

Aus dem Institut für Landwirtschaftliche Verfahrenstechnik der  
Christian-Albrechts-Universität zu Kiel  
Prof. Dr. Eberhard Hartung

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**Development and evaluation of methods to assess the  
machine milking process**

Dissertation zur Erlangung des Doktorgrades der Agrar- und Ernährungswissenschaftlichen  
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M.Sc. agr. Daniela Meyer  
aus Rotenburg (Wümme)

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Dekan:	Prof. Dr. Karl H. Mühling
1. Berichterstatter:	Prof. Dr. Eberhard Hartung
2. Berichterstatter:	Prof. Dr. Wolfgang Büscher
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Dekan:	Prof. Dr. Karl H. Mühling
1. Berichterstatter:	Prof. Dr. Eberhard Hartung
2. Berichterstatter:	Prof. Dr. Wolfgang Büscher

Mündliche Prüfung:	Prof. Dr. Eberhard Hartung
	Prof. Dr. Wolfgang Büscher
	Prof. Dr. Georg Thaller
	Prof. Dr. Joachim Krieter

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**Chapter One: General Introduction**

Milk production is a growing agricultural sector, due to a worldwide increasing demand for dairy products. According to calculations of the IFCN (2018), the worldwide milk production will increase by 304 million tons until 2030. The world's leading exporter of milk and dairy products with a relative market share of 27 % is the European Union (EU) (Deutscher Bauernverband, 2019). In line with a growing demand for milk products also the consumers' interest for a sustainable production species-appropriate husbandry systems and animal welfare grows.

Within the EU, the animal welfare and husbandry systems in dairy milk production and requirements on milk quality are generally determined by regulations of the EU and its implementations on national level. Additional requirements on European dairy production are made from trends in society, the market and upstream and downstream industries (BMEL, 2018). Especially the wish of politics and consumers for a sustainable production and high requirements on animal welfare and animal health accompanied by increasing production costs, currently challenges dairy farmers to work profitably, use their resources efficiently and improve animal health and welfare all at once. These fundamental requirements also need to be fulfilled by dairy farmers during the milking process, accounting for a large part of dairy cows' lifetime. For these reasons, the daily milking of dairy cows aims to be quick, complete and gentle – at the same time.

These requirements can only be fulfilled when the technical settings of the milking system, the choice of the milking equipment, the working procedures and characteristics of the dairy herd complement each other. Mismatches lead to losses in profits, decreases milk yield and animal health. The scale and complexity of this issue make dairy farmers searching for help from professional milking advisors. In Germany farmers are in the advantageous position to choose between official advisory services, advisory services offered by companies or self-employed private advisory services (Hoffmann, 2004). Irrespective of the provider, central requirements on advisors are a high level of professional, technical, methodology and social competency (Albrecht et al., 1987). Particularly with regard to the milking process and its complex interaction between the cow, the technical equipment, settings and the farmer, the consultant need to provide interdisciplinary knowledge and advanced methods to assess the machine milking process in respect of its quickness, completeness and gentleness. According to a survey of Gehrdau-Schröder (2017) milking advisors stated that the reason why farmers ask for help vary between farms and its specific requirements. Main reasons were the milk hygiene, the milking system and its settings, the milking routines and the dairy cows' udder health status. The milking advisors had very different educational qualifications.

Most of the respondents of the survey hold a diploma from universities or universities of applied sciences (62 %), 14 % of them in veterinary medicine. The remaining 38 % of the respondents completed an agricultural apprenticeship (19 %), an extension service training (9.5 %) or other non-agricultural apprenticeships (9.5 %). A problem encountered in this process is that there is no official apprenticeship for milking advisors. This could generate the issue that the quality of these services is not in all cases standardized and scientifically sound. Additionally, for milking advisors no standardized guideline is available.

In response to this current issue one main objective of this study was to pool and structure the knowledge about the milking process for advisors and farmers in order to assess the milking process as quick, complete and gentle on a scientific basis. A second main objective of this study was the development and evaluation of methods to assess the machine milking process. The developed methods, with the dairy cow in the focus of the investigation, aimed to be reliable and easy to handle for farmers or advisors in their practical application. The present thesis focuses on the machine milking process in milking parlours. For the above mentioned purposes, the following subtasks were created:

1. Development of a supportive concept to pool the knowledge about influencing factors on the milking process and its interdependencies
2. Development of a new, precisely defined method to assess the completeness of milking of dairy cows by 15 s of hand milking and comparison of this method with two other, already available methods.
3. The evaluation of the precisely defined method with regard to its milking system or cow related influencing factors, which need to be considered in practice use.
4. Evaluation of vacuum records during machine milking by observation of the dairy cows' hind leg activity, teat condition and machine-on time.

The results of these subtasks are described and evaluated in detail in four different chapters in this study.

Chapter Two explains the developed concept. The concept is based on literature review. It offers a guideline for milking advisors in order to obtain a faster overview and easier navigation to the central question what kind of data is needed to be collected and evaluated to solve the farmers' initial problem. Further it facilitates a better understanding of the various interactions between milking parlour and milking system, dairy cow and human. The concept is based on the three fundamental requirements on the milking process: quick, complete and gentle. Moreover this chapter points out occurring consequences when these three requirements are not adjusted to each other. The concept was the basis for the following subtasks, by indicating a need for further research.

In Chapter Three, the new developed, precisely defined hand-milking method was compared to a subjective visual assessment of the quarter filling degree and a method, by which the number of easy strips is counted to assess the completeness of milking. The three methods were compared with regard to its accuracy to predict the remaining rest milk in the udder after machine milking. The problem with existing manual methods was that they depend to a large extent on the accomplishing person and their subjective perception in case of the visual method. Therefore it was tried to develop a precisely defined and standardized hand-milking method in order to prevent the operators' influence. Further, the existing thresholds for hand-milking methods to assess the milking as completed or incompleted are based on an American hand-milking method, so-called "stripping" (Krömker, 2006), which is uncommon in Europe. Therefore, thresholds cannot be transferred and need to be redefined. The three methods were performed by three different evaluators in six herds each.

In Chapter Four, the possibilities and limits of the new, precisely defined hand-milking method are presented in its practical application. In a first step it was evaluated whether the hand-milking must be carried out at all four quarters of a cow or whether it is sufficient to hand-milk only one randomly selected quarter to predict the rest milk in the udder with highest accuracy. In a second step potential cow or the milking system related influencing factors were analysed for their influence on the hand-milked amount of rest milk and the remaining rest milk in the udder itself. This part was conducted in only one of the six herds, by one evaluator.

In Chapter Five, vacuum measurements were evaluated by observation of the dairy cows' hind leg activity and the dairy cows' teat condition. For this objective the vacuum levels in the short milk tube and mouthpiece chamber during milking times were investigated for its relationship with observations on the intensity of dairy cows' hind leg activity. In a second step, the machine-on time and length of single milking phases was investigated for its influence on the frequency of the dairy cows' hind leg activity and the dairy cows' degree of hyperkeratosis. The degree of hyperkeratosis in turn was also evaluated for its impact on the shown frequency and intensity of hind leg activity. Previous investigations have shown that the hind leg activity of dairy cows' is related to physical discomfort and stress, which indicates for ungentle milkings. The investigation was carried out in ten herds and two milking times per herd.

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**Chapter Two: Review of influences on the machine milking process**

The dairy cows' daily milking aims to be quick, complete and gentle. These three basic requirements go hand in hand with the farmers' aim to use resources efficiently, operate profitably and improve steadily the animal welfare and health of the dairy cows.

In order to fulfill and balance these three requirements, it is of great importance for milking advisors to know the various involved influencing factors on the quickness, completeness and gentleness of a machine milking process and its interdependencies.

The various influencing factors and possible methods for its data collection and assessment were carried out by an extensive literature research. In previous publications overviews on influencing and assessable factors of the machine milking process are given, but most publications focus only on segments of the milking process (Reinemann et al., 2001; Jones 1999; Mein 1998). No publication was found, which provides an overview on all conceivable influencing factors on the machine milking process and its available assessment methods.

In consequence of this, a general and new concept was developed, which identifies key areas and assessable influencing factors, called indicators, of the machine milking process. In addition to that, the new developed concept gives information on methodologies to collect the necessary data, the timing when the data need to be collected and the availability of thresholds which are necessary to assess the relevant indicator. The concept could help milking advisors to decide which indicators are necessary to take into account for their assessment.

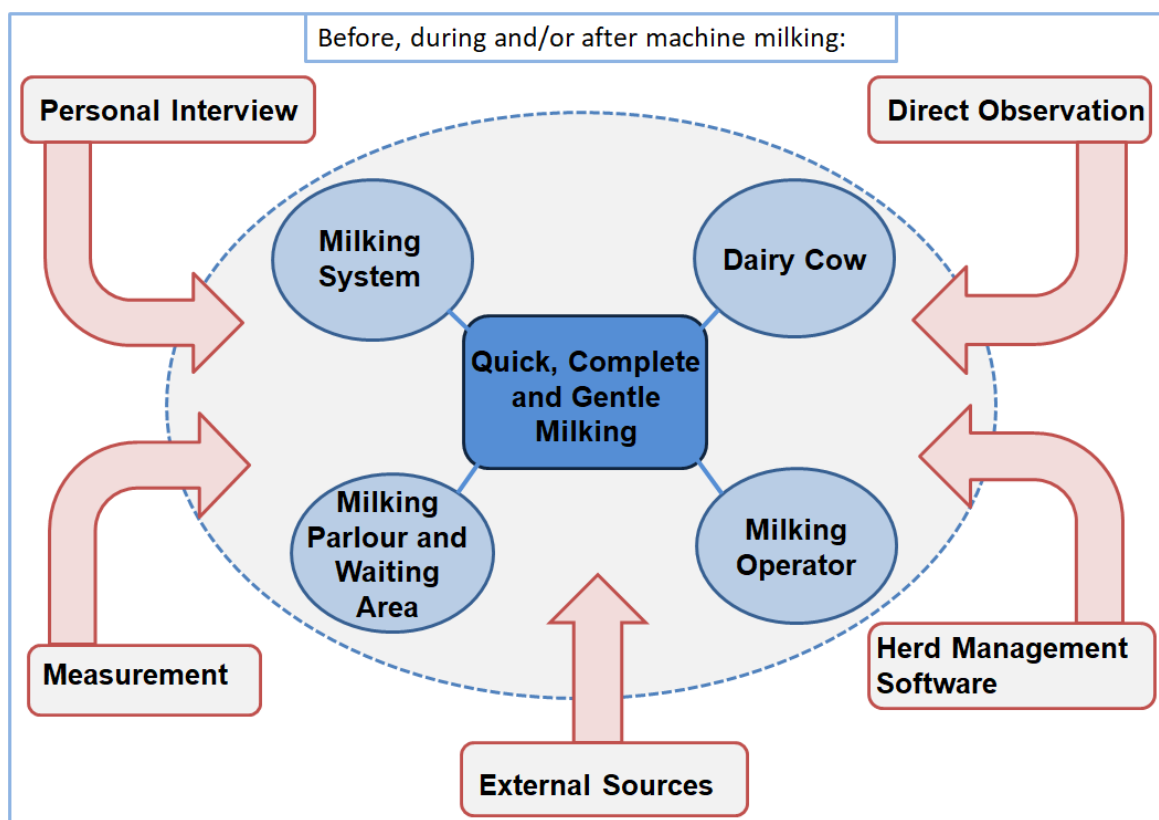
The concept is primarily developed for conventional machine milking in milking parlours and will be introduced in the following subsections of this chapter. Though, it is not a rigid system. Future research will potentially add indicators.

Publications were searched via Google Scholar and Science Direct including years from 1948 to 2020. Main key words used for literature research were: machine milking, milking system, milking vacuum, milking cluster, liner, over milking, mouthpiece chamber vacuum, teat-end vacuum, vacuum fluctuations, teat condition, udder health, automatic cluster remover, milking time test, dry test milking, ISO 5707, ISO 6690, machine-on time, milking routines, milk ejection, animal behaviour, waiting area, milking parlour performance.

## 2.1 Key areas of the machine milking process and methodologies for data collection

In consequence of the literature research and in order to develop a concept for milking advisors, it was started with the identification and determination of key areas of the milking process (Figure 1). For this purpose, it was deliberated who or what is basically needed to perform the machine milking process in milking parlours. In the end, four key areas were identified:

- (1) Milking System
- (2) Dairy Cow
- (3) Milking Operator
- (4) Milking Parlour and Waiting area



**Figure 1** Overview on methodologies and timing to gather information about the indicators of the four key areas: Milking System, Dairy Cow, Milking Operator, Milking Parlour and Waiting Area in order to assess the milking process

Starting from these four key areas, for milking advisors it is of prime importance to know how to collect the necessary information to assess the milking process. As a result from literature research, the following basic methodologies and data sources were identified (Figure 1):

- a. Personal Interview
- b. Direct Observation
- c. Measurement
- d. Herd Management Software
- e. External Sources

In some cases it is conceivable to make use of more than one or choose one from several methodologies, whichever is prioritized by the milking advisor.

In general the data collection can be carried out before, during or after machine milking. In the present study, “during” is understood as the point in time when the machine milking system is turned on and cows start to enter the milking parlour. It ends when the cows leaved the parlour. There are two further options: before milking cluster attachment and after milking cluster detachment. These two options mean to start the data collection immediately before or after the cluster was attached or detached.

## **2.2 Indicators of the four key areas to assess the machine milking process**

Each key area (Figure 1) includes a variable number of contributing indicators, which all have an influence on the successful milk removal and can be used for the assessment of the quickness, completeness and gentleness of the machine milking process by milking advisors. The indicators are summarized in Table A1 in the Appendix. Indicators were assigned to the belonging key area by the “polluter-pays” principle. For each indicator it was deliberated which key area is the relevant originator of the indicator. Timing of the data collection, references from literature for methodologies and thresholds, if available, to assess the indicators are also listed in Table A1 in the Appendix. For some indicators no concrete methodologies or thresholds are given by previous investigations, but constructive proposals were made, based on the literature research, how the assessment could be carried out. The proposals are set in italic font (Appendix Table A1).

In the following subsections of this Chapter, it will be explained how the four key areas and its belonging indicators influence the milking process and why the indicators were chosen to be part of the concept in order to assess the milking process. Short versions of Appendix Table A1 are included in the four subsections (Table 1-4). There are relationships and interdependencies among some indicators, which are of great importance to be understood by milking advisors. They will also be explained in the following. In addition to that, available

and missing research knowledge on required methodologies and thresholds for the assessment of the chosen indicators are summarized in each subsection.

### **2.2.1 Milking System**

The Milking System influences the machine milking process and the milk quality basically in four fundamental ways: by the cleanliness, the vacuum stability, the physical strain of the dairy cows' teat during milking and the automatic cluster remover and stripping arm settings (Table 1).

In a first step, milking advisors could gather general information about technical equipment used, technical settings and threshold values of the equipment, interval of technical services, dates of last service inspection, replacement of liners and certification according to ISO 6690 (2007). Long temporal intervals between technical services, replacements of liners or no certification with ISO 6690 (2007) could indicate for technical shortcomings which influence the milking characteristics (Table 1). Moreover the milking advisor gets a first overview on milking equipment and optional malfunction sources.

