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Swabbing milking machines – microbiological findings and posting behaviour of veterinary practitioners in Northern Germany

Zum Tupfern von Melkanlagen – mikrobiologische Befunde und Einsendeverhalten norddeutscher Tierarztpraxen

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Summary Milking machines are cleaned and disinfected on a regular basis, but since they are not sterilized, microorganisms grow inside it. Experience suggests that in veterinary practices, the difference between graduated monitoring via liquid samples to assess a hygiene problem of bulk milk on one side and surface swabs to detect mastitis pathogens in the proximity of the teat during milking on the other side, is not always understood. Using standard methods, a total of 2298 swabs was analyzed microbiologically. Environment-associated pathogens (mostly yeasts, coliforms, *Escherichia coli,* coagulase-negative staphylococci), opportunistic bacteria (enterococci) and contaminant flora (spore-formers and bacilli) were found almost ubiquitously in large quantities, even at locations which by being far from the teat cannot act as reservoirs for pathogens. Although 87 % of swabs originated from potential reservoir sites, 43 % of practitioners sent in swabs from unsuitable sites which would rather be sampled when addressing a hygiene problem for the bulk milk. These results support the assumption that corresponding practitioners have not fully understood the possibilities and limitations of swabbing the milking machine.

> **Keywords:** Milking machine, biofilm, mastitis pathogens, hygiene, bovine practitioner

Zusammenfassung alle in Melkmaschinen werden regelmäßig gereinigt und desinfiziert, doch da sie nicht sterilisiert werden, besteht die Gefahr des Wachstums von Mikroorganismen. Die Erfahrung lässt vermuten, dass der Unterschied zwischen einer Stufenbeprobung von Rohmilch zur Eingrenzung eines Keimzahlproblems und dem Tupfern von Oberflächen, um Reservoire von Mastitiserregern während des Melkens zu entdecken, in einigen Tierarztpraxen nicht immer erkannt wird. Die vorliegende Untersuchung beurteilt die in ein Speziallabor eingesandten Tupferproben für den Zeitraum 2004 bis 2010. Insgesamt wurden 2298 Tupferproben nach von der DVG vorgegebenen Standardmethoden mikrobiologisch untersucht. Umweltassoziierte Mastitiserreger (v.a. Hefen, coliforme Keime, *Escherichia coli,* coagulase-negative Staphylokokken), Opportunisten (Enterokokken) und Verderbnisflora (Sporenbilnder, *Bacillus* spp.) wurden annährend überall in großen Mengen angetroffen, auch in den Bereichen, die als Reservoir für die Zitze keine Rolle spielen. Obwohl 87 % der Tupferproben aus potentiellen Reservoirplätzen stammten, haben 43 % der einsendenden Praxen auch Proben an Lokalisierungen gezogen, die nicht als Reservoir für Mastitiserreger dienen können und eher im Rahmen einer hygienischen Überprüfung untersucht werden. Diese Ergebnisse unterstützen die Annahme, dass man sich in vielen Praxen nicht ganz über Möglichkeiten und Grenzen der Beprobung der Melkanlage mittels Tupfer bewusst ist.

> **Schlüsselwörter:** Melkmaschine, Biofilm, Mastitiserreger, Hygiene, praktizierender Tierarzt

Introduction

Milking machines are crucial to the success of a dairy herd. One important issue in this respect is milking technology and how it may eventually affect the udder health of an entire herd by compromising teat condition and, with that, the immune response. The other one is its sanitary status. Since the milking machine is cleaned and disinfected – but not sterilized – after each milking session, bacteria and other microorganisms will remain inside the device. Depending on the microorganism, this can in fact influence both bulk milk quality and the udder health of the cow (Reinemann et al., 2003).

