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Lessons learnt from strain types, milking order, and mastitis pathogen transmission

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1 Introduction

Mastitis is the inflammation of mammary glands and (one of) the most costly diseases in dairy cattle herds in developed countries (Halasa et al., 2007). The majority of antibiotics in dairy herds are used in the context of treating intramammary infections, which are the major cause of bovine mastitis (DANMAP, 2022). Current bio-economic simulation models used in the context of mastitis control generally assume that mastitis occurrence and pathogen transmission is a homogeneous process that may either originate from the environment (environmental) or the milking process (cow-associated transmission; e.g. Gussmann et al., 2018). However, research has shown that these assumptions are often wrong and unrealistic, as presumably purely cow-associated pathogens can also have reservoirs in the environment, and those pathogens categorized as environmental can behave in a cow-associated pattern and be transmitted via the milking process (Jørgensen et al., 2016; Zadoks et al., 2003). In addition, exposure to pathogens may not be random but governed by social interactions within the herd and the milking order of the cows. These assumptions may substantially impact the assessment of the cost-effectiveness of mastitis prevention and control strategies. This points out the need for data to properly parametrize these tools for accurate and economic assessment of mastitis prevention and control, to ensure sustainable dairy production.

The availability of technology to record social interactions between animals and the milking order of cows may allow parametrization of decision support tools more realistically, providing farmers with a valuable and accurate tool for decision support making. Precision livestock farming (PLF) opened a myriad of possibilities to monitor disease transmission patterns through cattle positioning systems, computer vision, and data collected via milking machines and robots. With a real-time location system (RTLS), cows

can be identified and tracked when being at a feeding trough (Ternman et al., 2019), alleys, or cubicles with high accuracy (Pastell and Frondelius, 2018; Tullo et al., 2016). Also, the order of cows entering the milking parlor can be monitored (Hansson and Woudstra, 2023).

One of the main goals of the research program on cow social interaction and disease transmission (CSI: DT) was to develop tools for minimizing disease transmission within dairy farms based on knowledge gained from continuous monitoring of the milking order of cows and the investigation of transmission patterns of frequently occurring mastitis pathogens. We summarize our publications (Table 1) and experiences from this research program in two opinion papers and give advice for future research within the field. In this opinion paper, we summarize our findings with regard to mastitis pathogen transmission and reservoirs as well as the milking order of cows.

2 Disease transmission

The disease we focused on in this project was mastitis. While mastitis is one of the most important diseases in dairy cow herds (Ruegg, 2017) and the prevalence of intramammary infections is usually high, direct contacts between individual cows are likely not playing a large role in transmission of mastitis pathogens. Instead, the consecutive use of milking equipment and pre-milking by milkers are thought to play a role in cow-associated transmission of pathogens from infected to uninfected quarters. In addition, there is also a transmission of pathogens that seem to have their primary reservoirs in the environment. Also, the consecutive use of infrastructure (e.g. cubicles) might be related to new infections. As described before, the general assignment of one transmission behavior to a certain pathogen species can be misleading as the transmission behavior within a species can vary between herds (Jørgensen et al., 2016; Zadoks et al., 2003).

Most existing models for spread of cow-associated pathogens assume homogeneous mixing of cows during milking [e.g. Gussmann et al. (2018)]. They do however not take hierarchy and social networks between cows into account which can influence which cows would like to enter a milking parlor together. Furthermore, other factors, such as housing group changes, that would influence transmission in the milking parlor are also not usually considered, as these would further complicate the models. Thus, the question arises how well these models can describe disease dynamics in a herd. Gussmann et al. (2018), for example, showed that infection rate had to be greatly reduced from rates estimated from field studies when some of these factors were taken into consideration more rigorously (such as, e.g., cow-specific susceptibility and decisions). However, they still assumed homogeneous mixing. In this project, we developed a similar simulation model where cow-associated mastitis transmission happens in the milking parlor and infection events are linked to the milking order of cows (Gussmann et al., 2024). This builds on the theory that the risk of infection from an infected cow is highest for the cow that is milked right after in the same place in the milking parlor (i.e. with the same milk liners), and then decreasing for the next cow being milked in the same place and so on. After calibrating the model, we tested if there was a difference when cows were milked in a random order and when cows with a clinical case of mastitis were milked first, or last. Our results showed that in a 'normal' dairy herd (i.e. a herd without an acute mastitis problem) there appeared to be no real difference between these three scenarios.

Within this project, we have, also, investigated what role a cow's parity and lactation stage, characteristics that are usually also associated with a cow's hierarchy and social interactions within a herd, have on the probability of a cow entering the milking parlor rather early or later during milking (Hansson and Woudstra, 2023). We found that both parity and lactation stage are associated with the order in which cows enter a milking parlor. Cows in early lactation or first parity cows entered the milking parlor earlier than herd mates in later lactation or

TABLE 1 Scientific publications published during the project.

