

Research Communication

Cite this article: Beckmann A, Barth K and Knappstein K (2025) Comparison of two sampling approaches to determine the prevalence of mastitis pathogens in dairy herds. *Journal of Dairy Research* **92**, 201–203. <https://doi.org/10.1017/S0022029925100940>

Received: 18 September 2024
Revised: 19 December 2024
Accepted: 21 March 2025
First published online: 14 October 2025

Keywords:
intramammary infection; mastitis; prevalence; udder health

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Comparison of two sampling approaches to determine the prevalence of mastitis pathogens in dairy herds

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Abstract

Improving udder health on dairy farms requires knowledge about the prevailing mastitis pathogens in order to take appropriate measures. The aim of this research communication was to evaluate the association between two sampling approaches for determining the prevalence of mastitis pathogens in dairy herds. Sampling approaches tested included (a) bacteriological investigation of randomly selected cows independent from stage of lactation (random sampling) and (b) sampling of cows two weeks prior to drying off (dry-off sampling). Using linear regression, the prevalence of mastitis pathogens were compared on herd-level for groups of specific pathogens. Associations between the prevalence estimated by the two approaches were found for *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis* and Gram-negative bacteria but not for other esculin-positive streptococci. This study indicated that both sampling approaches provide farmers with an overview of the prevalence of mastitis pathogens in their herds, with the dry-off results also being used to target antibiotic use to infected quarters.

Knowledge on causative agents of intramammary infections is important for udder health management in dairy herds. However, farmers often only take samples of clinical mastitis cases (Falkenberg, 2022), making it difficult to assess the true state of the herd while missing subclinical cases. Herd investigations of all cows provide highest accuracy on the prevalence of mastitis pathogens, but sampling and analyses are time-consuming and expensive. Therefore, alternative sampling approaches are desirable. Different sampling strategies have been compared for lameness (Hoffman *et al.*, 2013) or dairy cattle welfare in general (van Os *et al.*, 2018), but less is known about best practices identifying the prevalence of mastitis pathogens in dairy herds. In scientific studies it is quite common to sample a number of randomly selected cows independent from herd size (Tenhagen *et al.*, 2006; Groh *et al.*, 2023).

As part of a study on pathogen-based dry cow therapy (Beckmann *et al.*, 2025; Beckmann, A., K. Barth and K. Knappstein, *accepted*), quarter milk samples were collected per farm for bacteriological analyses at two different time points. A random sampling approach was conducted once on herd-level on lactating cows regardless of lactation stage and lactation number, while a dry-off sampling was conducted two weeks prior to dry-off. This research communication aimed to compare and discuss these two sampling approaches in terms of determining the prevalence of mastitis pathogens in dairy herds.

Materials and methods

A total of 16 dairy herds interested in quarter-selective dry cow therapy voluntarily participated in the study. Farms varied in herd size, ranging from 79 to 1,280 cows, and breed, with German Holstein dominating (for details see Beckmann A., K. Barth and K. Knappstein, *accepted*). Eleven farms milked in a parlour and 2 farms in a rotary, while 2 farms used an automatic milking system and one farm used both a parlour and an automatic milking system.

Quarter milk samples of approximately 75 cows per farm were collected at two different time points (a) at a single sampling day during lactation (random sampling) and (b) two weeks prior to drying off (dry-off sampling). The random sampling was conducted between October 2020 and June 2021, with one of the farms examined in January 2022. Milk samples were collected by technicians and the research team once from randomly selected cows in each herd, regardless of lactation number, stage of lactation or udder diseases. Sterile 10 mL test tubes were used, only containing boric acid (final concentration 0.5%) if overnight transport was necessary. In milking parlours and rotaries, sampling was performed throughout one milking time. On farms

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with automatic milking, cows were sampled independently from the milking procedure, either confined to the feed fence or standing in the cubicles. The sampling before dry-off was conducted during a time period of 5 to 18 months per farm, with a time lag between the random sampling and the start of dry-off sampling of 1 to 5 months per farm. Dry-off samples were collected two weeks prior to the scheduled dry-off date by trained farm personnel. As these samples were mailed, the sterile 12 mL vials contained boric acid. Resulting of the different sampling approaches, it was possible that cows were sampled twice, at the random sampling approach and the dry-off sampling approach (ranging from 8.9% to 87.7% of cows sampled twice per farm), or only at one of the two sampling time points.