On one hand, milk quality may be decreased because of bacteria which colonize the interior of the milking machine and the outlet valve that connects to the tank lorry. According to Cousin and MacKinnon (1977), jetter plates, milk inlet ports, rubber parts of the milk receiver and its overflow control, filters of vacuum-regulating valves as well as metering systems for detergents and disinfectants are the most important weak points regarding the hygiene of a milking machine. All these localisations are characterized by a change in the vessel diameter and of the material which makes effective cleaning and disinfection difficult. This promotes the formation of biofilms which in turn may lead to increasing bacterial counts in milk. In order to localize the origin, milk or water samples may be drawn at the entrance of the tank, inside it and at the outlet (graduated monitoring), and checked for total bacterial (mesophillic aerobic) counts (TBC), coliforms, thermoduric bacteria (Reinemann et al. 2003) as well as pseudomonads. In Germany, TBC beyond 100 000 cfu/ml (calculated as the geometric mean over two months) lead to a reduction of the price for milk by 2 cents/kg (§ 3 regulation for milk quality; *"Milch-Güteverordnung"*).

On the other hand, those components of the machine which are within reach of the teat, or from which a reflux of contaminated milk towards the milking teat is possible, may serve as a reservoir for pathogenic bacteria, if the cleaning and disinfection routine is insufficient (Reinemann et al., 2003).

For graduated monitoring, liquid samples are recommended, as they allow a precise determination of bacterial counts (i. e. cfu/ml). Swabs however may suffice to detect

typical bacteria associated with udder health regardless their actual counts. Since these counts do a major impact on the development of the mastitis and 50 cfu of pathogen may infect an udder quarter just as 5000 cfu, this disadvantage may be neglected. Swabs are easy to handle and their analysis is economical. However, as most structures are tubular with diameters smaller than a human hand, their physical reach is limited by the length of the swab, and results cannot be extrapolated to TBC values (Pfannenschmidt, 2003). Regarding swabs in the milking environment, no practical standard method has been established, yet. Although dry-moist swabs are recognized as a standard for surfaces (DIN ISO 6887-1:1999), practitioners usually apply swabs stored in a dry container or in a container filled with medium, as their handling is easier. Many other issues contribute to the lack of standardisation, e. g. the type of swab used, the area swabbed, the level of hygiene applied while swabbing, the condition of the surface (cleaned and disinfected vs. not cleaned and disinfected), and the duration of and temperature at transport (Feldmann et al., 2008).

Existing flora might contribute to increased total bacterial counts (bulk milk hygiene) and may be source for contamination of teats with mastitis pathogens if in reach of them (udder health). While swabs may suffice to detect mastitis pathogens in the proximity of teats, a graduated sampling with liquid milk samples is mandatory to asses a problem of bulk milk hygiene. Yet, bovine practitioners send in swabs to specialized laboratories, and the intention of doing so is not always clear. The present paper evaluates the samples handed in between 2004 and 2010 to such a laboratory. Swabs taken from milking machines by customers are analysed at the Institute for Food Quality and Food Safety on a regular basis. The present paper sums up the results of this analysis activity, focusing on a) microbiological findings on biofilms in milking machines and b) client sampling behaviour.

Material and methods

Between 2004 and 2010, a total of 2298 samples from milking machines was analysed. All but six samples (bedding material; Table 1) were swabs. Swabs were taken from 40 different veterinary practices or dairy consulting agencies which represented 170 customers. Farms were located in Northern Germany. Completeness of data accompanying the samples depended on the person handing in the samples and ranged between the description of the exact sampling location (58.7 %) and none (41.3 %).

Upon arrival, each swab was streaked onto half a blood agar plate and a quarter of a yeast glucose chloramphenicol (YGC) plate. This corresponds to the procedures recommended for quarter foremilk samples by the German Veterinary Association (DVG, 2009). Then the swab was immersed in liquid culture medium (10 g Liebig's extract of meat, 10 g peptone, 3 g NaCl, 2 g Na₂HPO₄·2H₂O, 10 g dextrose, 100 ml water, adjusted to a pH of 7.4 via NaOH) used for mastitis pathogens (the area of contact of the swab with the fingers cut off while immersing the swab). All

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media were incubated aerobically at 37 °C for 24 h after which a first identification took place and one loop of the liquid medium was streaked onto another half of a blood plate. Final diagnosis occurred after another 24 hours of incubation at the same temperature using the International Dairy Federation (IDF) recommendations (DVG, 2009). Bacterial counts were recorded semi-quantitatively on the plates using a score system $(x = 10$ cfu or less, $xx = 11$ to 50 cfu, $xxx = > 50$ cfu), based on DVG (2009) recommendations. Findings were also categorized according to their relevance for udder health (Table 3). For each sample, the most numerous finding was recorded. In case two or more findings were equally numerous, the samples was rated as "coequal".