In a second step, the jettors, milking units, rubber and silicon parts can be visually checked before machine milking for its grade of cleanliness. Organic contaminations on the surfaces indicate for a deficient disinfection which is closely related to the risk of bacterial transmissions and cross contaminations during the milking times (Jones, 1999) and must be prevented in order to maintain and improve the dairy cows' udder health and the gentleness of the milking. In general, the effectiveness of the milking systems' cleaning and disinfection is also decisive for milk quality in order to prevent high bacterial cell counts in bulk milk (Bava et al., 2011) and can be checked by a cleaning-time test (Rasmussen et al., 2003). Therefore, the bacterial cell count and the cleaning-time test can be found as indicators in Table 1.

**Table 1** Objective of the assessment, chosen indicators and its influence on the machine milking process for the key area Milking System

<b>Objective of assessment</b>		<b>Indicators</b>	<b>Influence on</b>
Risk analysis for technical shortcomings	Quickness Completeness Gentleness	<ul style="list-style-type: none"> <li>- Milking equipment, settings and thresholds</li> <li>- ISO 6690 certification</li> <li>- Last date and regularity of technical services</li> <li>- Last change and regularity of change of liners</li> </ul>	<ul style="list-style-type: none"> <li>- Potential sources of disturbances and mismatches</li> </ul>
Cleanliness	Gentleness	<ul style="list-style-type: none"> <li>- Visual grade of cleanliness</li> <li>- Bacterial cell count in bulk milk</li> <li>- Settings of cleaning and sanitation</li> <li>- Cleaning-time test</li> </ul>	<ul style="list-style-type: none"> <li>- Bacterial transmission</li> <li>- Milk quality</li> </ul>
Vacuum stability	Quickness Gentleness	<ul style="list-style-type: none"> <li>- Dimensions and slope of milk line, dimensions of milk tubes and claws, unblocked vents of milking claws</li> <li>- Visible cracks and audible leakages</li> <li>- Vacuum measurements:               <ul style="list-style-type: none"> <li>o Operating vacuum level</li> <li>o Fall-off test</li> <li>o Cyclic and irregular vacuum fluctuations</li> </ul> </li> <li>- Liner slips (irregular vacuum fluctuations)</li> </ul>	<ul style="list-style-type: none"> <li>- Milk transport</li> <li>- Bacterial transmission</li> <li>- Bacterial invasion</li> </ul>
Physical strain of dairy cows' teat and body	Quickness Completeness Gentleness	<ul style="list-style-type: none"> <li>- Liner choice</li> <li>- Liner condition</li> <li>- Vacuum measurements               <ul style="list-style-type: none"> <li>o Teat-end vacuum</li> <li>o Mouthpiece chamber vacuum</li> <li>o Pulsation settings</li> </ul> </li> <li>- Vacuum/ milk flow measurements:               <ul style="list-style-type: none"> <li>o Length of over-milking phases</li> </ul> </li> <li>- Teat condition</li> </ul>	<ul style="list-style-type: none"> <li>- Teat tissue changes and natural defense against udder pathogens</li> <li>- Milk-out degree</li> <li>- Duration of physical strain</li> <li>- Risk for teat cup crawls/liner slips</li> <li>- Animal behaviour</li> </ul>
Automatic cluster remover/ stripping arm settings & thresholds	Completeness Gentleness	<ul style="list-style-type: none"> <li>- Actual threshold of cluster remover and stripping arm</li> <li>- Milk-out degree               <ul style="list-style-type: none"> <li>o Hand-milking (cf. Chapter Three and Four)</li> <li>o Machine-milking</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>- Milk composition</li> <li>- Milk production</li> <li>- Duration of physical strain of the teat</li> </ul>

In a third step, the slope and dimension of the milk line, the dimensions of the milk tubes and claws should be noted and evaluated (Table 1). A correct dimensioning of milk transporting parts, claws with unblocked vents and a suited vacuum level is important for quick and continuous milk transportation and vacuum stability (Mein and Reinemann, 2014). If the milk is not transported fast enough and continuously away from the udder, the risk for bacterial transmission and udder infections rises (Krömker, 2006). Also the vacuum pump capacity must fit to the chosen dimensions of the milking system (Table 1). Visible cracks or audible leaks in the vacuum system affect the vacuum stability (Jones, 1999). In consequence of this, the risk of bacterial invasion into the dairy cows' udder is increased (Thompson et al., 1978). Moreover leaks could reduce the turbulences during the cleaning and disinfection after machine milking (Mein and Reinemann, 2014). In order to keep the vacuum stable during the course of machine milking, the vacuum pump reserve capacity and regulator response should be able to response quick enough to sudden or unintended air ingresses to ensure a vacuum stability. This can be tested in a fall-off test (Table 1) without milking (dry). Sudden air ingresses may result in respray effects or even impacts by which the milk follows the pressure gradient back to the teat orifice or even into the teat canal. This can cause serious udder infections (O'Shea and Walshe, 1970). The vacuum stability should also be tested while machine milking (milking-time test). The operating vacuum of the milking system should meet the manufacturers' recommendation and be high enough to maintain a quick milk transport and high milk flow rate. Further, it should ensure the adhesion of the teat cups on the dairy cows' teats. Cyclic vacuum fluctuations, caused by the pulsation and irregular vacuum fluctuations, caused by sudden air ingresses (Thiel et al., 1973), can be checked by vacuum measurements (Rønningen, 2017) (Table 1). Audible liner slips or abrupt unit fall-offs during the course of milking are a valuable indicator for an initial troubleshooting by milking advisors and give hints for obvious mismatches. Causes should be searched in a further analysis (Table 1). Liner slips indicate for a poor friction between liner barrel and the cows' teat, due to a mismatching liner size, cluster weight, teat-end and mouthpiece chamber vacuum level (Mein and Reinemann, 2014). Liner slips lead to vacuum fluctuations and increase the risk of udder infections and bacterial transmissions. The sudden air inrush through one (or several) teat cup(s) can generate transient pressure differences by which milk droplets can be transported with great speeds towards the teat orifice and into the teat canal of adjacent teats (Nyhan & Cowhig, 1967).

In a fourth step, the choice and condition of the liner should be evaluated by milking advisors (Table 1). It represents the direct connection between the dairy cow and the milking system and influences the quickness, completeness and gentleness of the milk removal by its size and massage efficiency. The milking vacuum induces an accumulation of blood and lymph in

the teat apex, which needs to be returned by the massage of the liner. This can only be achieved when the liner is regularly checked for twists and potential damages. Also the liners' fit into the teat cup shell should be checked to ensure the right tension of the barrel. A poor condition and proceeded age of the rubber liner of a milking system lead to slower milking, poor teat condition (Boest et al., 2008), higher vacuum values in the mouthpiece chamber and also higher strip yield (Davis et al., 2000) and liner slips (Reinemann et al., 2002), which goes hand in hand with ungentle and incomplete milking. This can be prevented when the liner is regularly changed (Table 1). Generally, the choice of the liner size should be based on the dairy herds' average teat length and width (cf. Dairy Cow). The liners' movement during the course of milking is determined by the settings of pulsation rate and pulsation ratio. These settings are decisive for a quick and gentle milk removal. The liners' material and its tension in accordance with the teat-end vacuum level and its pressure difference compared to pulsation chamber has an important influence on the compression of the dairy cows' teat. The liners' movement during the course of milking, from closed to opened, can be divided into four phases (a, b, c and d). In phase a the liner start to (re-) open, in phase b the liner is open, in phases c the liner closes and in phase d the liner is closed. Phases a and b together are the milking phase, phase c and d the massage phase. The ratio between the liners' milking and massage phases and the forces which are applied to the teat in the four phases are decisive for the quickness and gentleness of the milking process. Beside the pulsation settings and the liners' choice, the teat-end vacuum level and the vacuum level in the mouthpiece chamber are important indicators for the physical strain of the dairy cows' teat (Table 1). The teat-end vacuum level is dependent from milk flow, the change in elevation from teat cup to milk line (Mein and Reinemann, 2014) and the liners' fit (Newman et al. 1991). In periods of high milk flow rates, the teat-end vacuum level drops, while in periods of low milk flow the vacuum level rises (Ambord and Bruckmaier, 2010). Particularly the teat-end vacuum influences the quickness of the milking. According to Dodd and Clough (1959), a raising vacuum level increases the peak milking rate. Though, the milking rate is not continuously rising with increase of the vacuum level. At some point there is a negative return and the milk flow rate is reduced by teat congestion in early periods of low milk flow (Mein and Reinemann, 2014).

The level of the mouthpiece chamber vacuum is dependent from the liners' fit and the teat length and width (Borkhus and Rønningen, 2003). Further, it affects primarily the adhesion of the milking cluster at the dairy cows' teat. Moreover, it has been associated with increased hind leg activity (cf. Chapter Five) and risk for teat congestions (Penry et al., 2017). In periods of low milk flow rates, often occurring in the end of the milking process and sometimes also in the beginning, due to bimodality, the vacuum levels in the mouthpiece

chamber rise. At this point, the teat cistern cannot re-fill with milk fast enough, the teat shrinks slightly and the friction between teat and barrel is reduced. The teat cups are at risk to slip down or crawl up the teat (Mein et al., 1973). Teat cup crawls can result in visible teat tissue changes and congestions at teat base (Neijenhuis et al., 2001a), which can have impact on the completeness of milking (Mein and Reinemann, 2014). The influences of the teat-end and mouthpiece chamber vacuum on the milking process and the dairy cows' behaviour are described in Chapter Five.

The milking phase of low or absent milk flow near to the end of the milking is named over-milking. This milking phase should be kept as short as possible. While in high milk flow periods the teat-end vacuum is reduced, in low milk flow periods the teat-end vacuum level is nearly the operating vacuum (Ambord and Bruckmaier, 2010). Over-milking is associated with higher degrees of hyperkeratosis and higher incidence of mastitis (Natzke, 1978; Cerqueira et al., 2018). The lengths of over-milking phases can be derived by vacuum measurements (Rønningen, 2017) or milk flow measurements (Tančín et al., 2006). According to Rasmussen (2004), conclusions to the presence of over-milking can be drawn from palpable rings at teat base after milking and restlessness or kicking behaviour shown by the cows near the end of milkings.

The physical strain of the teat during the machine milking can be evaluated best by the assessment of the teat condition (Table 1). The teat condition sums up short- and medium-term effects as changes of the teat tissue in form of colour, swellings and firmness and also medium- and long-term effects as changes in teat skin and teat-end hyperkeratosis (Mein et al., 2001). In exception of the dairy cows' teat skin changes (cf. Milking Operator), main causes for poor teat condition are the teat-end and mouthpiece chamber vacuum level, length of over-milking, liners' fit and pulsation settings (Mein et al., 2001). Poor teat condition affects the natural defense of the teat negatively (Krömker, 2006) what can result in deteriorated udder health status of dairy herds and finally more cases of mastitis. Additionally, hyperkeratosis can increase the possibilities of adhesion for pathogens as *E.Coli* and *Sc. uberis* at teat-end (Paduch et al., 2012) and teat disinfection could be less effective with high degrees of hyperkeratosis (Neijenhuis et al., 2001a). Hyperkeratosis also can increase the SCC of dairy cows (Emre and Alaçam, 2015) and change with parity and stage of lactation (Neijenhuis et al., 2001a). The teat condition influences not only the gentleness, but also the quickness of milking. Congestions in the teat apex lead to lower milk flow rates (Hamann and Mein, 1990), what in turn increases the milking time and decreases the milking parlour performance. The teat condition is a valuable indicator for an initial troubleshooting and can be followed up by a further analysis of causes to improve the gentleness and quickness of the milking process. In Chapter Five, the influence of hyperkeratosis on the dairy cows' hind

leg activity during milking and the influence of the machine-on time and the vacuum level in short milk tube on the degree of hyperkeratosis are investigated.

In a fifth step, the additional milking equipment as the automatic cluster remover and automatic stripping arm, if available, should be checked for its threshold values. The automatic cluster remover is beside other factors which are presented in the section “Dairy Cow” and “Milking Operator”, decisive for the milk-out degree of the dairy cow. Moreover, the automatic cluster remover settings can shorten the periods of low milk flow in the end of the milking, resulting in shorter physical strain of the dairy cows’ teat. The settings and thresholds of some automatic cluster removers and dipping arms can be evaluated for its functions by a wet test with water instead of milk, depending on the measurement principle. The implications of the automatic cluster remover settings on the milking process, udder health and milk production and methods to determine the dairy cows’ milk-out degree are reported and investigated in Chapter Three.

#### *Available methods and thresholds to assess the chosen indicators*

Requirements on temperature, concentrations of disinfectants, water quality, time and vacuum level for cleaning and disinfection of the milking system are recommended by the manufacturer. The results from the milking advisors measurements and cleaning-time tests can be compared with these requirements. The visual assessment of the grade of cleanliness is based on a subjective impression of the milking advisor. No thresholds were found in literature. In Reinemann et al. (2000), it is explained which conclusions can be drawn from the visual inspection of biofilms on the milking equipment. The calculation of needed relations between vacuum pump capacity and dimensions of milk line, milk tubes and claws is given by ISO 6690 (2007). In addition to that also manufacturer’s recommendations are given. The operating vacuum, pulsation settings and regulator response can be tested without machine milking (dry). Procedures for testing, recommendations and thresholds are given by the manufacturer or ISO 6690 (2007) (cf. Appendix Table A1). Cracks or leakages should not be visible or audible (Jones, 1999), thresholds are not given for this indicator. Vacuum fluctuations can be derived by vacuum measurements of some vacuum measurement devices, as explained by Rønningen (2017), but no thresholds are available. Though, vacuum fluctuations should be as low as possible (Nyhan and Cowhig, 1967). Hearable and visible liner slips indicate for mismatches. According to Mein and Reid (1996) less than 5 % of cow milkings should need corrections by the milking operator. There are instructions available to choose the right size of the liner. The barrel diameter of the liner should be 1 mm or 2 mm less than the average herds’ teat

diameter (Reinemann et al., 2001). For the visual assessment of liners' condition no threshold was found. Though, damages and twisted liners should be avoided (Jones, 1999). Further, the useful life should not be exceeded (Davis et al., 2000). Currently, there is a method missing for practice use to measure the compression load of the liner. Nevertheless, an insufficient compression load could lead to a visible poor teat condition. According to ISO 6690 (2007), the teat-end vacuum should be in a range between 32 kPa and 42 kPa. According to Rønningen (2011) the mouthpiece chamber vacuum should be in a range between 10 kPa and 30 kPa. Thresholds for the evaluation of the pulsation settings are given in ISO 6690 (2007). According to Reinemann et al. (2001), d-phase should be at least 150 ms. There are no thresholds available for the length of over-milking phases, but over-milking should be kept as short as possible, due to its impact on teat condition (Cerqueira et al., 2018) and udder health (Natzke, 1978). The evaluation of the liners' fit, pulsation settings, teat-end and mouthpiece chamber vacuum can also be done by evaluation of the teat condition. There are clear instructions and thresholds for the assessment of the teat condition available in Mein et al. (2001): less than 20 % of the cows should be classified with changes of the teat tissue.