Milk samples were analysed for mastitis pathogens by standard culture according to the guidelines of the German Veterinary Medical Society (2018). Briefly, 0.05 mL of milk per quarter was inoculated onto a Columbia agar plate with 5% sheep blood and incubated 18–24 h at 35°C and another 24 h. If no bacteriological growth was observed after 48 h, the quarter was defined as not infected. Major pathogens were considered if at least 20 cfu/mL of milk were detected, whereas minor pathogens were only considered with > 400 cfu/mL (for details see Beckmann A., K. Barth and K. Knappstein, *accepted*).

All statistical analyses were done with SAS Version 9.4 (SAS Institute Inc., Cary, NC). Data of cows treated with antibiotics or within the withdrawal period were excluded ($n = 8$). First, data evaluation was carried out descriptively on quarter and herd-level. For subsequent analyses, herd was set as statistical unit. For cows with 2 dry periods during the study ($n = 49$), one dry period was randomly excluded. The association between the herd-level prevalence estimated by dry-off sampling and random sampling was assessed using linear regression (SAS procedure REG) with the herd-level prevalence at random sampling as target variable for the most frequently detected pathogen groups (Non-*aureus* staphylococci, *Corynebacterium* spp., *Staphylococcus* (*S.*) *aureus*, *Streptococcus* (*Str.*) *dysgalactiae*, *Str. uberis*, other esculin-positive streptococci (including enterococci) and Gram-negative bacteria (including *Escherichia coli*, *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp., *Proteus* spp., *Pasteurella* spp. and *Pseudomonas* spp.)). An association was considered as significant at $P \leq 0.05$. Herd size and proportion of cows which have been subject to both random sampling and dry-off sampling were tested as covariates in the regression models, but were excluded from the final model as no association with the prevalence estimate of any group of pathogens ($P > 0.05$) was observed.

Results

In the random sampling approach, a total of 4,809 quarter milk samples from 1,221 randomly selected cows were analysed. The number of cows sampled per farm varied from 68 to 85 cows (mean \pm standard deviation (SD): 76 ± 4). This represented $41.3\% \pm 25.4\%$ of all cows – 6.3% in the largest herd and 90.4% in the smallest. Proportion of sampled cows in parity 1, 2 and ≥ 3 were 29.2%, 26.2% and 44.6%, respectively. The cows were sampled at 174 ± 116 days in milk indicating that all lactation stages were covered. For the dry-off sampling approach, the initial data set of Beckmann A., K. Barth and K. Knappstein (*accepted*) was used, containing a total of 5,663 quarters from 1,444 dry periods (from 1,395 cows). This corresponded to 90 ± 10 sampled dry periods per farm, ranging from 61 to 106 dry periods. Proportion of sampled cows in parity 1, 2 and ≥ 3 were 32.8%, 25.9% and 41.3%,

Table 1. Regression coefficients for the association between the herd-level prevalence estimated by dry-off sampling and random sampling (target variable) for specific pathogen groups ($n = 16$ German commercial dairy farms)

Pathogen group	Coefficient	SE ^a	R ²	P value
Major pathogens				
<i>Staphylococcus aureus</i>	0.8	0.2	0.54	<0.01
<i>Streptococcus dysgalactiae</i>	0.4	0.1	0.78	<0.01
<i>Streptococcus uberis</i>	0.2	0.1	0.34	<0.05
Other esculin-positive streptococci ^b	0.2	0.1	0.12	0.19
Gram-negative bacteria ^c	0.7	0.3	0.26	<0.05
Minor pathogens				
Non- <i>aureus</i> staphylococci	0.4	0.1	0.35	<0.05
<i>Corynebacterium</i> spp.	0.9	0.2	0.64	<0.01

^aSE = standard error.

^bIncluding enterococci.