Data recording included the date of submission, sender and their client (encoded), the sampled localisation, the bacteriological finding and its semiquantitative score ("x" to "xxx"). Data was processed using Excel ©.

Results

Posting behaviour

During the survey, a total of 2298 swabs were received. The localisations where the swabs were taken (if indicated) were grouped into major areas, i. e. the stall (all structures contained in the place where the cow is milked [including the teat surface, but excluding the milking unit]), the milking unit (all components, from the liner to the long milk tube), the milking pipeline (structures between the milking tube and the milk filter), and the cleaning and

disinfection devices. Furthermore, scattered samples from other localisations were grouped into an "other" category. Due their small sample size, these data were not considered further. Finally, many samples were sent in without any specifications on the precise sampling site ("not specified"). Table 1 details the source and the amount of swabs. It also groups the localisations with regard as their potential to serve as a reservoir for mastitis pathogens that could actually contaminate teats during milking. The milking unit was

the most prominent site sampled, followed by structures along the milk pipeline and the stall.

Due to lacking information, 949 samples could not be localized more precisely ("not specified"). The majority of traceable samples (86.7 %; i. e. n = 1128) originated from localisations that actually can act as a pathogen reservoir for the teats. However, samples from areas far from the teat (i. e. with no potential to contaminate it during milking) were posted by 17 practices (42.5 %).

Table 2 shows that samples were posted at varying quantities during the survey. It shows the heterogeneity of the data, reflecting the needs of the practitioners; e. g. stall samples usually amounted for 4 to 6 % of the total annual samples. However,

year	stall	milking unit	milk ipeline	cleaning and disinfection	not specified
2004	4.2(10)	79.2 (190)	6.3(15)	(5) 2.1	(12) 5.0
2005	(7) 2.1	83.2 (282)	6.5(22)	3.5(12)	(3) 0.9
2006	4.4(13)	77.9 (232)	14.1 (42)	(5)	0.3 (1)
2007	23.1(27)	(75) 64.1	(6) 5.1	(6) 5.1	(3) 2.6
2008	5.0(10)	58.2 (117)	7.0(14)	8.0(16)	(35) 17.4
2009	5.9(32)	27.3 (148)	1.9(10)	1.9(10)	63.1 (342)
2010		(3) 0.5		(3) 0.5	98.6 (553)

TABLE 3: *Categorisation of microbiological findings in relation to their relevance as mastitis pathogen.*

extremes range between 2 and 23 %. The incidence of unspecified samples increased over the years, particularly during 2009 and 2010. Likewise, the amount of samples properly labelled dropped (particularly those of milking units). Since the majority of senders realized the importance of milking unit samples, it may be suggested that many of the unspecified samplesin fact originated from this site.

FIGURE 1: *Microbiological findings in relation to major structure areas of the milking machine; AMS = automatic milking system*

TABLE 4: *Occurrence [n] of pathogenic and opportunistic microorganisms in swab and bedding samples.*

localisation	stall	milking unit	milk pipeline	cleaning and disinfection	not specified	total
Pathogenic microorganisms						
CNS	3	62	5	$\overline{2}$	73	145
Stapyhlococcus aureus					5	$6\overline{6}$
coliforms	14	88		9	70	188
E. coli		40			76	125
Pantoea agglomerans						
Citrobacter freundii						
Enterobacter aerogenes		8				8
Pseudomonas aeruginosa		13		2	13	28
Pseudomonas spp.	5	63	13	5	40	126
yeasts	14	128	12	5	115	274
Opportunistic microorganisms						
Corynebacterium spp.		4				4
Enterococcus spp.	9	115	12	5	96	237
Proteus spp.					6	
Klebsiella spp.		9	5			15
Klebsiella p. pneumoniae					$\overline{2}$	3
Klebsiella p. ozanae						
Serratia marcescens						
Serratia spp.						
total	54	534	56	31	497	1172

CNS = coagulase-negative staphylococci

Microbiological findings

Bacteriological results were categorized according to their relevance for the udder health (Table 3). Based on this categorisation, Figure 1 sums the findings in relation to the major areas that were sampled.