Beside other factors, the automatic cluster remover settings influence the milk-out degree of the dairy cows after machine milking. The determination of the milk-out degree is the only method to determine the completeness of the machine milking process. There are hand-milking methods and machine-milking methods described in literature (cf. Chapter Three). Also thresholds are available (Davis and Reinemann, 2001; Reinemann et al., 2001; Joe et al., 2010). Nevertheless, it was doubted, if hand-milking methods and described thresholds in literature and studies from America can be transferred to European conditions. While in American countries often the hand-milking is performed by so-called "stripping" (Krömker, 2006), the Europeans often use the "full-hand" method for hand-milking (Krömker, 2006). The stripping method uses only two fingers and can be faster performed than the full-hand method. Therefore by stripping it could be milked more milk per second. Furthermore in literature, an influence of evaluators on the amount of strip yield collected by hand-milking methods is reported (Davis et al., 2000). In consequence of this it was decided to develop a hand-milking method with a precisely defined handgrip frequency (Chapter Three) and compare this method with a visual method to assess the milk-out degree by the quarter filling and a method, which counts the numbers of easy strips with regard to its ability to predict the remaining milk in the udder. Both methods are already used in practice. Based on this, it was evaluated whether the influence of the evaluators on the outcome of the new precisely defined hand-milking method can be avoided by the given definitions for the hand milking handgrip and grip frequency. In continuation of this research, it was further investigated

whether milking system and cow related factors influence the remaining amount of milk in the udder after machine milking and which factors influence the new developed method to collect the strip yield (Chapter Four).

### 2.2.2 Dairy Cow

Beside the milking system, the dairy herd and individual cows of a herd are essential to take into account for the assessment of a successful milk removal. Only if milking system and dairy herd are harmonized with one another, the milk removal is gentle for the cows and do not affect their udder health negatively.

The dairy cow influences the milking process to a large extent by its genetic predisposition, which is essentially dependent from its breed. The breed and genetic roots affect the milk composition, milk production, body size (Dillon et al., 2003), teat morphology (Chrystal et al., 1999) and probably the dairy cows' individual character. In addition to that, the stage of lactation and parity affects the milk production, milk composition (Rook and Campling, 1965) and animal behaviour (Szentléleki et al. 2015). Also the somatic cell count is affected by the stage of lactation and parity in dairy cows with prior udder infections (Laevens et al., 1997). Therefore, it is helpful for milking advisors to start in a first step with getting an overview on these characteristics of the observed dairy herd (Table 2).

**Table 2** Objective of the assessment, chosen indicators and its influence on the machine milking process for the key area Dairy Cow

Objective of assessment		Indicators	Influence on
Herd characteristics	Quickness Gentleness	- Overview on herd characteristics <ul style="list-style-type: none"> <li>○ Breed</li> <li>○ Parity</li> <li>○ Days in milk</li> </ul>	- Character - Milk production - Milk composition - Somatic cell count - Morphologies
Udder health status	Gentleness	- Somatic cell count (herd/cow) - Visual inspection of milk filter	- Milk quality
Sizes and morphologies	Completeness Gentleness	- Teat length and width - Udder and teat morphologies - Body length and width	- Liners' choice - Milking cluster positioning - Size of milking positions
Milking characteristics	Quickness	- Peak milk flow - Average milk flow - Milk yield	- Dimensions of milk transporting parts - Milking parlour performance
Stress level	Completeness Gentleness	- Animal behaviour: <ul style="list-style-type: none"> <li>○ Hind leg behaviour</li> <li>○ Rumination</li> <li>○ Defecation and urination</li> </ul> - Other: <ul style="list-style-type: none"> <li>○ Heart rate</li> <li>○ Cortisol concentrations in milk or blood</li> </ul> - Disturbed milk ejection	- Animal comfort - Milk production - Immune system - Milk ejection - Work safety of milking operator

In a second step, by help of the somatic cell count (SCC) per ml milk of the dairy herds' bulk milk and milk samples of individual dairy cows, information of the udder health status can be derived (Table 2). The somatic cell count is used in Europe as an essential assessment parameter of the milk quality by dairy factories (EU Regulation 1662, 2006). Somatic cells are part of the dairy cows' natural immune reaction and primary cells of the immune system (75 % leucocytes) (Sordillo et al., 1997). Leucocytes increase in response to bacterial infection, injuries of the udder tissue and stress (Sharma et al., 2011). Previous studies reported that healthy, uninfected udder quarters have a mean SCC of approximately 70.000 cells/ml milk (Scheepers et al., 1997). The dairy cow influences the variability of the SCC by its breed, its lactation number and its lactation stage (Scheepers et al., 1997). The reports of the official milk control in Germany with analyses of the individual dairy cows' and the herds' somatic cell count offer a good opportunity to assess the current udder health status and its developments over the last months. Further it gives initial pointers for potential weaknesses in the dairy production system with regard to udder health. For this reason it is chosen as indicator (Table 2). Nevertheless, it must be taken into account, that the milk control report considers only the SCC on udder level. According to DVG (2012), not only the SCC should be determining in order to distinguish between infected and uninfected udders, but also the evidence of present udder pathogens. The dairy cows' mastitis resistance is probably genetically influenced (Carlén et al., 2006). In order to get information about the udder health status of the herd also the milk filter can be visually inspected after milking for the presence of flakes or clots.

As mentioned above, the udder and teat morphologies and the dairy cows' body size depend to a large extent on the breed and its genetic ancestry. For milking advisors, it is necessary to determine the dairy herds' average sizes and morphologies in a third step. The choice of the liner should depend on the herds' average teat diameter and teat length, as already mentioned in the section "Milking System". Only when the liner fits the dairy herds' demands, the milk removal can be performed gently. This could result in difficulties to milk individual cows, when the herds' teat morphology varies enormously. For example, due to different breeds housed in one farm. The chosen liner size can be seen as a compromise and should be regularly assessed. The teat length and diameter of dairy cows change also with advancing lactation (Seykora and McDaniel, 1986). Inappropriate liners, beside other factors mentioned in the previous subsection, can lead to negative changes in teat condition. This can result in higher intensities of hind leg activities and presumably restrictions in physical well-being of dairy cows, as reported in Chapter Five. The degree of hyperkeratosis seems to be influenced by the genetic predisposition of the cow (Neijenhuis et al, 2001a). Furthermore, dairy cows with long and pointed teats, slow milkings or high milk yields seem to be more

affected by high degrees of hyperkeratosis (Emre and Alaçam, 2015). Also teats with pointed teat-ends seem to be at higher risk for teat callosity (Neijenhuis et al., 2001c). The udder and teat morphology can influence the probability of occurrence of liner slips. According to Rogers and Spencer (1991) wider teats and tilted udders are associated with increased liner slips and manual adjustments by the milking operator. The udder and teat morphology should also be taken into account with regard to the assessment of milking cluster positioning. Due to the connection of the four teat cups with the claw in most milking clusters, the alignment of the milking cluster depends decisively on the morphology of the four individual quarter and teats. A correct milking cluster positioning, evenly under the udder, might be difficult in very inhomogeneous quarters. The influence of the milking cluster positioning on the completeness of milking is explained in the second part of Chapter Three. These impacts of the dairy cows' udder and teat morphology on the teat condition and milking cluster positioning are important to take into account for milking advisors. In addition to that, also the dairy cows' body size is an important indicator (Table 2). The size of the milking positions should be adapted to the dairy cows' size (Gómez et al., 2017) in order to avoid discomfort or skin abrasions (cf. Milking Parlour and Waiting Area).

In a fourth step, the milk yield and peak milk flow of the dairy herd is important to know. It determines the needed dimensions of milk lines, milk tubes and claws and the pump capacity. The average milk flow influences the milking parlour performance. The peak milk flow is individual, varies among quarters and is potentially influenced by the length (Weiß et al., 2004) and diameter of the teat canal (Williams and Mein, 1982). High yielding cows are at higher risk for hyperkeratosis (Neijenhuis et al., 2001c).

In a fifth step, for milking advisors it is necessary to understand the behaviour of dairy cows and factors which influence the dairy cows' physiological functions. Generally, negative stressors need urgently to be avoided during the milking procedure. Stressors can be summed up as environmental and social factors or changes of factors which disturb the homeostasis and neuroendocrine system of dairy cows (Johnson and Vanjonack, 1976). In general, the dairy cow answers to stressors with immediate neural and hormonal changes, which can cause increased heart rates, blood pressures and behavioural changes of the animal (Johnson and Vanjonack, 1976). The behavioural changes depend on the dairy cows' previous experiences and its genetic inheritance (Bobić et al., 2011). In previous studies the stress level of cows was assessed by indicators as the heart rate (Bruckmaier et al., 1993), concentration of stress hormones as cortisol in milk or blood (Bruckmaier et al., 1993), rumination (Dijkstra et al., 2012), urination and defecation (Rushen et al., 2001) and hind leg activity (Wenzel et al., 2003) (Table 2). In Chapter Five, the vacuum level in short milk tube and mouthpiece chamber was investigated for its relation to the animals' hind leg activity, as

a sign of discomfort. The observation of the dairy cows' behavioural changes could be used as an indicator of the gentleness of milking and potentially in future as an alert system for mismatches in the milking process (cf. Chapter Five). Persistent stressors can result in long-term effects on reproduction and (milk) production (Johnson and Vanjonack, 1976). Based on these facts, the assessment of the dairy cows' well-being in the milking parlour during the course of milking is not only important with regard to its health and the gentleness of the milking process, but also from the farms' economic point of view. In addition to that, the dairy cows' behaviour is an important aspect for the milking operators' work safety (Cavallina et al., 2008).

The smallest amount of dairy cows' milk, only about 20 %, is stored in the milk cistern. The largest amount is stored in alveoli and can only be milked by milk ejection, a neuroendocrine reflex. By tactile stimulation of the mammary gland, oxytocin is released off the neurohypophysis and transported through the cows' blood system to the myoepithelial cells, which surround the alveoli. The myoepithelial cells' contraction leads to the release of milk from the alveoli to the udder cistern. Only then, the total amount of milk can be milked. In order to initiate this milk release a certain level of oxytocin needs to be exceeded (Bruckmaier and Blum, 1998). The time span between start of the tactile stimulation and milk ejection is between 40 s and 120 s (Bruckmaier, 2005) and increases with the dairy cows' udder filling degree. Therefore, in late stages of lactation dairy cows need longer periods of pre-stimulation than in early stages of lactation (Bruckmaier, 2005), supporting the importance of knowledge on the dairy cows' days in milk, as described in the first step. The milk ejection reflex can be disturbed in stressful situations and unfamiliar environments (Rushen et al., 2001), especially in primiparous cows (Bruckmaier et al., 1992). This could lead to incomplete milking, which is associated with udder infections, which will be explained in Chapter Three.

#### *Available methods and thresholds to assess the chosen indicators*

The informative value of the somatic cell count is well explored and also thresholds to assess the udder health status of a herd and the milk quality are either given by the EU Regulation 1662 (2006), the dairy company or official milk control. The somatic cell counts in bulk milk are measured by the dairy company. Somatic cell counts of individual animals are given by the official milk control report or can be estimated by the California Mastitis Test (Paduch et al., 2013).

Teat width and length of the dairy cows' body and teats do not need examples or thresholds and can be measured by the milking advisors. The classification of different types of udder

and teat morphologies is also already described in literature (Chrystal et al., 2001; Hickman, 1964; Bakken 1981; Grunert, 1990). For the measurement of peak milk flow rate and milk yield methods and target values are known (Mein, 1998; Marony et al., 2004). The average milk flow can be calculated by the division of the milk yield by the length of the machine-on time (Reinemann et al., 2001).

Several studies have tried to evaluate the dairy cows' stress level, not all of them in the milking parlor. The stress was either measured by cortisol concentrations in blood (Hopster et al., 2002) and milk (Gygax et al., 2006) or the heart rate (Rushen et al., 1999). The measurement of hormone concentrations in the animals' blood must be carried out by a veterinary and also the analysis of cortisol concentrations in the milk is linked with relatively great effort. The measurement of the heart rate needs costly equipment and preparation time. Attempts were made to find indicators to assess the dairy cows' stress and physical discomfort by obvious external signs. This includes the urination and defecation rate (Rushen et al., 2001), rumination (Bristow and Holmes, 2007) or the hind leg activity (Rousing et al., 2004). While the observation of urination and defecation rate and the dairy cows' rumination could be difficult, depending on the type of the milking parlor, its design and the availability of manure gutter, the hind leg activity can be observed easily in all types of milking parlours. Currently, there is no threshold for the animals' behaviour available by which it can be differentiated between stressed and unstressed dairy cows in the milking parlour. Previous studies indicated that the hind leg activities stepping and kicking increase in cases of physical discomfort (Rousing et al., 2004; Cerqueira et al., 2017) and stress (Rushen et al., 2001). Rasmussen (2004) and Cerqueira et al. (2017) reported that an increase of hind leg activity can be observed in over-milking phases. During over-milkings the teat-end vacuum is nearly at the same height as the operation vacuum level. In Cerqueira et al. (2017), the over-milking phase was derived by milk flow curves. No study was found, which has done the observation of hind leg activity and real-time vacuum measurements during the course of milking simultaneously. As explained in the subsection "Milking System", quicker milking can be achieved by higher teat-end vacuum levels. High vacuum levels in turn might result in reduced gentleness of the milking process, due to the physical strain of the teat in low milk flow periods. Also extended milking times could reduce the gentleness of the milking process (Mein and Reinemann, 2014). In consequence of this, with regard to the gentleness and quickness of the milk removal, it was evaluated in Chapter Five, whether the intensity or frequency of dairy cows' hind leg activity is related to the vacuum level in the short milk tube and mouthpiece chamber or the machine-on time. The machine-on time was also evaluated for its impact on the dairy cows' hyperkeratosis. Additionally, the grade of hyperkeratosis in turn was also tested for its influence on the frequency and intensity of shown hind leg activity.

### 2.2.3 Milking Operator

The milking operator can be seen as an essential link between dairy cow and milking system. Effects on the machine milking process are basically due to his/her chosen procedures with respect to the prevention of bacterial transmission, improvement of the milk-out degree and vacuum stability, interactions with the dairy cow, the milking time management and his/her working efficiency (Table 3).

The biggest influence on the milk removal has the milking operator by the chosen milking procedures with respect to bacterial transmission, milk-out degree and vacuum stability. In a first step, the prevention of bacterial transmission between cows during the milking time could be assessed. The dairy cows with udder infections should be milked in a separate group and last (Ruegg et al., 2000) (Table 3). Further, bacterial transmission can be prevented best by the performance of the following milking routines: wearing gloves and keeping the gloves clean (Olde Riekerink et al, 2008), carry out the fore stripping (Wagner and Ruegg, 2002), clean the teats wet (Galton et al., 1988) and use intermediate disinfection of the milking clusters, at least after cows udder health issues (Table 3). The teat cleaning can be optimized by the usage of pre-dips (Galton et al., 1988). In Europe, the fore-stripping and teat cleaning is mandatory (EU Regulation 1662, 2006). The fore stripping before teat cleaning has positive impacts on the milk ejection reflex due to the tactile appeal. This can result in reduced machine-on time (Bruckmaier and Blum, 1996). Pankey (1989) reported a relationship between teat and udder cleanliness and the risk of bacterial transmission during the milking process. According to Zucali et al. (2011), the cleaning routines can affect the bacterial and somatic cell count in milk. Schreiner and Ruegg (2003) reported a relationship between udder cleanliness and intramammary infections. For these reasons, the assessment of the effectiveness of the teat and udder cleaning by the milking operator is chosen as indicator (Table 3).