| Topic | Title | Year | DOI |
|----------------------|--|------|--------------------------------|
| Data quality | Where do we find missing data in a commercial real-time location system? Evidence from 2 dairy farms | 2021 | 10.3168/JDSC.2020-0064 |
| Data quality | Interpolation Methods to Improve Data Quality of Indoor Positioning Data for Dairy Cattle | 2022 | 10.3389/FANIM.2022.896666 |
| Social interactions | Parity and days in milk affect cubicle occupancy in dairy cows | 2021 | 10.1016/J.APPLANIM.2021.105494 |
| Social interactions | Cow characteristics associated with the variation in number of contacts between dairy cows | 2023 | 10.3168/JDS.2022-21915 |
| Social interactions | New insight into social relationships in dairy cows, and how time of birth, parity and relatedness affect spatial interactions later in life | 2023 | 10.3168/JDS.2023-23483 |
| Social interactions | Social network analysis to predict social behavior in dairy cattle | 2023 | 10.3168/JDSC.2023-0507 |
| Social interactions | Multiple factors shape social contacts in dairy cows | 2024 | 10.1016/J.APPLANIM.2024.106366 |
| Milking behavior | Associations of parity and lactation stage with the order cows enter the milking parlor | 2023 | 10.3168/JDSC.2023-0491 |
| Infectious pathogens | Strain diversity and infection durations of <i>Staphylococcus</i> spp. and <i>Streptococcus</i> spp. causing intramammary infections in dairy cows | 2023 | 10.3168/JDS.2022-22942 |
| Infectious pathogens | Reservoirs of <i>Corynebacterium</i> spp. in the Environment of Dairy Cows | 2023 | 10.3390/PATHOGENS12010139 |
| Infectious pathogens | Reservoirs of <i>Staphylococcus</i> spp. and <i>Streptococcus</i> spp. Associated with Intramammary Infections of Dairy Cows | 2023 | 10.3390/PATHOGENS12050699 |

higher parity. Parity and lactation stage are therefore associated with the risk of getting in contact with liners which were contaminated with cow-associated pathogens during milking by a previous cow and therefore probably also with the risk of getting infected with these pathogens. This influence of hierarchy and social interactions on the milking order means that the milking order is not really random, as it has been assumed in previous simulation models. It is conceivable that such a “structured randomness” could have an influence on the transmission dynamics of mastitis pathogens in dairy herds, particularly as parity and lactation stage are considered risk factors for intramammary infections. We therefore hypothesize that milking first-parity cows, which have a lower risk of being infected with contagious organisms, at the beginning of the milking process would reduce transmission of contagious pathogens during milking. This has, to our knowledge, not been tested yet in simulation models and should be explored in the future.

Due to the high cost associated with strain typing, many studies investigating mastitis epidemiology of different pathogens focused on the species-level only. However, in order to study the actual transmission of pathogens, strain level studies are required to investigate if cows are actually infected with the same strain of a species and to distinguish ongoing from new infections. Within the present project we therefore used strain typing to investigate the transmission routes of different staphylococci and streptococci, namely *Staph. aureus*, *Staph. epidermidis*, *Staph. haemolyticus*, *Strep. uberis* and *Strep. dysgalactiae* (Woudstra et al., 2023a, 2023b). Most studies using strain comparisons, including our own, found that *Staph. aureus* seems to be largely transmitted contagiously from quarter to quarter. Other pathogens such as *Strep. dysgalactiae* and *Strep. uberis* however, behave differently from herd to herd (Leelahapongsathon et al., 2020; Wente et al., 2019; Wente and Krömker, 2020).

In this project, a farm with around 200 lactating cows was visited ten times at 14-day intervals and individual quarter foremilk samples were collected at each of the ten visits from all lactating cows. We found few cases of new infections due to contagious pathogen strains making it hard to estimate accurate pathogen transmission parameters, e.g. the rate of transmission through consecutive contact with milking liners. To study the level of importance of the different fomites (e.g. liners, milkers' hands), a large number of new infections needs to be observed, meaning studies have to be conducted either in herds with high prevalences, or studies have to include a large number of animals. Herds with high prevalences cannot be expected to represent an average herd, while large studies are expensive, and it is hard to get funding for these. Similar limitations are valid when studying the importance of consecutive use of infrastructure (e.g. cubicles) for pathogen transmission. We could, for example, demonstrate that cows of different parities prefer different areas in the barn (Churakov et al., 2021). At least in theory, also milk leakage in the cubicles between milkings could lead to transmission of pathogens from udder to udder.

3 Discussion

Milking order, social interactions and area utilization can play a crucial role in the transmission of infectious diseases. The sequential

use of different areas in the barn and the close proximity between certain individuals can facilitate the spread of organisms between animals. In fact, the likelihood of disease transmission between individuals can be computed as a function of contact intensity (de Freslon et al., 2019). In this regard, PLF information, such as RFID tags providing milking order information or UWB sensors providing social interaction and area utilization information, offer the opportunity to automatically monitor the social interactions and area utilization of dairy cows. The use of this information might contribute in the future to facilitating the adoption of preventive measures to minimize the spread of pathogens on dairy farms.

3.1 Ideas and practical advice to farmers

In our study, the prevalence of mastitis pathogens was low, making it hard to estimate accurate parameters that could be used in models. Furthermore, the simulation model we developed showed that in situations, where mastitis was not a big problem in the herd, changes in the milking order did not have a large influence on pathogen transmission. However, with increasing prevalence and transmission, changing the milking order had an increased influence on pathogen spread (Gussmann et al., 2024). We therefore suggest that measures connected to social contacts (induced for instance by milking order) should be considered by farmers depending on the main transmission patterns of pathogens with significant impact on milk quantity and quality in the herd making preventive measures economically meaningful. We also recommend that strain typing to become more widely used in mastitis investigations in the future, as transmission patterns can in some cases not be concluded from a mere species identification.

Author contributions

SW: Writing – original draft, Writing – review & editing. MG: Writing – original draft, Writing – review & editing. HM: Writing – review & editing. IH: Writing – review & editing. CK: Writing – review & editing. VK: Writing – review & editing. PN: Writing – review & editing. KR: Writing – review & editing. LR: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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