de Leemput & Zadoks, 2007).

SCC and DSCC were measured using a flow cytometry-based method (FACS) as described by Widmer et al. (2022). This analysis was performed on all quarter milk samples from cows that were positive for *S. uberis* (N=67). Quarters from the same cows that were negative for *S. uberis* were used as negative controls (N=89). Furthermore, all microbiologically positive *S. uberis* milk samples were confirmed by PCR targeting the *pauA* gene (Raemy et al., 2013).

To investigate antimicrobial resistance, strains isolated from different environmental sources from two farms were compared using Minimum Inhibitory Concentration (MIC) testing with the commercial MICroSTREP plus Panel Type 6 (Beckman Coulter), which includes 23 antibiotics. Further analysis, including IR Biotyper and Whole Genome Sequencing (WGS), is planned to test more strains for phenotypic and genotypic antimicrobial resistance.

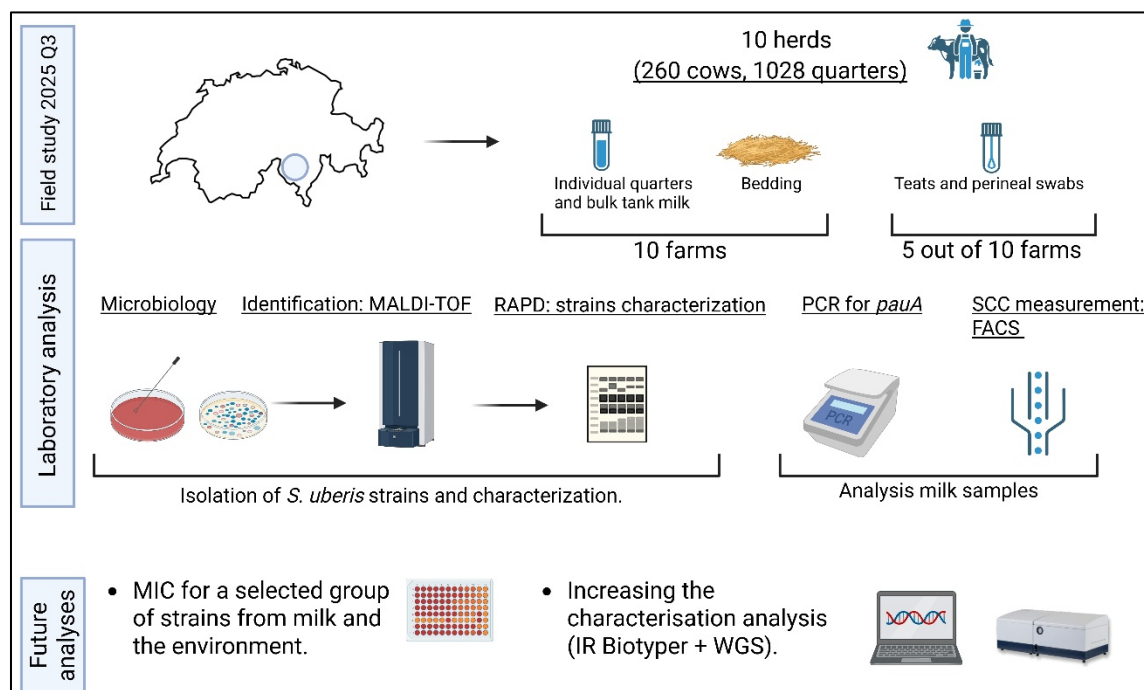


Figure 1: Graphical representation of the research project performed in the Swiss region of Tessin (Created by Biorender).

Results & Discussion

The study revealed that 8 out of the 10 farms were positive for *S. uberis*. The prevalence ranged from 5% to 25.8% at the cow level and from 1.3% to 13.2% at the quarter level.

Body sampling indicated a low prevalence of the pathogen on teat skin (1/129 samples; 0.7%) and the perineal region (6/127 samples; 4.7%). This suggests that these specific body sites are not likely primary reservoirs for *S. uberis* mastitis in these herds. In contrast, bedding materials were a significant source, with 15 out of 36 samples (41.6%) testing positive for *S. uberis*. The bedding samples comprised of 29 straw, 4 straw pellets, and 3 manure-based samples.

SCC in *S. uberis*-positive quarters was highly elevated, with a median of 1.06×10^6 cells/mL, and varied widely, from 1.85×10^3 to 7.31×10^6 cells/mL. Differently, SCC in control quarters (negative to *S. uberis*) showed a median of 1.85×10^5 , with a range from 1.54×10^3 to 4.52×10^6 cells/mL. A p-value < .001 showed statistical significance between the two groups of milk samples.

Genotypic characterization using RAPD demonstrated that multiple *S. uberis* genotypes could be isolated from a single quarter, reinforcing existing knowledge about the high strain diversity involved in mastitis pathogenesis (Käppeli et al., 2019).

Preliminary MIC data from two farms revealed distinct AMR patterns: strains isolated from milk samples shared a common AMR profile, which distinctly differed from the profile observed in strains isolated from environmental samples. These analyses will be expanded to all remaining farms to corroborate this observed discrepancy.

Conclusion

Our study demonstrates that teat skin and manure (perineal samples) are not primary reservoirs for *S. uberis* mastitis and confirms bedding as a major reservoir for *S. uberis*. The lack of direct strain overlaps between bedding and milk samples, coupled with the high strain diversity found within quarters, underscores the complexity of transmission routes. These findings highlight the need for rapid, high-resolution genotyping methods, such as IR-Biotyper, to handle the large number of strains required for a precise and meaningful evaluation of *S. uberis* epidemiology. Furthermore, whole genome sequencing is needed to study the genetic differences among genotypes and their virulence factors.

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