**Table 3** Objective of the assessment, chosen indicators and its influence on the machine milking process for the key area Milking Operator

Objective of assessment		Indicators	Influence on
Bacterial transmission	Quickness Gentleness	<ul style="list-style-type: none"> <li>- Milking order</li> <li>- Milking routines:               <ul style="list-style-type: none"> <li>o Wearing gloves</li> <li>o Washing hands</li> <li>o Fore-stripping</li> <li>o Choice and concentration of pre milking teat disinfection</li> <li>o Cleaning of udder and teats</li> <li>o Intermediate disinfections</li> <li>o Choice and concentration of post milking teat disinfection</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>- Prevention of bacterial transmission</li> <li>- Prevention of udder infections</li> <li>- Milk ejection reflex</li> </ul>
Milk-out degree	Completeness Gentleness	<ul style="list-style-type: none"> <li>- Milking routines:               <ul style="list-style-type: none"> <li>o Duration of stimulation</li> <li>o Duration of tactile appeal</li> <li>o Milking cluster positioning</li> </ul> </li> <li>- Milk flow curves:               <ul style="list-style-type: none"> <li>o Bimodality</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>- Milk ejection reflex</li> <li>- Milking in absence of milk flow – physical strain of the teat</li> </ul>
Vacuum stability	Quickness Gentleness	<ul style="list-style-type: none"> <li>- Milking routines:               <ul style="list-style-type: none"> <li>o Attachment routine</li> <li>o Attachment interval</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>- Vacuum fluctuations</li> <li>- Risk for udder infections</li> </ul>
Interactions with the dairy cow	Quickness Gentleness	<ul style="list-style-type: none"> <li>- Human-animal-interaction in milking parlour and while herding of the cows into the parlour</li> </ul>	<ul style="list-style-type: none"> <li>- Stress for dairy cows</li> <li>- Milking parlour performance</li> </ul>
Milking time management	Gentleness	<ul style="list-style-type: none"> <li>- Milking intervals</li> <li>- Milking times per day</li> </ul>	<ul style="list-style-type: none"> <li>- Milk production</li> <li>- Milk composition</li> </ul>
Efficiency	Quickness	<ul style="list-style-type: none"> <li>- Cows per milker</li> <li>- Working-time measurements</li> </ul>	<ul style="list-style-type: none"> <li>- Milking parlour performance</li> <li>- Work load of operators</li> </ul>

Milking advisors should assess the effectiveness of udder and teat cleaning performed by the milking operator. The teat should be dry before milking cluster getting attached, in order to decrease the risk of bacterial transmission (Galton et al., 1982) and to avoid crawling of the teat cup or increased liner slips (Spohr, 1998). After machine milking the teat canal need some time to recover and close (Neijenhuis et al., 2001b). The needed time for recovery is longer, as more the teat tissue was congested during the milking process, for example due to a lack of massage by the liner (Neijenhuis et al., 2001b). For this reason, immediately after machine milking cows are at higher risk for udder infections from pathogens on its teat skin or from pathogens in its beddings. Udder infections generate pain, physical discomfort and a serious live risk for dairy cows. For farmers, mastitis lead to higher costs (Krömker, 2006; Heikkilä et al., 2012) and probably the use of antibiotics, what should be avoided for a sustainable milk production. In addition to that, the milking and treatment of cows with mastitis decrease milking parlour performance (Armstrong and Quick, 1986). The risk of udder infections can be reduced by applying disinfectants on the dairy cows' teat after machine milking (Natzke, 1977) (Figure 3). The disinfectant can be applied by dipping or spraying. The choice of the disinfectant can improve or worse the degree of hyperkeratosis, due to high concentrations of disinfectants or emollients (Neijenhuis et al., 2001a). The dairy cows' teat skin condition is influenced by harsh weather conditions and the choice of disinfectant. Low temperatures and high humidity can increase the skin roughness. Thereby skin pathogens as *S. aureus* can easily colonize in skin cracks and increase the risk for udder infections (Neijenhuis et al., 2001a). Disinfection with high concentrations of emollients can improve the skin hydration and elasticity. The skin gets smoother and becomes more resilient to the adhesion of udder pathogens (Neijenhuis et al, 2001a). In consequence, teat disinfection, respectively teat care, as part of the milking routine is chosen as indicator and should be evaluated by the milking advisor (Table 3). The milking routines should remain as constant as possible over time to maintain positive effects on milk yield and milk fat (Rasmussen et al. 1990) – therefore the implementation of standard operating procedures can be helpful in farms with more than one milking operator. The choice and duration of milking routines combined with the number of cows per milking operators and the milking parlour design affects the milking parlour performance (O'Brien et al., 2012).

In order to enable the milking of alveolar milk, the release of oxytocin is mandatory (cf. Dairy Cow). Therefore, the milking routines with regard to teat stimulation should be assessed by the milking operator in a second step. As reported in Bruckmaier and Blum (1996) there is a positive correlation between the temporal length of the tactile appeal and the release concentration of oxytocin in the dairy cows' blood. The stimulation needs to be continuous during the whole course of milking, applied by the liner, to ensure elevated concentrations of

oxytocin and to enable the milk removal. An interruption of milk flow after removal of the cistern milk and time lag until milk ejection can be seen in bimodal milk flow curves. In consequence of this, the bimodality in milk flow curves is an indicator of great importance for milking advisor (Table 3). In case of bimodal milk flow curves, the teat cups are at risk to crawl, due to a rising mouthpiece chamber vacuum level in the teat cup. Milking is at risk to be incomplete (Mein and Reinemann, 2014). The positioning of milking cluster should be positioned correctly by the milking operator, to ensure complete milking (Figure 3). As reported in Chapter Four, poorly positioned milking clusters can increase the amount of rest milk in the udder.

In a third step, the influence of the milking operator on the vacuum stability during the course of milking by unit attachment practices can be assessed. During the unit attachment as less air as possible should be admitted to the milking system. The number of simultaneously attached milking units is decisive for the needed vacuum reserve. In addition to that, the milking operator should monitor the attached milking units during the whole course of milking and if necessary adjust and re-attach immediately.

Dairy cows are social animals and the milking operator or farmer is an important contact person for them. They can remember and avoid humans who treat them badly (Rushen et al., 1999) and also calm down and relax in the presence of humans they linked to positive experiences (Hemsworth et al., 1989). As already mentioned above (cf. Dairy Cow), stress of dairy cows can have negative effects on animal welfare, milk production, milk ejection and work safety of the milking operator, resulting in negative impacts on the milking parlour performance, the gentleness and completeness of the milking process. According to Hemsworth et al., (2000), negative interactions can reduce milk yield, fat and protein content of the milk and increase cortisol concentrations in milk. These findings are profound reasons that milking advisors should observe and assess the human-animal-interactions in milking parlours and also during the herding of the dairy cows into the milking parlour in a fourth step (Table 3).

In a fifth step, also the milking time management is an important indicator for milking advisors (Table 3). The milking should be performed in equal intervals. Prolonged milking intervals between milking lead to decreased milk production rate. Increased udder fillings, due to long milking intervals, reduce the milks' fat content (Weiß et al., 2002), which is decisive for the payout price for milk.

The milking parlour performance and efficiency of milking operator can be assessed by milking advisors in a sixth step with help of working time measurements (Table 3). On this basis it can be evaluated how many cows can be milked per operator and whether there are options to save valuable time through the milking process in order to improve milking parlour

performance. The milking parlour performance is also influenced by the number of cows which are prepared and attached per milker. The procedure of simultaneous milking cluster attachments is also decisive for the dimensions of milk transporting parts of the milking system (cf. Milking System) (Table 3).

*Available methods and thresholds to assess the chosen indicators*

The indicators of the bacterial transmission are well investigated. The milking order and milking routines can be observed by milking advisors and compared with claimed standard operations procedures by the farmer, if available. Otherwise, they can be noted and assessed by its consistent performance. The accuracy of the teat cleaning and teat dipping, respectively spraying, can be assessed by milking advisors and is described in previous investigations (Reinemann et al., 2001; Ostendorf, 2017; Drieling, 2017). The duration of teat stimulation can be measured and assessed by comparison with given targets by literature or assessed by the presence or absence of bimodal milk flow curves (cf. Appendix Table A1). The milking operators' attachment practices can be observed and compared to the chosen dimensions and slope of the milk line (ISO 6690, 2007). Air admissions during unit attachment and milking operators' reactions to unit fall-offs should be as low and short as possible (Reinemann and Mein, 1996; Reinemann et al., 2001). The human-animal-interaction is observed in Prüser (2017) and Hemsworth et al. (2000), though no threshold values are available and Prüser (2017) reported difficulties encountered with the observation and its interpretation. The milking time management and milking parlour performance can be assessed by direct observations or by information from herd management software (cf. Appendix Table Table A1). There are examples for working-time measurements of milking operators available in literature (Armstrong and Quick, 1986), thresholds are not available but generally results could be compared with results of other farms or previous investigations.

### **2.2.4 Milking Parlour and Waiting Area**

The milking process and animal welfare is influenced by the design of the milking parlour and waiting area, the attendance time of the cows, the work environment of the milking operator and the milk-out degree, enabled by the parlour construction (Table 4).

In a first step, the milking advisor can collect information about the milking parlor type, its number of milking positions and its exit design (Table 4). All three have an essential impact on the milking parlour performance (Armstrong and Quick, 1986). The dimensions of the entrance of the milking parlour and the milking positions should ensure a relaxed body and working posture for the dairy cows in order ensure a quick and gentle milk removal. Many milking positions in combination only one exit in the end of the row will increase the needed time for cows to reach the milking positions and to leave the milking parlour after milking (Armstrong and Quick, 1986). The size of the milking positions should be adapted to the average body size of the herd (Gómez et al., 2017). Wounds, bald spots and skin abrasions could give valuable hints in an initial troubleshooting for too small dimensions of the milking positions (Figure 4). Gómez et al. (2017) indicated that large milking positions could increase the animal welfare.

**Table 4** Objective of the assessment, chosen indicators and its influence on the machine milking process for the key area Milking Parlour and Waiting Area

Objective of assessment		Indicators	Influences
Milking parlour design	Quickness Completeness Gentleness	<ul style="list-style-type: none"> <li>- Type of parlour; number and size of milking positions; exit type (one-end/rapid exit)</li> <li>- Wounds, bald spots, skin abrasions</li> <li>- Design:               <ul style="list-style-type: none"> <li>o Ramps, steps and obstacles, narrow curves, sharp edges</li> <li>o Lighting; Volume level; Flooring; Insects; Temperature</li> <li>o Drinking troughs</li> <li>o Concentrated feed</li> </ul> </li> <li>- Electrical current</li> <li>- Willingness to enter and to leave the parlour</li> </ul>	<ul style="list-style-type: none"> <li>- Milking parlour performance</li> <li>- Animal welfare</li> <li>- Animal behaviour</li> <li>- Risk for injuries</li> <li>- Body posture of cows during milking</li> </ul>
Waiting area design	Quickness Completeness Gentleness	<ul style="list-style-type: none"> <li>- Size and number of cows waiting simultaneously</li> <li>- Antagonistic behaviour</li> <li>- Injuries/Wounds</li> <li>- Passageway from stable to waiting area and waiting area itself:               <ul style="list-style-type: none"> <li>o Ramps, steps and obstacles, narrow curves, sharp edges</li> <li>o Lighting; Volume level; Flooring; Insects; Temperature</li> <li>o Drinking troughs</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>- Milking parlour performance</li> <li>- Animal welfare</li> <li>- Animal behaviour</li> <li>- Risk for injuries</li> </ul>
Attendance time	Gentleness	<ul style="list-style-type: none"> <li>- Attendance time while milking</li> <li>- Attendance time while waiting</li> </ul>	<ul style="list-style-type: none"> <li>- Animal behaviour</li> <li>- Milk production</li> </ul>
Work environment for milking operator	Quickness Gentleness	<ul style="list-style-type: none"> <li>- (Variable) operating height for milking operator</li> <li>- Body posture while attachment of milking cluster</li> <li>- Stairs; Flooring; Temperature; Lighting; Volume level; Insects</li> </ul>	<ul style="list-style-type: none"> <li>- Body posture while working</li> <li>- Safety at work</li> <li>- Health protection</li> <li>- Efficiency</li> </ul>
Milk-out degree	Completeness Gentleness	<ul style="list-style-type: none"> <li>- Constructional possibilities for correct milking cluster positioning</li> </ul>	<ul style="list-style-type: none"> <li>- Milk composition</li> <li>- Milk production</li> <li>- Udder health</li> </ul>

Steps, obstacles or narrow curves could slow down the dairy cows' will to enter or leave the milking parlour, which in turn could decrease the milking parlour performance. Due to the fact, that dairy cows can adapt from brightness to darkness not as quick as humans (Dimberton, 1999), the lighting in the passageway from waiting area into milking parlour and from milking parlour to stable should be uniform and adjusted to the cows' needs. A missing or wrong lighting can be the reason why the cows enter or leave the milking parlour hesitantly. According to Abrosimova (1978), a higher light intensity can shorten the milking process by 8 % to 12 %. The flooring in entrance and exit areas should be absolutely slip-resistant in order to prevent the cows from linking negative experiences with the milking parlour or the milking process and in order to prevent injuries and pain. Cows are able to learn and are unwilling to return to areas they link with negative experiences (Pajor et al., 2000). A slippery floor and the experience of slipping, possibly accompanied by pain and fear, can cause an unwillingness to enter the milking parlour and result in poor milking parlour performance (Table 4). In addition to that, Gudaj et al. (2009) reported that the installation of rubber mats in the milking parlour can increase the dairy cows' motivation to enter the parlour. Also the temperature and humidity in waiting area and milking parlour are valuable indicators for the gentleness of the milking process (Table 4). Heat stress should urgently be avoided and the climate should be adjusted to the cows' preferences. In case of German-Holstein dairy cows its thermal comfort zone is in a range between 5 C° and 25 C° (McDowell, 1972). High temperatures can cause changes in milk composition, reduce milk yield (Gorniak et al., 2014) and increase hind leg activity (Cerqueira et al., 2017). The presence of insects as stable flies is chosen to be an indicator for the gentleness of the milking process (Table 4). They affect the cows' welfare and health negatively. According to Vitela-Mendoza et al. (2016), stable flies influence the cortisol concentration in the blood, tail movements and leg activity of beef cattle. An increased hind leg activity of dairy cows represents a risk for the milking operator and can influence the milking parlour performance negatively by more defensive behaviour of the cows. Increased animal hind leg activity, during machine milking could also be caused by electrical current, which should be urgently be avoided in machine milking systems (Henke Drenkard et al., 1985; Reinemann et al., 2002). According to Kauke and Savary (2010), dairy cows react to loud noises with signs of fear, increased defecation and heart rate. A missing willingness of the dairy cows to voluntarily enter the milking parlour could indicate for high stress levels due to the indicators mentioned above and could be used as an indicator for an initial troubleshooting to assess the quickness and gentleness of the milking process.

In a second step, the waiting area should be considered in the assessment of the milking process. During the waiting time and later in the milking parlor, the cows are standing close

to each other and low-ranking cows have no possibility to avoid antagonistic behaviour of high-ranking cows (Dijkstra et al., 2012). Confined space conditions, especially in the waiting area can cause stress and increase antagonistic behaviour (Donaldson et al., 1972) and in turn higher risks of injuries. According to Irrgang et al. (2015), small space allowance in the waiting area lead to increased heart rates, which are still elevated during the milking process. Therefore, the observation of antagonistic behaviour is chosen as indicator (Table 4). Stress can have negative impacts on the milk ejection (cf. Dairy Cow). For those reasons, waiting areas should offer enough space, to enable low-ranking cows to evade from high-ranking cows. Furthermore, drinking trough(s) should be installed in the waiting area. After machine milking dairy cows have an increased need for water intake (Cardot et al., 2008). Therefore, the installation of drinking troughs in the parlour or in the stable is important for animal welfare and health. Though, the installation in the passageway from milking parlour to stable could lead to crowds and congestions and decreased milking parlour performance. The passageway from stable to waiting area and from milking parlour back to the stable should be also considered in evaluation. It should be appropriate for lame and ill dairy cows with slip-resistant flooring and without steps or obstacles and an appropriate lighting.