^c*Escherichia coli*, *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp., *Proteus* spp., *Pasteurella* spp., *Pseudomonas* spp.

respectively. On average, cows were sampled when 351 ± 64 days in milk.

Of all quarters sampled in the random sampling approach, 5.5% showed intramammary infections with major pathogens, while 13.2% were infected with minor pathogens. The quarter-level prevalence of intramammary infections before dry-off was in a similar range, with 7.5% major pathogen infections and 13.3% minor pathogen infections (Supplementary Table S1).

The prevalence of each group of pathogens at the random sampling approach or at dry-off varied widely between the herds (Supplementary Table S1; Supplementary Figure S1; Supplementary Figure S2). Using linear regression, associations between the prevalence of the two approaches were observed for the most important groups of major pathogens per herd, the contagious pathogens *S. aureus* and *Str. dysgalactiae*, and the environmental pathogens *Str. uberis* and Gram-negative bacteria (Table 1). Only for the prevalence of other esculin-positive streptococci no association was found.

In general, an increase in dry-off prevalence of 1% was associated with an increase in random sampling prevalence lower than 1% for all pathogens (Table 1), meaning that none of the prevalence during lactation were underestimated with regular dry-off sampling.

Discussion

Given the lack of knowledge regarding the true prevalence in the herds, as is common in other prevalence studies (Hoffman *et al.*, 2013; van Os *et al.*, 2018), this study only allowed to compare the prevalence estimated by two different approaches, rather than evaluating them. In addition, the number of herds and the repeated sampling in the same herd have to be considered when interpreting regression results. However, there was no effect of the proportion of cows sampled at both sampling times on the prevalence estimate of any group of pathogens in the herds. Moreover, all farmers were aware of the random sampling results and thus were able to make culling decisions until dry-off sampling. Nevertheless, there was an association between the prevalence of pathogens, particularly in the cases of *S. aureus* and *Str. dysgalactiae*.

As the farmers had special interest in strategies focusing on antibiotic reduction and participated voluntarily, the prevalence of mastitis pathogens compared to other herds might be biased. Especially the higher prevalence estimated for *S. aureus* at dry-off is in accordance with prevalence data from Tenhagen *et al.* (2006) and Nitz *et al.* (2021), whereas their findings for other mastitis pathogens differ in dependence on study design (e.g. sampling time, parity). Compared to other German studies, the general prevalence of *S. aureus* was lower in our study and the prevalence of *Str. uberis* was higher (Tenhagen *et al.*, 2006; Nitz *et al.*, 2021; Groh *et al.*, 2023). A long-term focus on the predominant mastitis pathogens, a targeted use of antibiotics and an improvement of udder health management, for example through good milking hygiene, may have contributed to a reduction in the proportion of contagious pathogens in the study herds.

Sampling all cows two weeks prior to dry-off provides an analysis per cow and lactation that can be used to target dry cow treatment to infected quarters only. It also has the advantage of detecting subclinical cases of mastitis. Combined with the practice of sampling clinical cases when they occur, this approach can provide a comprehensive insight into the infection situation of a herd.

In conclusion of our study, for the most relevant groups of mastitis pathogens in dairy herds, significant associations were found between the prevalence estimated by random sampling and dry-off sampling, with the dry-off results also being applicable for targeting antibiotic use to infected quarters only. The results of the bacteriological analyses can be used to monitor changes in the prevalence of certain mastitis pathogens and to monitor the success of management strategies for mastitis control.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0022029925100940>.

Acknowledgements. This work (Model- and Demonstration Project for Animal Welfare) was financially supported by the Federal Ministry of Food and Agriculture based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food, grant number

2819MDT211/212. We are grateful to all participating dairy farmers for their cooperation. We thank the laboratory staff of the Max Rubner-Institute (Kiel, Germany) for excellent technical assistance. Further, we wish to thank the animal and udder health services from Bavaria, Hesse, North-Rhine Westphalia and Baden-Württemberg for their support in the random sampling approach. For the support in preparing the manuscript, we would like to thank Christina Umstätter and Eberhard Hartung.

Competing interests. The authors have not stated any conflicts of interest.

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