The list in Table 3 corresponds largely to the "classical" mastitis pathogens usually encountered in quarter foremilk samples. However, some groups (streptococci, *Trueperella pyogenes, Citrobacter koseri* and *Enterobacter cloacae*) could not be detected by swabs.

With almost 40 %, mastitis-relevant pathogens were the most frequently isolated group of microorganisms. Still, approx. 30 % of samples contained innocuous bacteria (contamination flora), and almost 15 % were negative to

(present in >100 samples) at selected sampling sites (site represented by >30 swab samples)

routine culture procedures. Within the milking unit, the percentage of negative samples of short milk tube, long milk tube, cluster and liner was 14.0, 12.6, 13.1, and 24.5 %, respectively. Finally, opportunistic bacteria accounted for less than 15 % of all samples. In general, frequencies did not vary markedly among areas.

Mastitis and opportunistic pathogens were isolated from a total of 1172 samples. Table 4 details the distribution of these microorganisms according to the localisation, while Figure 2 presents the corresponding percentage values of selected localisations. To be considered for this Figure, the pathogen had be isolated in >100 swabs, and the location had to be represented by >30 swab samples.

Yeasts, coliforms, CNS, and enterococci were the most frequent findings. Yeasts dominated in all sampled parts of the milking units, the milk pipeline and the bulk milk tank, while coliforms were particularly present in the cleaning and disinfection samples. In the stall, both pathogens were equally present. Enterococci were found ubiquitously. More

than 90 % of innocuous microorganisms referred to bacilli and/or spore-forming bacteria.

Regarding bacterial count scores, the overall percentages of results scored as "x", "xx", and "xxx" were 29.0, 14.2, and 56.9, resp., i. e. most samples yielded >50 cfu/ml. This basic pattern was observed regardless the swab location and, as can be seen in Figure 3, regardless the relevance of the microbiological diagnosis for the udder health. Yet, slightly varying patterns among microorganisms types were observed. The ratios (mean \pm standard deviation) between these three scores $(x : xx : xxx)$ for all positive findings (n = 2132) were $1:0.4 \pm 0.1:2.0 \pm 1.5$ (contamination flora), $1: 0.5 \pm 0.3: 1.8 \pm 1.1$ (opportunistic pathogens), and $1: 0.7 \pm 0.2.$: 3.8 ± 0.7 (mastitis pathogens).

> On species respectively group level, swabs rated with 'xxx' generally amounted between 56 (CNS) and 82 % *(Pseudomonas aeruginosa). S. aureus* only occurred in counts <50 cfu ("x" and "xx"), and non-*aeruginosa* pseudomonads were present almost equally in low $(x; 43\%)$ and high (xxx; 42 %) bacterial scores.

Discussion

The present paper describes a survey with swab samples drawn by veterinary practitioners in Northern Germany during their routine work and sent to the authors' microbiological laboratory. As such, a series of limitations have to be mentioned that make the difference to an epidemiological study. One is the lack of information about sampling procedures. This includes exact location of swabbing,

92 Journal of Food Safety and Food Quality 65, Heft ⁴ (2014), Seiten 85–112

swabbing technique, surface area swabbed and the hygienic status of the milking machine at sampling, i. e. whether the sample was drawn before or after cleaning and disinfection. Accompanying information was scarce and was limited, at best, to the localisation where the swab was applied. No data on previous mastitis or hygiene problems was supplied, so that results have to stand alone. The same is true for the instructions to the laboratory; although the downloadable accompanying letter requests more detailed information, data provided by the practitioners were frequently limited to the addresses of the sender and of the owner, eventually the swab localisations and the request for what kind of microbiological analysis was given. Another limitation is the varying transport conditions as the samples were usually sent by mail and eventually cooled; shipping time varied between one and three days. Swabs were stored either in dry containers or in a transport medium. Thus, the results highlight the practical needs of practitioners as well as their professional