The attendance time of cows in the milking parlour and waiting area before machine milking, could be assessed by the milking advisors in a third step. In parlour and waiting area, the dairy cows are kept away from feeding, drinking and possibilities for lying down. The dairy cows' frustration about inability to carry out its desired behaviours can cause stress and loss of well-being (Dijkstra et al., 2012). Furthermore, dairy cows feel uncomfortable when forced to stand for long periods (Cooper et al., 2007).

In a fourth step, the work environment for the milking operator can be evaluated. In case of changing milking operators with different body heights it might be useful to plan the milking parlour with a height-adjustable floor in order to improve the body postures and prevent joint or back pain (Cockburn et al., 2015). While attachment of the milking cluster, the milking operator should be protected by the milking parlour construction from dairy cows' kicking behaviour. The floor and potential stairs should be slip-resistant and the volume level of the machine milking system should be as low as possible. Air quality, temperature and lighting should be optimized for a healthy and pleasant working environment. The number of insects as stable flies should be reduced to a minimum.

In a fifth step, the milking parlour design should be checked for its ability to position the milking clusters correctly. If the milking system installation offers no possibilities for a correct positioning of the long milk tube or the ability to fix the long milk tube in its desired position, the milking cluster is not prevented from twisting or pushing/pressing upwards/downwards the milking cluster.

*Available methods and thresholds to assess the chosen indicators*

There are previous investigations available on the effects of the milking parlour size, type and design on the milking parlour performance (Armstrong et al., 1990; Hillerton et al., 2002). The size of milking positions can be measured and compared to the average body size of the herd as basically done in Gómez et al., 2017. The observation of wounds can be a valuable indicator for discomfort due to wrong sizes of milking places, but no method or threshold is available in previous literature. Light intensity and temperatures can be measured and compared to target values (Appendix Table A1). The preferred temperature of dairy cows is known and mentioned above (McDowell, 1972). According to Phillips (2009), thresholds for discomfort with regard to the volume level could be set between 90 dB and 100 dB. As reported by the American Society of Agricultural and Biological Engineers (ASABE, 2006), the light intensity should be at least 500 lx in milking parlours. Insects should be as few as possible, but no thresholds are given. The flooring could be assessed by observation of slipped cows per milking or the step length as done in Telezhenko and Bergsten (2005). Electrical current during machine milking can be measured and need to be avoided (Reinemann et al., 2002). The assessment of the milking parlour design could also be derived by the observation of the dairy cows' willingness and quickness to enter and to leave the milking parlour as for example done in Gómez et al. (2017), but no thresholds are given for this assessment by literature. Congestions, due to the buildings' design or installations of drinking troughs at inappropriate places decrease the milking parlour performance and can be easily observed by the milking advisor. Approaches to observe antagonistic behaviour are described well in literature, for example in Rousing and Wemelsfelder (2006). The dairy cows' attendance time should be kept as short as possible in waiting area and milking parlour. The working environment of the milking operator could be assessed by a personal interview with the milking operators and the observation of the milking operator during his/her work procedure. The positioning of the milking cluster can be observed and assessed for its correct position. An example for the definition of correct cluster positioning is given in Chapter Four. No thresholds are given in literature for a maximum of incorrect milking cluster positions during the milking process, but incorrect positions should be kept as low as possible, due to its effect on the completeness of milking as described in the subsections above.

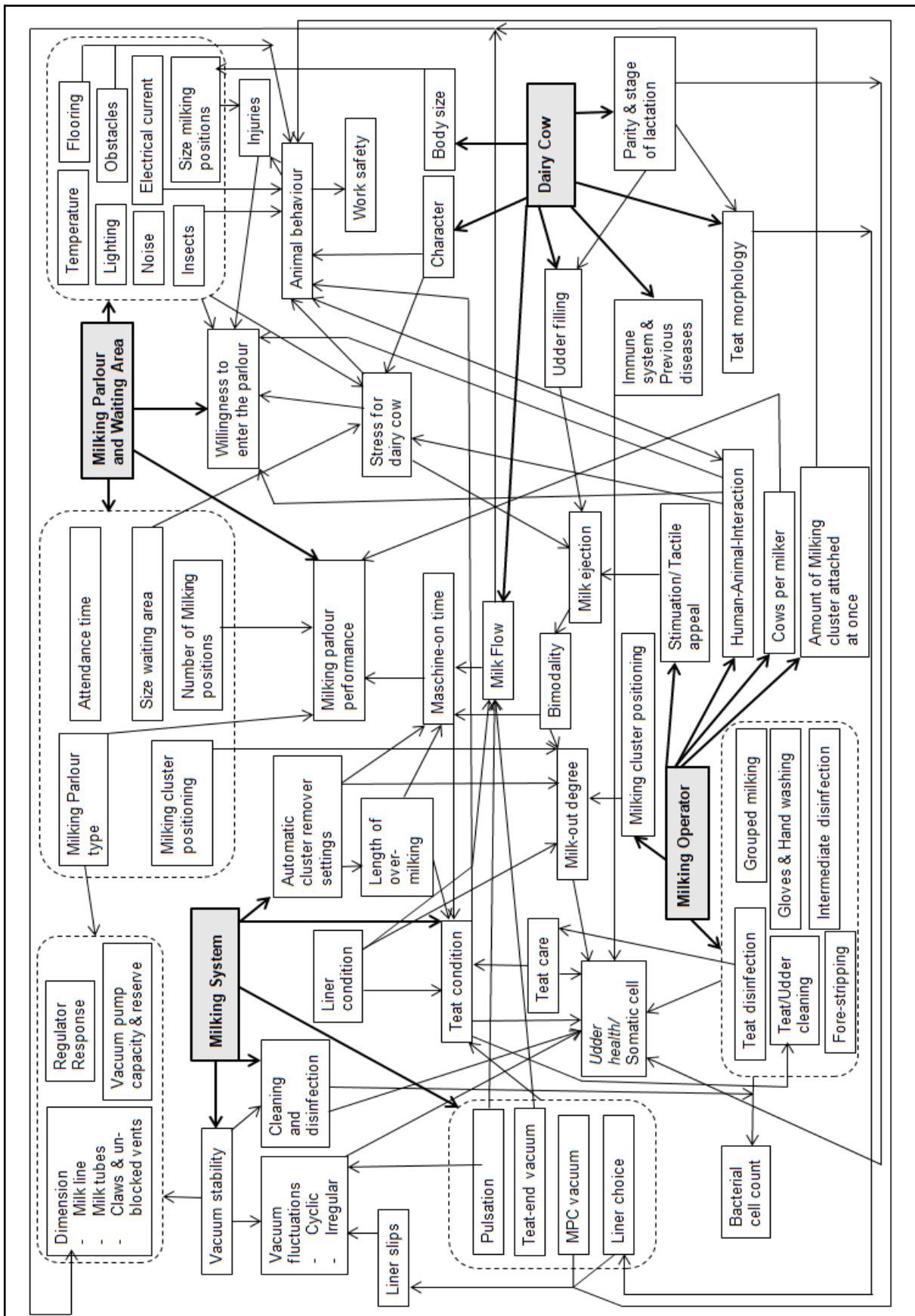
### 2.2.5 Summary

In summary of this chapter, it can be noted that the quickness of the milk removal is basically influenced by the adjustment of the average teat-end vacuum, the liners' fit and material, pulsation settings, automatic cluster remover settings, sufficient teat stimulation, calm and stress-free handling of the animals, the size and design of the milking parlour and waiting area and efficiency of the milking operators' work.

The completeness of milking is influenced by the liners' fit and material, the pulsation settings, teat-end vacuum, the automatic cluster remover settings, sufficient teat stimulation before machine milking, a correct milking cluster positioning, a stress-free surrounding and interaction with the dairy cows.

The gentleness of the milking process is dependent from the liners' fit, the teat-end vacuum, the pulsation settings and the machine-on time which all result in the physical strain on the dairy cows' teat. Moreover, the vacuum stability and the cleanliness of the milking system influence the gentleness by its potential impacts on the dairy cows' udder health. The milking routines can prevent bacterial transmission. Further the gentleness is dependent from a calm handling of the animals by the milking operator and species-appropriate conditions and constructions in milking parlour and waiting area with short attendance time.

The indicators were assigned to the belonging key areas by the "polluter-pays" principle. This was done by the central question: which of the four key areas mainly determine the indicator. The main originator ("polluter") of bimodal milk flow curves, for example, is mainly the milking operator due to an insufficient teat stimulation or time span between teat stimulation and milking cluster attachment. The main originator of the dairy cows' teat condition is the Milking System. Only rough teat skin condition could also depend on the Milking Operator and its milking routines after machine milking. These interrelations are important to know for milking advisors. In consequence of this importance, the influences and interdependencies of the four key areas and its chosen indicators are illustrated in Figure 2 on the following page.



**Figure 2** Interdependencies between indicators of the four key areas Milking System, Dairy Cow, Milking Operator and Milking Parlour and Waiting Area

The illustration of interdependencies of indicators in Figure 2 is new developed from the results of the literature research. It aims to improve the understanding for milking advisors. For milking advisors it is essential to know, that observed indicators can affect other indicators and how they relate to each other. Furthermore, by help of Figure 2, milking advisors get informed on which indicators they need to take into account for their assessment procedure. It becomes visible at first sight which indicators need to be checked, when the milk-out degree, udder health, teat condition or animal behaviour should be assessed. According to the survey of Gehrdaus-Schröder (2017), one of the main reasons why farmers ask milking advisory services for help is in case of udder diseases. For this reason there will follow an example in the subsection 2.3 of this Chapter for the approach of Figure 2 as a helping device for milking advisors in case of dairy herds with udder health issues.

In best case milking advisors should have prior knowledge about dairy cows' physiology and a good technical understanding. In addition, for the evaluation of some indicators technical equipment as vacuum loggers or a volume flow meter are mandatory. In consequence of this it is aimed to evaluate and develop simple methods in Chapter Three, Four and Five, which need only basic equipment and put the dairy cow in the center of the investigation.

In order to simplify and standardize the assessment of the machine milking process it could be helpful to develop a digital concept which works with illustrates and simple instructions for the assessment, similar to the concept "Cows and more" (Dahlhoff, 2017). The work on a digital tool for the assessment of the milking process had already began in the project of the operating group "InnoMelk", founded by EIP-Agri and processed by the Institut für Landwirtschaftliche Verfahrenstechnik of Kiel University. The programming of the digital tool could be supported by the developed basic structure of the concept, Table A1 in Appendix and Figure 2.

### **2.3 Milking advisory service in dairy herds with udder health issues**

In Figure 2, the box “Udder health” is directly connected with different indicators. By help of these connections and the explanations in Chapter 2, it can be concluded that the dairy cows’ udder health can be negatively influenced by four ways during the machine milking process:

- (1) Bacterial transmission and invasion
- (2) Intensity and length of the physical strain of the teat and its impact on the teats’ natural defense system
- (3) Milk-out degree
- (4) Dairy cows’ immune system and previous diseases

In a first step, the milking advisor could check potential causes of bacterial transmission, respectively bacterial invasion, during machine milking. For this purpose, the milking routines of the Milking Operator can be directly observed for an initial troubleshooting (Figure 2). As in the section “Milking Operator” explained, the risk of bacterial transmission among cows and udder quarters during machine milking can be reduced by: wearing gloves, washing hands, make use of fore-stripping, being thoroughly in teat cleaning and apply a post-milking teat dip after milking which is disinfectant and skin-caring at once and milk the cows with udder infection grouped and last. Bacterial transmission or even bacterial invasion into the teat canal or udder could also be caused by the Milking System by an insufficient cleaning and disinfection or vacuum fluctuations (Figure 2), especially irregular vacuum fluctuations. In an initial troubleshooting, hints for an insufficient cleaning and disinfection are given by dirty milking equipment, which can be observed before machine milking. In a further step, the efficiency of the cleaning and disinfection can be checked by a cleaning-time test. A first hint for irregular vacuum fluctuations can be perceived by the milking advisor by hearable liner slips during the milking process. In case of hearable liner slips, a further cause analysis can be started, which includes the inspection of the dimensions of milk transporting parts, vacuum capacity, vacuum reserve and fall-off test by vacuum measurements (Figure 2).

In a second step, the physical strain of the dairy cows’ teat during machine milking can be evaluated by the teat condition. It can be used as indicator for an initial troubleshooting and indicates for potential mismatches of the teat-end and mouthpiece chamber vacuum, the pulsation settings and the chosen liner (Figure 2). These parameters should be inspected in detail if more than 20 % of the dairy herds’ teat condition is classified as poor. The assessment of the teat skin offers also information of an insufficient teat care as part of the milking routines (Figure 2), which could enable the colonization with udder pathogens. Generally, the physical strain of the teat during machine milking should be kept as short as

possible and as low as possible. Hyperkeratosis can indicate for long periods of over-milking. The over-milking phases then should be determined by vacuum measurements. In consequence of that, it could make sense to increase the automatic cluster remover settings in order to shorten the average time of over-milking of the dairy herd.

The automatic cluster remover settings can be assessed by the milk-out degree of dairy cows, which is step three (Figure 2). A method to measure the milk-out degree is presented in Chapter Three. The milk-out degree can affect the udder health and milk production (cf. Chapter Three). Though, the dairy cows' milk-out degree is not only determined by the automatic cluster remover settings, but also the influenced by the milking routines. This is illustrated in Figure 2. Insufficient duration of stimulation and tactile appeal can lead to bimodality (cf. Milking Operator). As described in the section "Milking Operator", the absence of milk flow in bimodal milk flow curves can lead to teat cup crawlings and incomplete milkings. In consequence of an insufficient milk-out degree, the presence of bimodality can be derived by vacuum measurements (cf. Milking Operator and Chapter Five). If bimodal milk flow curves are recorded, the stimulation time and duration of tactile appeal by the milking operator should be extended. Further, the milking operator affects the milk-out degree also by its milking cluster positioning. In case of insufficient milk-out degrees this indicator can be assessed (Figure 2).

In a fourth step, also the dairy cows' individual immune system and previous udder diseases affects the present udder health status of a cow (Figure 2). From this point of view the stress level, human-animal-interaction and Milking Parlour and Waiting Area should be taken into account. The size, design of the milking parlour and its conditions inside should not restrict the dairy cows' well-being. The milking and waiting times per day should not last too long, because it prevents the dairy cow from lying down and feed intake, resulting in stress. In addition to that, also the housing conditions and feed quality plays a role in this context, but are not part of the present study.

The chronological order for the approach can be varied. Though, in an initial troubleshooting indicators should be assessed which are directly connected to "udder health" in Figure 2. Afterwards, in case of detected mismatches, the error search can be extended by further connected indicators in order to find causes of the present problem.

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**Chapter Three:**

**Evaluation of three methods to assess the degree of milk-out in dairy cows**

Daniela Meyer<sup>1</sup>, Angelika Häußermann<sup>1</sup>, Kerstin Barth<sup>2</sup>, Stefan Lingner<sup>1</sup>,  
Eberhard Hartung<sup>1</sup>

<sup>1</sup>Institute of Agricultural Engineering, Kiel University, 24118 Kiel, Germany

<sup>2</sup>Johann Heinrich von Thuenen-Institute, Federal Research Institute for Rural Areas,  
Forestry and Fisheries, Institute of Organic Farming, 23847 Westerau, Germany

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**Abstract**

The assessment of the completeness of milk-out in dairy cows is one of the indicators used to evaluate and optimize the milking process. A number of different methods and thresholds are available for this purpose, but procedures and validation of the methods are not always described in detail, and may vary between studies. The objective of this study was to introduce and evaluate a new, precisely defined hand-milking method (DEFINED) and to compare its outcome with two commonly applied methods to assess completeness of milking: visual scoring of the degree of quarter filling (VISUAL) and quantitative assessment of number of easy strips (EASYSTRIPS). Each of the three methods was applied in 131 Holstein cows of six dairy herds in northern Germany. Assessment of milk-out was carried out by three experienced but non-regular milkers (evaluators). Each evaluator visited the six herds once during afternoon milking. To avoid any transitions, the interval between visits of two evaluators was at least two days. Maximum hand-milking time per cow was set to 60 s. The total strip yield collected in 60 s (SY60) by application of a strip frequency of 1 Hz was used as a reference for the amount of milk left in the investigated quarter after machine milking. The three methods were evaluated by analysing their statistical relationship with SY60, and by ranking their suitability for quantitative or qualitative assessment of milk-out. VISUAL and SY60 were not related, indicating that VISUAL was unsuitable for estimating the amount of milk left actually in the udder quarters. The strip yield in 15 s (DEFINED) and SY60 were significantly related, but results varied among evaluators. With regard to EASYSTRIPS, a significant relationship with SY60 was found, but the results were influenced by evaluator and herd. The findings of this study imply that DEFINED allows a rapid and farm-independent quantitative estimate of the post milking strip yield. Likewise, EASYSTRIPS was meaningful for assessing milk-out of quarters in a given herd, whereas VISUAL did neither allow quantitative nor qualitative assessment of post milking strip yield or milk-out. Thresholds for complete or incomplete milk-out by DEFINED must be lower than those commonly applied in 15 s of post-milking.

**Keywords: strip yield, udder filling, handgrip, settings, cattle**

**Implications**

Methods and thresholds to record and assess the amount of rest milk in the udder vary among studies and operators in literature. The comparison and evaluation of three different methods and implementation of a new hand-milking method with a defined handgrip and strip frequency to record and assess the amount of rest milk in the each udder quarter

offers help for further researchers, consultants and farmers to make choice of one of these methods for their specific scope of application.

### **Introduction**

In order to record and assess the completeness of milking in dairy cows, usually the post milking strip yield is collected. It represents the amount of rest milk which remains in the udder cistern and large milk ducts after milk flow rate drops below a given threshold (Wehowsky, 1972), or after milk flow has ceased due to temporary closing of the milk passageway between the udder and the teat cistern (Mein et al., 1973). This closure of the milk passageway towards the end of milking is inherent in machine milking and can be explained by the narrowing of the surrounding tissue followed by peaks in flow velocity, which results in temporary negative pressure in the milk passageway, typically around 0.7 to 1.3 kPa (Bothur and Wehowsky, 1978). The extent to which incomplete milking negatively affects the milk production rate and the probability for new intramammary infections depends on the filling of the alveolar and cisternal udder compartments and the preceding udder health status of the cow, and is not yet conclusively studied. Milk remaining in the alveoli, so called residual milk, reduces the milk production rate through autocrine inhibition of milk secretion with serotonin as one main regulator (Weaver and Hernandez, 2016). In addition, the alveolar pressure building up during the filling of the alveoli (Stelwagen et al., 1996) and concomitant leaky tight junctions (Stelwagen et al., 1998) likely induce a decline in milk secretion once the alveolar capacity is reached (Stelwagen, 2001). The amount of residual milk in the alveoli will be high after milking if the oxytocin release from the pituitary was inhibited, e.g., due to failed stimulation or unfamiliar surroundings (Bruckmaier et al., 1993). On the contrary, rest milk in the udder cistern will not necessarily affect milk secretion. Large amounts of rest milk might have an indirect effect however. Penry et al. (2017) found that considerable leftovers of milk in the udder cistern resulted in a reduction of the milk production rate by about 25 %. The authors assumed that the remaining milk in the udder cisterns reduced the time until alveolar capacity was exceeded. Likewise, Stelwagen et al. (1996) reported a higher milk yield in catheterized cows, i.e. with infinite cisternal capacity, compared to non-catheterized cows both milked once daily. Although, large amounts of rest milk in the udder cistern and rather unusual in common milking operation, the information about the leftover in the cistern can be suitable information for the evaluation of the milking process itself. Its main use is to improve the milking process by adapting the settings and the equipment of the milking system, such as automatic cluster remover (ACR) settings and liner fit, in order to optimize milk flow characteristics, milking duration and teat condition.

In order to prevent negative effects of incomplete milking, traditional threshold settings for ACR were set at low levels (~ 200 mL/min). Low threshold settings in turn present a higher risk of over milking at quarter level that has negative impacts on parlour efficiency and teat condition, including impacts on udder health (Rasmussen, 1993). Particularly the incidence of hyperkeratosis increases the probability of intramammary infections (Neijenhuis et al., 2001). Previous research showed that raising threshold settings for ACR from 200 mL/min to 400 mL/min (Rasmussen, 1993; Reid and Stewart, 1997) and setting a limit for milking time (Clarke et al., 2004; Jago et al., 2010) lead to a higher milking efficiency and not necessarily to milk yield losses. Besier and Bruckmaier (2016) concluded, that ACR settings up to 600 mL/min would not cause a considerable loss of milk. This seems to change if the threshold setting for the ACR is raised up to 800 mL/min when Magliaro and Kensinger (2005) recorded a reduction of the milk yield by about 2.5 %. According to Edwards et al. (2013) an earlier removal of the milking cluster led to more rest milk in the udder, leading to higher post milking strip yields.

In addition to the threshold settings of the ACR, the completeness of milking is affected by further factors, such as mismatches of technical settings of the milking machine, cluster weights, milking clusters hanging unbalanced at the udder, liner type and liner condition (Jones, 1999). For example, Davis et al. (2000) found a significant higher machine strip yield with aged milking liners compared to new liners. The post-milking strip yield can be harvested by either giving extra weight to the teat cup or by hand-milking after teat cup removal (Wehowsky, 1972). A key issue of hand-milking methods is the high variation among operators (Davis et al., 2000). Furthermore, hand-milking methods are often not described in detail, which is presumably the main reason for the variation in the results among operators in previous studies.

The aim of this study was to evaluate a new, precisely defined hand-milking method (DEFINED) to collect quarter strip yield, using a typical northern Europe milking handgrip and a predefined handgrip interval. The hypothesis was that the outcome of a precisely defined method depends mainly on the rest milk in the cistern and only to a lesser extent on external influences such as evaluator or herd. This method was compared with two other methods: visual scoring of the quarter filling degree (VISUAL) and assessment of the number of easy strips (EASYSTRIPS). The evaluation comprised analysis of the statistical relationship of each method to the hand-milked strip yield in 60 s. Effects of evaluators and herds on the outcome were included in the evaluation, and the suitability of the three methods for quantitative or qualitative assessment of milk-out was finally ranked.

## Material and Methods

The study was conducted from July to August 2017 in six German-Friesian dairy herds in northern Germany. Herd size varied between 81 and 390 cows. Herd B belonged to an organic research farm. All animals were kept in cubicle housings and milked in different milking parlours (Appendix Table A2).

### *Experimental cows*

From each dairy herd 22 to 25 experimental cows were selected based on (1) being Holstein-Friesian breed, (2) in first to sixth lactation, (3) more than 50 days in milk (DIM) to a maximum of 200 DIM, and (4) four intact udder quarters. In total, 148 cows entered the experiment. Due to clinical mastitis ( $n = 7$ ), claw disease ( $n = 4$ ), acute death ( $n = 2$ ), aggressively defend ( $n = 2$ ) and loss of their marking ( $n = 2$ ), 17 cows were excluded from the experiment. Therefore, the final data set consisted of 131 cows, between 20 and 24 per herd. The experimental cows were in average 124 DIM and mean daily milk yield (DMY) was  $33.7 \pm 9.3$  L/d (Table 1). With regard to parity, 43 % of the cows were in their first lactation, 45 % in their second or third lactation, and 12 % in their fourth or higher.

**Table 1** Daily milk yield (DMY) and lactation characteristics of the cows included in the evaluation of completeness of milk-out at the six German Holstein dairy herds

	Herd					
	A	B	C	D	E	F
Milking times per day	2	2	2	2	2	3
Cows in experiment	20	20	22	23	24	22
DMY Mean (L/d) (SD)	37.4 ( $\pm$ 5.9)	22.7 ( $\pm$ 4.4)	38.9 ( $\pm$ 7.0)	27.2 ( $\pm$ 3.6)	32.9 ( $\pm$ 5.8)	43.1 ( $\pm$ 8.0)
DIM <sup>1</sup> Mean (d)	152	136	117	110	125	111
Cows in 1 <sup>st</sup> lactation	6	8	4	13	12	9
Cows in 4 <sup>th</sup> or a further lactation	4	3	5	2	1	2

<sup>1</sup> DIM = days in milk

### *Milking systems and technical settings*

The cows were milked in parallel, herringbone and tandem parlours with 2x4 to 2x20 milking places. Operating vacuum settings in the milking parlours were between 37 kPa and 44 kPa. Pulsation rate was 58 to 60 cycles/min and pulsation ratio settings in the milking parlours were 60:40 or 65:35 (Appendix A3). All milking parlours were equipped with an ACR. In addition to this, the milking parlour of herd 2 was equipped with a mechanical teat stimulation with a preset stimulation time of 30 s (300 cycles/min, 37 kPa, GEA Farm Technologies GmbH, Bönen, Germany) and an automatic stripping arm, starting when milk flow drops below 700 mL/min. The switch point settings for the ACR were between 250 mL/min and 480 mL/min with a delay time of 25 s to 30 s for herds milked twice daily (n = 5) and 750 mL/min with a delay time of 15 s for herd F for which milking was performed three times daily (Appendix Table A2).

### *Experimental Design*

Assessment of completeness of milking was done by three female evaluators. They have experience with different types of milking parlours, but practiced milking only in irregular intervals. In order to align the procedure for hand-milking between the three evaluators, they were trained in hand-milking in herd A during one milking time 1 week before the experiment started. Each evaluator sampled herd 1 to 6 once; the herds were visited in six consecutive weeks (Appendix Figure A3). Farm visits were scheduled in the afternoon at the second milking of the day. The time interval between two visits at the same farm was 2 days to minimise effects of the preceding visit on the actual milk-out. The three evaluators visited the herds in a randomised order to account for interactions between visit and evaluator or evaluator sequence. Hence, the chronological order of the evaluators visiting the herds changed from week to week (Appendix Figure A3).

### *Visual scoring of the degree of quarter filling*

Immediately after the milking cluster was removed, the evaluator started to assess the completeness of milk-out of the right rear quarter using a visual scoring method adopted and modified from Joe et al. (2010) and Mein et al. (2010). The evaluator assessed the degree of quarter filling of the rear quarter visually with a straight point of view from behind the cow. A flashlight was used, if necessary.

The completeness of milk-out was assessed as follows: good (quarter visibly wrinkled), poor (obvious filling of the quarter, quarter appears slightly plump, not visibly wrinkled) and

uneven (one rear quarter appears plumper and less wrinkled, relative to the other quarter). Cows with natural anatomical unevenly formed udders were noted before the milking process started, to avoid an erroneous classification as “uneven” milked-out. Concerning these cows, the evaluators only distinguished between “good” or “poor” milked-out. This method is named VISUAL in the further course.

#### *Hand-milking using a defined handgrip and handgrip interval*

After visual scoring of the udder, the evaluator started to hand-milk the right rear quarter of the cows for 15 s. The procedure of hand-milking was clearly defined for all evaluators, involving the following steps: (1) four finger behind the teat, the thump in front; (2) the four finger encircle the teat; (3) the thump is used to compress the teat on the upper end to prevent the milk in the teat canal and teat cistern from flowing up in the milk cistern of the udder; (4) the remaining four finger apply downward directed pressure on the teat by forming a fist, beginning with the index finger and end with the little finger. This milking handgrip was applied once every second. The three milkers used a stopwatch to ensure a consistent rhythm for the hand-milking. If no milk was milked for at least 5 seconds, hand-milking was stopped. This method is named DEFINED in the further course.

#### *Assessing number of easy strips*

During the first 15 s of hand-milking of the right rear quarter (see DEFINED), the evaluator counted the number of easy strips to a maximum of six easy strips. The completeness of milk-out was assessed as “good”, if the evaluator counted four or fewer strips, and as “poor” if the evaluator counted five or more easy strips. This method was adopted and modified from Mein et al. (2010).

An easy strip was predefined by three criteria, which all needed to be fulfilled. The jet of milk needed to be: (1) uninterrupted, (2) with a clear direction of the milk strip/flow and (3) needed to produce a hearable sound, when hitting the surface of the collecting container. This method is named EASYSTRIPS in the further course.

#### *Reference value*

The total strip yield collected in 60 s (SY60) was used as a reference for the amount of milk left in the udder after machine-milking. For this purpose, the first 15 s of hand-milking of the right rear quarter of the cows was followed by another three periods of 15 s, adding up to a total hand-milking time of 60 s. The gained strip yields per 15 s were collected in four

separate containers and weighed afterwards. A digital scale with a measurement resolution of 1 g was used (measuring accuracy:  $\pm 1$  g, KA7-DE, Amir, Shenzhen). The milking handgrip was applied once every second using the same procedure as described in the DEFINED hand-milking method. The reference value is named SY60 in the further course.

### *Statistics*

In order to analyse the statistical relationship between the three distinct methods - the herd, the evaluator and SY60 - a separate full model was fitted for each of the three methods. The first full model (VISUAL), a logistic regression model, had VISUAL as dependent variable and herd, evaluator and SY60 as independent variables (Appendix Material A4). The second full model (DEFINED) included the strip yield gained in the 15 first seconds as dependent variable and herd, evaluator and SY60 as independent variables (Appendix Material A4). In the third full model (EASYSTRIPS), the number of easy strips was the target variable; and herd, evaluator and SY60, the independent variables (Appendix Material A4). The two last models were linear regression models. In principle, all these models rely on independent data points, but the cows were sampled repeatedly during the experiment. To avoid pseudoreplication, the cow was included in all models as random effect (Appendix Material A5). Another assumption of linear regression models is a homogeneous variance of the residuals throughout all values of the independent variables. However, neither herd nor evaluator had a homogeneous variance. This problem can be solved by allowing for different variances per variable value (Zuur et al., 2009). Therefore, a fixed variance structure based on herd and evaluator was implemented in the models of DEFINED and EASYSTRIPS (Appendix Material A5). To find the minimum adequate model, backward elimination was performed. Independent variables in the minimum adequate model were selected based on a significance level of 5 % ( $\alpha = 0.05$ ).

To compare the different methods,  $R^2$  was calculated. For  $R^2$  calculation, single-parameter models were fitted to avoid having different numbers of parameters. The single parameter for the first and second model was SY60. Data handling, statistics and graphics were performed in R (R-Core Team, 2017) using the packages xlsx (Adrian et al., 2018), nlme (Pinheiro et al., 2018), ggplot2 (Wickham, 2009) and plyr (Wickham, 2011).