FIGURE 3: Occurrence of bacterial count scores [%] according to localisation and relevance of the microbiological finding (for description of the categories see Table 3); $x = \le 10$ cfu; $xx = >10$ to ≤ 50 cfu; $xxx = >50$ c

performance rather than representing an epidemiological study. This all translates in a marked degree of heterogeneity which only allows a qualitative and semi-quantitative description of the findings as such.

Practitioners' sampling effectiveness

Reinemann et al. (2003) recommend to sample milking units, the milk pipeline, receiver, filters, pre-coolers and the bulk tank. Their analysis includes both hygienic and udder health issues, but their sample material was milk or diluted milk. Most locations were sampled also in the present survey, but at different intensities.

Swabs were collected from many different locations of the cows' environment (Table 1). Of those, 15 sites could be a reservoir to contaminate the teats during milking, while 18 were too far away from the teat to act as a contamination source. Together with the fact that more than 40 % of practitioners sent in swaps from those inappropriate sites to detect reservoirs for udder health problems, it may be deduced that the function of swabbing milking machines is not clearly understood by all clinicians. This is also supported by the relative high amount of samples originating from the cleaning and disinfection devices. Still, 87 % of samples could be used to detect mastitis pathogens, whether this was the original intention of the senders or not. The risks associated with this behaviour lies in the misinterpretation of data leading to false guidance, e. g. in deriving hygienic measures to reduce the bacterial load of bulk milk.

Cannas da Silva et al. (2006) postulated a change in the attitude of modern bovine practitioners, away from the clinician that treats single problems of single cows towards the herd manager that understands the herd as the basic unit which has to be promoted and taken care of as such. During an US survey, bovine practitioners rated the "problem-solving ability" (Cannas da Silva et al., 2009, p. 349) asthe most important competence a veterinary practitioner should demonstrate in this field (Miller et al., 2004), and understanding the difference between these two common problems in dairy herds along with adequate sampling procedures is part of this requirement. Besides that, areas like 'milk production', 'milking technology', 'hygiene status of milk' and 'milk quality' are part of the curricula in German (DVG, 2007) and in most European veterinary faculties, and most of them teach sampling and microbiological analysis also practically (Fischer, 2010).

So, the question remains whether the practices employ veterinarians with varying degree of knowledge or the difference between the two diagnostic goals is not clearly understood in general. In reply to that, milk hygiene training at the authors' institution has been stressing on that subject for the last 15 years.

Confronting the facts with these requirements at veterinary training, the discrepancy between what is and what should be becomes apparent. The increasing negligence at filling in accompanying letters does not necessarily mean that the practitioners themselves do not keep a register of the localisation of the swab; they simply do not share this data with the laboratory.It becomes clearthat the role of the analysing laboratory has been reduced to a mere provider of microbiological results. Adding to that, the frequency at which costumers call at the laboratory to discuss the results has also decreased. If at least the localisation of the swab is known, the laboratory could contact the sender when a sample originates from a site which has no relevance for the udder health, in order to save costs and time.

Microbiological findings

A basic pattern was observed in almost all areas, i. e. 40 % pathogens, 12 % opportunistic and 33 % innocuous

microorganisms, and 14 % of culture-negative samples (Figure 1). Liners and milk pipes were the areas with the highest incidence of culture-negative samples, while the stall yielded most culture-positive results.