**Results**

The dataset consists of the results of a total of 131 cows, their identification number, the herd (1 to 6) they belonged to, information about the number of the evaluator (1 to 3) and the day when the data were collected. Every evaluator carried out each method - VISUAL, DEFINED, EASYSTRIPS and the reference value (SY60) - in every herd and on every cow that entered the experiment (Table 2). Concerning the method of EASYSTRIPS, the evaluators milked in 30 % of the quarters 6 easy strips and in 16 % of the quarters 0 easy strips. The results achieved by the three evaluators were approximately even (Table 2). Due to the fact, that only one cow was scored as unevenly milked-out, this cow was excluded from further investigations. The number of easy strips was significantly higher in herd 4 in comparison to herd 1 ( $p < 0.001$ ), herd 2 ( $p < 0.001$ ) and herd 3 ( $p < 0.001$ ). Concerning the quantity of milk hand milked in 15 s (DEFINED), the results varied from 0 g to 57 g. With regard to the strip yield in 60 s (SY60), the measured quantity of milk varied from 0 g to 214 g (Table 2).

**Table 2** Herd and evaluator dependent outcome of the applied methods (VISUAL, DEFINED, EASYSTRIPS) and the reference (SY60) for assessing completeness of milk-out in dairy cows (n = 131 German Holstein dairy cows)

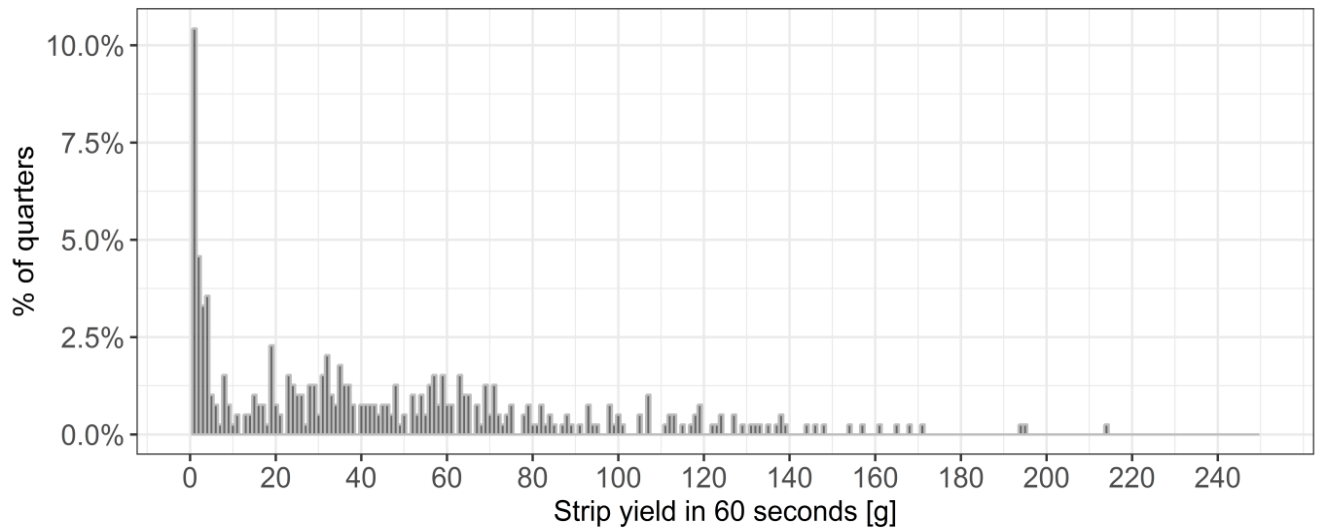
Methods	Herd					
	A	B	C	D	E	F
<b>VISUAL<sup>1</sup>:</b>						
Good (%); Poor (%)						
Evaluator 1	50; 50	60; 40	45; 55	69; 31	63; 37	54; 46
Evaluator 2	40; 60	65; 35	68; 32	65; 35	70; 30	50; 50
Evaluator 3	65; 35	65; 35	45; 55	56; 44	66; 34	45; 55
<b>DEFINED<sup>2</sup>:</b>						
Min; Median; Max						
Evaluator 1	1;9;27	1;4;25	1;6;31	1;10;29	3;8;51	1;11;29
Evaluator 2	1;7;57	1;9;47	0;9;47	1;15;46	1;11;55	2;16;50
Evaluator 3	1;5;22	0;2;21	1;5;32	1;6;29	1;7;35	1;18;46
<b>EASYSTRIPS<sup>3</sup>:</b>						
<5 (%); ≥ 5 (%)						
Evaluator 1	80; 20	80; 20	73; 27	44; 56	67; 33	59; 41
Evaluator 2	75; 25	60; 40	64; 36	31; 69	46; 54	55; 45
Evaluator 3	85; 15	99; 1	78; 22	61; 39	63; 37	46; 54
<b>SY60<sup>4</sup>:</b>						
Min; Median; Max						
Evaluator 1	1;61;139	1;32;119	1;20;111	1;34;144	1;48;127	1;39;137
Evaluator 2	2;59;214	1;11;195	1;29;194	0;32;148	1;59;135	1;33;171
Evaluator 3	2;69;161	1;24;113	0;14;93	1;30;133	1;35;107	1;41;157

<sup>1</sup> VISUAL = visual scoring of the degree of quarter filling;

<sup>2</sup> DEFINED = defined hand-milking method;

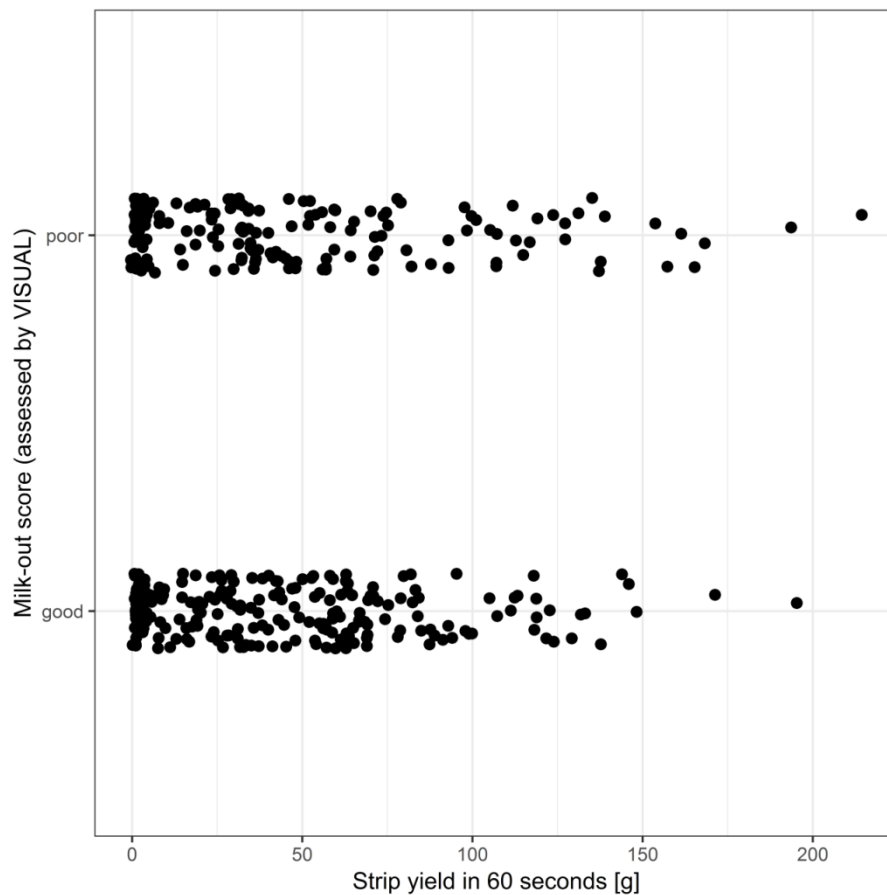
<sup>3</sup> EASYSTRIPS = quantitative assessment of number of easy strips; <sup>4</sup> SY60 = strip yield of the right rear quarter in 60 s

Evaluator 2 collected slightly higher amounts of rest milk in 60 s than evaluators 1 and 3 ( $p < 0.001$ ). The strip yields in 60 s gained by hand-milking showed a left-leaning distribution (Figure 1). Most quantities were measured between 20 g and 50 g.



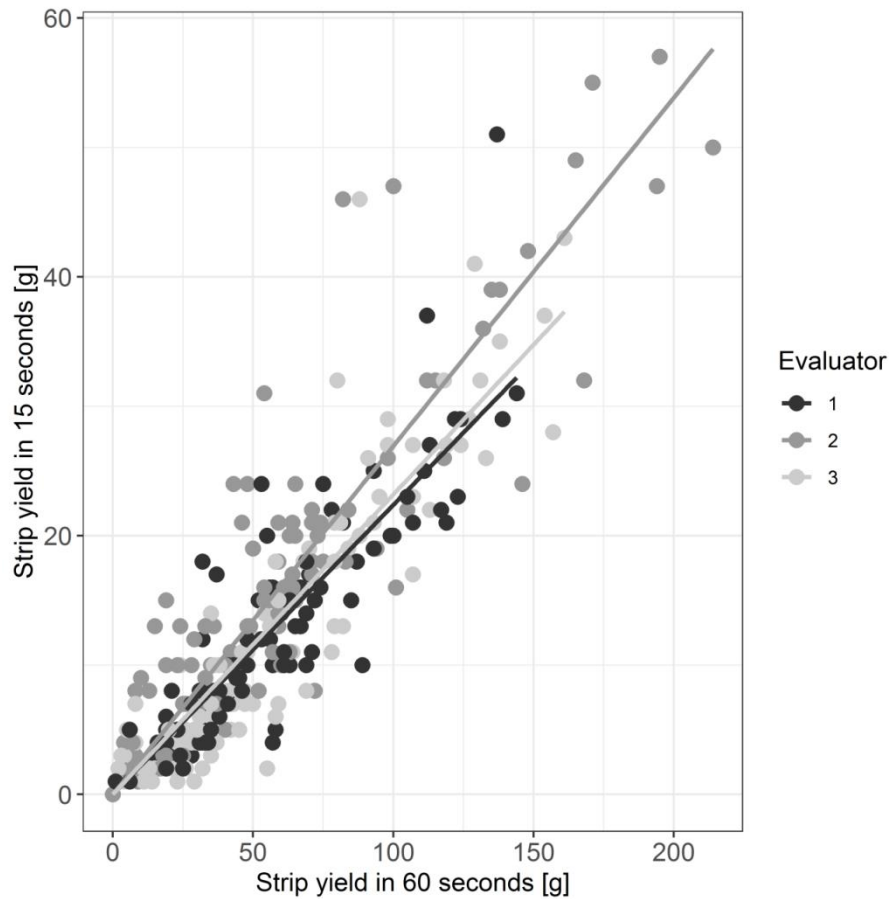
**Figure 1** Frequency distribution of strip yields in dairy cows gained immediately after cluster removal by hand-milking of right rear quarters for 60 s (g) (n = 131 German Holstein dairy cows, 6 farms, 3 evaluators)

The influence of the herd, the evaluators and the reference value (SY60) on the three methods (VISUAL, DEFINED and EASYSTRIPS) were analysed in minimum adequate models. Neither the herd ( $p = 0.24$ ), nor the evaluator ( $p = 0.82$ ), nor SY60 ( $p = 0.6$ ) had a significant influence on the score of milk-out given by VISUAL scoring. The relationship between SY60 and VISUAL scoring of the degree of quarter filling is shown in Figure 2.



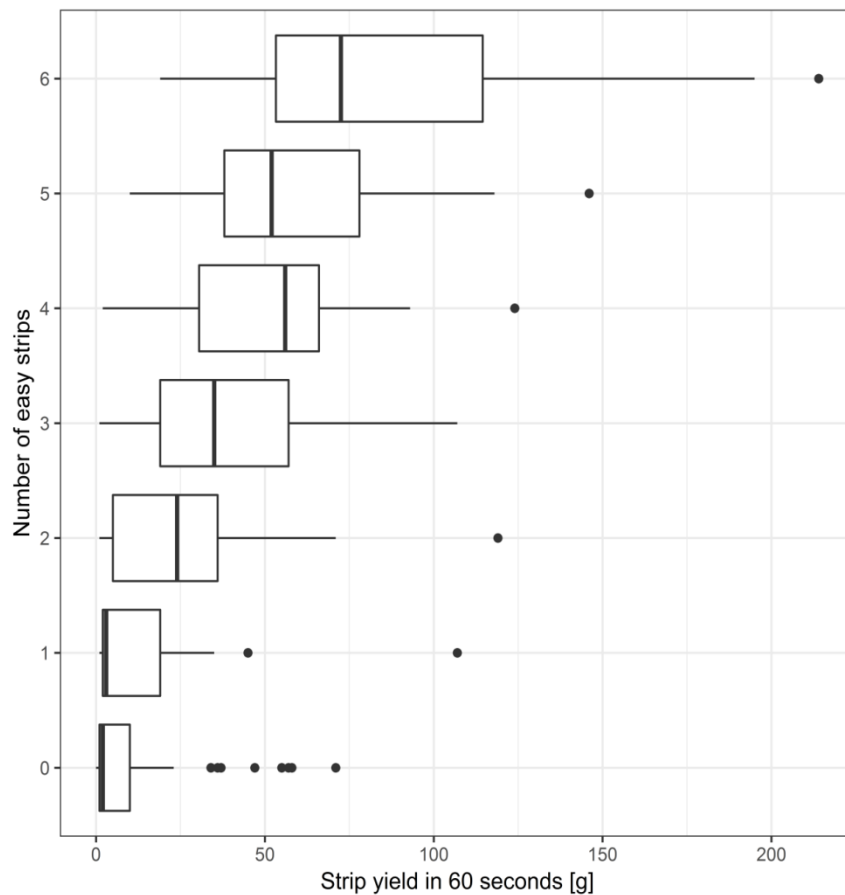
**Figure 2** Completeness of milk-out of the right rear quarter of dairy cows assessed by VISUAL quarter filling degree after cluster removal and related strip yield in 60 s (g) (n = 131 German Holstein dairy cows, 6 farms, 3 evaluators)

In the minimum adequate model of DEFINED, the strip yield in 15 s were significantly influenced by SY60 ( $p < 0.0001$ ) and evaluator ( $p < 0.0001$ ), while the herd had no influence ( $p = 0.26$ ). The full model of DEFINED, consisting of SY60 and evaluator, was significant ( $p < 0.0001$ ). The relationship between SY60, evaluator and the strip yield in 15 s (DEFINED) is shown in Figure 3.



**Figure 3** Strip yield of dairy cows in the first 15 s (g) gained by three different evaluators by DEFINED hand-milking of the right rear quarter after cluster removal and related strip yield in 60 s (g) (n = 131 German Holstein dairy cows, 6 farms, 3 evaluators)

In the minimum adequate model of EASYSTRIPS, SY60 ( $p < 0.0001$ ), herd ( $p < 0.0001$ ) and evaluator ( $p = 0.04$ ) had a significant influence on the estimation of the number of easy strips. The full model of EASYSTRIPS, consisting of SY60, herd and evaluator, was significant ( $p < 0.0001$ ). The relationship between SY60 and EASYSTRIPS is shown in Figure 4.



**Figure 4** Maximum number of easy strips (EASYSTRIPS) milked from dairy cows after cluster removal by hand-milking of the right rear quarter and related strip yield in 60 s (g) (n = 131 German Holstein dairy cows, 6 farms, 3 evaluators)

The single variable models EASYSTRIPS and DEFINED were compared by analysis of their coefficient of determination  $R^2$ . Thereby, the single input variable SY60 could explain the output achieved by the DEFINED method ( $R^2 = 0.84$ ) to a higher extent than the number of strips achieved by the method EASYSTRIPS ( $R^2 = 0.54$ ).