These results reflect a common situation in the environment of a milking machine (Reinemann et al., 2003). Since the system is cleaned, but not sterilized, environmental bacteria and yeasts commonly colonize the surfaces milk gets into contact with. However, most pathogens encountered are either environment-associated or opportunistic. *S. aureus* is the only cow-associated pathogen that was detected. These low rates are also reflected in the literature, as Paduch et al. (2009) isolated *S. aureus* in merely 4.1 % of operator hands. Feldmann et al. (2008) did not detect any cow-associated pathogens at all, although a testing of swabs and quarter foremilk samples in parallel showed that cows were infected with these bacteria. Zadoks et al. (2002) typed different strains and deduced that the machine may act as a reservoir for pathogenic *S. aureus* from both operators' hands and bovine milk. Many strains of *S. aureus* are capable of producing biofilms (Darwish and Asfour, 2013), but current cleaning devices seem capable of reducing the load of this pathogen in the immediate surroundings of the teat. Thus, the data supports the assumption of Feldmann et al. (2008) that the milking machine is a not predominant source of contamination, at least not between milking times.

Yeasts were the most common colonizers of milking machines. This predominance might be due to the fact that these microorganisms are not inactivated by typical milking machine disinfectants to a degree comparable to that of bacteria (Reinemann et al., 2003). Feldmann et al. (2008) detected less yeasts and assumed that the generation time of yeast tends to be longer than the time between one cleaning and disinfection step and the next. Pfannenschmidt (2003) made similar observations and argued that yeasts might accumulate in dead ends and other areas which are inaccessible for swabs for which they are flushed out in liquid samples, but do not occur so frequently in swabs. Both papers

were designed as epidemiological studies, but the present work reflects the naturally-occurring cases, so the samples were predominantly drawn in enterprises that displayed a problem of any kind. So, yeasts may be typical for problematic farms, but rather uncommon on a general level. A similar explanation can be given to the high percentages of pseudomonads encountered in this study which also contrasts to the findings of Feldmann et al. (2008).

CNS were also a common diagnosis; however it is difficult to evaluate this result further, as CNS comprise a series of different staphylococci with a varying degree of relevance to the udder health. The same is true for *Corynebacterium* spp.

The analysis of swabs also included a semi-quantitative approach (scores "x" to "xxx"). Due to the nature of the samples, only tendencies rather than concise results may be drawn from that. Still, the surfaces of milking machines are disinfected, but not sterilized, so a certain amount of microorganisms may be encountered readily. In any case, Pfannenscmidt (2003) and Feldmann et al. (2008) demonstrated that different types of swabs and swabbing techniques yielded different bacterial counts, and if reliable bacterial counts are the goal, liquid samples must be drawn. For udder health considerations however, bacterial counts beyond the limit of relevance (i. e. what is the bacterial count of a given pathogen on a plate to consider a sample positive and capable of infecting the quarter, ususally between 3 and 8 cfu, depending on the species) do not play a major role, so that scoring may be used to determine the most prominent pathogen of a sample.

Results showed that most microorganisms were equally distributed throughout the sampled areas of the milking machine. However, these sites not always represent a direct source of contamination for the teat. A direct relation between the existence of pathogen reservoirs in the proximity of the teat during milking and the development of an infection does not exist mandatorily (Feldmann et al., 2008).

Conclusion

The results of this survey indicate that most swab samples from milking machines in Northern Germany sent in by bovine practitioners contained environment-associated pathogens, opportunistic pathogens or contamination flora. Almost all microorganisms were ubiquitous. In this sense, swabbing areas of the milking machines close the animals' teats during milking, in search of mastitis pathogens may be a viable technique. As with any technique, the possibilities and limitations must be known to the operator.

- \blacksquare precise data on bacterial counts will not be possible, i. e. swabbing may contribute to understanding an udder health disorder but not to solving a hygiene problem; for the latter, a graduated monitoring (tank inlet, tank inside, tank outlet) using liquid milk samples should be performed
- the findings represent a potential reservoir for mastitis pathogens which may eventually infect an udder quarter; however the existence of a reservoir does not imply an infection
- cow-associated pathogens, e. g. *S. aureus* or streptococci are unlikely to be detected via this method.

Those practitioners who participated in this survey have not fully internalised the use and the limitation of swabbing milking machines, as many sent in samples from sites that pose no direct risk of contaminating the teat during milking. When swabs are drawn keeping in mind the key facts mentioned above, it will be easier to evaluate the results provided by the laboratory, and in returning to fully filled-out preliminary reports, the latter in turn will be able to offer a more goal-oriented service.

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