## Discussion

The present study evaluated three methods to record completeness of milk-out by visual scoring or hand-milking. The aim was to examine the relationship between the outcome of the three methods and SY60 of hand-milking. The latter was defined as independent variable based on the assumption that it represents the rest milk in the rear quarter and the expectation that the recorded indicators depend to a large extent on the rest milk and only to a minor extent on external influences like evaluator or herd. This expectation proved to be true with regard to the methods DEFINED and EASYSTRIPS. In consideration of the single variable model, SY60 explained 84 % of the statistical variation in the data gained by the DEFINED hand-milking method, and 54 % in the data gained by EASYSTRIPS. The expectation, that a high amount of rest milk in the quarters cause a less wrinkled and plump appearance of the quarter, was not confirmed by the data in this study.

The reference strip yield, SY60, was generally lower in this study than the strip yield gained by hand-milking over 60 s in Davis and Reinemann (2001). The maximum recorded SY60 was 241 g, and only 0.2 % of the records exceeded 200 g. Davis and Reinemann (2001) found a maximum of > 450 ml, and in 28% of the cases at least 200 ml per 60 s milking. These differences can be explained, to some extent, by the fact that the strip yield in Davis and Reinemann (2001) comprised four single strip yields of four different quarters of a cow. In the present study, SY60 comprised the strip yield of only one quarter per cow. In addition, Davis and Reinemann (2001) used a 'quick method of hand-stripping' to collect the strip yield. The hand-stripping method uses two fingers and two alternate hands and allows fast action at the teat if the strip frequency is not restricted or predefined. In comparison, concerning milking handgrip typical for northern Europe, the thumb is used to constrict the upper end of the teat and the remaining four fingers apply downward directed pressure to the teat (Krömker, 2006). Furthermore, in the present study, the frequency of the milking hand grip was fixed to 60 times per minute or 1 Hz, which was particularly important to ensure comparable results between evaluators and days. It is reasonable that, given the same amount of rest milk in the udder, milking by hand-stripping or by using a full-hand method will not result in the same amounts of milk per unit of time. Likewise, Davis and Reinemann (2001) concluded that the amount of rest milk harvested by machine-stripping will be much higher than by hand-stripping.

The maximum of hand-milking time was set to 60 s in order to prevent the results from being affected by another milk let-down, caused by the stimulation effect of the hand grip. According to Bruckmaier and Hilger (2001), the start of milk ejection following tactile stimulation depends on the degree of udder filling. For degrees of filling of 0 % to 20 %,

which might be also assumed for udders post milking, the expected mean delay until alveolar milk ejection occurs is 107 s, while for degrees of filling of 20 % to 40 % the latency until milk ejection is reduced to 78 s (Bruckmaier and Hilger, 2001).

The influence of evaluators on hand-milking methods to record strip yield was already mentioned in previous studies (Davis et al., 2000; Davis and Reinemann, 2001; Mein et al., 2010). On the basis of the fact that in this study clearly defined instructions for the hand-milking handgrip and time interval were given, influence of the evaluators was expected to be least. However, that expectation was not completely fulfilled. The evaluator had an influence both using the DEFINED method and the method EASYSTRIPS. Possible explanations for the observed effects of the evaluator on the DEFINED hand-milking method might be variations in the applied pressure during hand-milking or the hand size in relation to the morphology of the teat. Milking short teats with large hands might be a problem as well. With regard to the EASYSTRIPS method, the quantity of milk in distinct easy strips was unfortunately not recorded. It might be that the amount of milk harvested by one easy strip varies enormously among cows and evaluators. Likewise, the influence of the herd on EASYSTRIPS could possibly be explained by teat morphology or by a higher internal pressure in the teat cistern in cows with higher amounts of rest milk.

The assessment of the completeness of milk-out is described in different ways. According to Joe et al. (2010), it can be scored by a visual assessment of udder fill and ease of hand-stripping using the following three criteria: very good (udder visibly wrinkled and very little milk stripped, < 100 ml), good (udder with some wrinkles and some milk able to be stripped, 100-500 ml) and poor (obvious filling of the udder and very easy to strip milk, > 500 ml). It remains unclear whether the mentioned thresholds (100 ml and 500 ml; Joe et al., 2010) referred to hand- or machine-stripping. Thresholds for rest milk harvested by machine-stripping are in general higher than for hand-stripping, and thresholds ranging from 500 ml (Mein and Reid, 1996; Mein et al., 2010) to 1500 ml (Davies and Reinemann 2001) are suggested in machine-stripping. The present study used the following three visual criteria to score the completeness of milking: good, poor and uneven milked-out. The requirements on 'good' and 'poor' milked-out udders were adopted from Joe et al. (2010). As can be seen from the results, the rest milk in the udder could not be derived by these assessment criteria. Even more, almost similar quantities of the reference strip yield, SY60, were measured for udders that were assessed as 'good' or 'poor' milked-out. As noted in Mein et al. (2010) the visual assessment method is recommended for herds where a hand-stripping method would cause unacceptable disruption of milking or an unacceptable risk from kicking cows. Further, it can improve the reliability of diagnosis, when practiced in

combination with a quantitative method (Mein et al., 2010). The current findings, however, contradict these recommendations.

Davis and Reinemann (2001) compared the strip yield of two hand-milking methods and two machine milking methods. One of the hand-milking methods was to strip the quarter of a cow's udder for 15 seconds, whereby 21 % of the measured strip yields was equal or higher than 100 ml, and 71 % of the strip yields were below 40 ml. On basis of these findings, Davis and Reinemann (2001) concluded that a hand-milking threshold should be set between 40 ml and 100 ml for the assessment of the milk-out on quarter level. Reinemann et al. (2001) recommended that less than 20% of quarters should yield more than 50 ml of rest milk when hand stripped. Using the northern European milking handgrip with a predefined frequency of 60 per min, the recommendations for the threshold should rather orientate on lower threshold levels. Only 3 % of DEFINED were equal or higher than 40 g, with a maximum of 57 g, and 12 % of SY60 were equal to or above 100 g. With regard to the median of the sixth easy strip in the EASYSTRIPS method, the findings of this study suggest that a possible threshold for milked-out quarters could be set at 75 g quarter strip yield per 60 s of hand-milking, corresponding to a quarter strip yield of 20 g per 15 s of hand-milking, both with a strip frequency of one hand grip per second. In that case, a total of 19 % of quarters would have been not milked out completely. Further investigations are required to confirm this threshold and to exclude the possibility that lower quantities of the collected strip yield in this study were found due to the fact that the cow's quarter were sufficiently milked-out.

### **Acknowledgements**

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**Chapter Four:**

**Effects on strip yield collected by a defined method after  
machine milking of dairy cows**

Daniela Meyer<sup>1</sup>, Angelika Häußermann<sup>1</sup>, Kerstin Barth<sup>2</sup>, Eberhard Hartung<sup>1</sup>

<sup>1</sup> Institute of Agricultural Engineering, Kiel University, 24118 Kiel, Germany

<sup>2</sup> Johann Heinrich von Thuenen-Institute, Federal Research Institute for Rural Areas,  
Forestry and Fisheries, Institute of Organic Farming, 23847 Westerau, Germany

Submitted in

*Animal*

**Abstract**

Hand-milking methods to assess the completeness of milking in dairy cows need to be reliable as well as simple and fast to avoid delays in group milking parlours. A previous study comparing different methods demonstrated that a defined milking hand grip with a strip frequency of 1 Hz was most suitable to assess the completeness of milk-out in dairy cows. The present study aimed to investigate which and how many udder quarters of a cow need to be hand-milked by the defined method for a reliable prediction of the actual rest milk in the udder (Experiment A). Further, the effects of the cow and the milking system on the amount of rest milk were investigated (Experiment B). Experiment A comprised 28 German Holstein cows of one herd. The cows were hand-milked after cluster detachment by an experienced milker using the defined milking handgrip. All four quarters per cow were hand-milked during nine consecutive milking times. The strip yield per quarter per 15 s was collected in four different containers and weighed with a digital scale. Afterwards, the remaining milk of all four quarters was collected and weighed in a fifth container with a maximum volume equivalent to a net weight of 540 g milk. The analysis showed that neither the position of the quarter, nor the chronological order, in which hand-milking was carried out, had an influence on the strip yield in 15 s. The remaining milk could be estimated best by hand-milking all four quarters.

Experiment B included 131 German Holstein cows of six dairy herds in Northern Germany. Three experienced milker collected the data each during one afternoon milking per herd. The right rear quarter of the cows was hand-milked with the defined hand-milking grip. Duration of hand-milking per quarter was four times 15 s, i.e. 60 s in total. The amount of hand-milked strip yield in 15 s was influenced by the herd, the evaluator, cluster positioning during machine milking, teat length and occurrence of palpable swellings at the teat base. The herd and the cluster positioning affected the amount of hand-milked strip yield in 60 s. The results have shown that hand-milking with a defined hand grip is a suitable method to compare completeness of milking among herds. Best results will be achieved if all four quarters are hand-milked for 15 s and if the hand-milked strip yield at different farms is collected by the same person.

**Keywords:** udder filling, incomplete milking, rest milk, cluster positioning, quarter position

## Implications

A previous study introduced a precisely hand-milking method with a predefined milking hand grip and hand grip frequency to collect the post milking strip yield and assess the completeness of milk-out of dairy cows. The current study helps researchers, consultants and farmers to use this method, to be aware of influences on the collected strip yields, and to relate them to the remaining rest milk in the udder. Further, the study indicates major influences on rest milk after machine milking of dairy cows.

## Introduction

Post milking by hand or machine is a common method to estimate the amount of rest milk in the cow's udder after cluster detachment and to assess the completeness of milking. Several previous studies used this indicator to modify or monitor the automatic cluster remover (ACR) settings (Clarke et al., 2004; Clarke et al., 2008; Jago et al., 2010; Edwards et al., 2013). Additionally to the ACR settings, the amount of rest milk in the udder can be influenced by the milking process itself, for example by the milking cluster position (Jones, 1999), the operating vacuum (Thiel and Mein, 1979) or poor condition of the liner (Mein and Reinemann 2007), and hence can be used as an indicator for rapid, complete and gentle milking.

The rest milk in the cow's udder is understood as the milk that remains in the udder cistern and large milk ducts after the milking time ends, caused by the temporary closure of the milk passageway between udder and teat cistern (Mein et al., 1973). This milk is not to be confused with the residual milk, i.e. the milk fraction that remains in the alveolar cells after milking (Bruckmaier and Blum, 1998). While the residual milk directly affects milk secretion through autocrine inhibition (Weaver and Hernandez, 2016), this is not necessarily true for common amounts of rest milk.

The post milking strip yield can be collected either by machine or hand-milking. The machine milking methods are based on the principle that a downwards directed pulling force on the milking cluster either by an additional weight or rope will re-open the milk passageway between udder and teat cistern, and thus allows the remaining milk in the udder cistern to be harvested (Wehowsky, 1972). In studies using machine milking methods often a detailed description of the technique and on the weight used to weigh down the milking cluster is given. By contrast, in studies using hand-milking methods, detailed information about the applied hand-milking procedure is often missing. Basically, two hand-milking methods can be differentiated (Krömker, 2006): first, the "full hand method" and second, a two fingers method, named "stripping". In the full hand method, the teat cistern is blocked from the udder cistern by mechanical compression of the teat at the teat base with the thumb and index

finger. Afterwards the milk is pressed downwards by closing the hand to a fist, one after the other finger. The second method, stripping uses also the thumb and the index finger to block the teat cistern from the udder cistern, but the downward pressure is only applied by the thumb and index finger, the other fingers of the hand are uninvolved (Krömker, 2006). But even if the applied hand-milking method is described, varieties among the operators are common (Davis et al., 2000; Meyer et al., 2020). While the milking speed in machine milking methods is set by the vacuum level and the pulsator rate and ratio, the information about the grip frequency and yield per grip in hand-milking methods is often missing. It is reasonable to assume that, depending on the applied hand-milking method and duration of the hand-milking, the relationship between the amount of collected strip yield and the actual rest milk in the udder varies, and that the interpretation whether a record is classified as high depends largely on the method and target. Thus, previously assumed thresholds of 40 ml to 100 ml per quarter (Davis and Reinemann, 2001; Joe et al., 2010; Mein et al., 2010) need to be verified or adapted. In order to achieve a comparability between investigations which use hand-milking methods to record and assess the strip yield, we introduced a defined full hand-milking method with a given grip frequency of 1 Hz (Meyer et al., 2020). The current study builds on the full hand-milking method defined in the previous study and aims to answer three fundamental questions concerning the practical application and informative value of this method. In the first step, the aim was to investigate whether 15 s of hand-milking with the defined milking handgrip is a suitable method to estimate the actual rest milk, i.e. the remaining milk in the udder cistern and large milk ducts. In a second step, the aim was to clarify whether it is necessary to hand milk all four quarters of a cow or whether it would be sufficient to hand milk only one or two quarters to get reliable information on the amount of rest milk. The aim in the third step of this study was to answer the question whether the amount of rest milk and the collected strip yield in 15 s hand-milking are influenced mainly by the ACR settings of the herds or if additional factors such as udder and teat morphology, teat-liner interaction and the positioning of the milking cluster have a relevant effect on the amount of rest milk.

## Materials and Methods

The study was split into two parts, Experiment A (Exp A) and B (Exp B). Exp A focused on the selection of quarters to be hand-milked and on the relationship between the strip yield per quarter per 15 s and the actual rest milk in the udder. This experiment was conducted in one dairy herd in Northern Germany (Herd 1) in August 2017. Herd size was 303 German-Holstein cows.

Key focus in Exp B was the variation in the results between and within farms and how they are influenced by herd or milking. This experiment was conducted from July to August 2017 in six dairy herds (Herd 1- Herd 6) also located in Northern Germany. Thus, Herd 1 was part of both experiments but with different evaluators and cows involved. Herd sizes for Exp B varied between 81 and 390 German-Holstein cows. Cows were kept indoors, in cubicle housings, only Herd 2 had access to pasture. The farms milked in different types of milking parlours: herringbone (Herds 1, 4, and 5), parallel (Herds 3 and 6), and auto-tandem (Herd 2). The cows were milked two times (Herds 2 to 6) and three times daily (Herd 1) (Appendix Table A6).

### *Experimental cows*

In both experiments, the experimental cows were selected based on (1) being German-Holstein breed, (2) in first to sixth lactation, (3) more than 50 days in milk (DIM) to a maximum of 200 DIM, and (4) four intact udder quarters.

In Exp A, 30 cows entered the experiment, randomly selected out of all cows fulfilling the selection criteria. Two of the cows were excluded from the data analysis as they had a second milk let down during at least one post milking. The final data set thus included 28 cows. The experimental cows were in average 142 DIM and mean daily milk yield (DMY) was  $38.6 \pm 10.7$  L/d. With regard to parity, 30 % of the cows were in their first lactation, 53 % in their second or third lactation, and 17 % in their fourth or higher.

In Exp B, in total 148 cows entered the experiment, selected out of all cows fulfilling the given selection criteria. The cows were marked with a coloured leg band in order to be easily identified during the milking times. Due to clinical mastitis ( $n = 7$ ), claw disease ( $n = 4$ ), acute death ( $n = 2$ ), aggressively defend ( $n = 2$ ) and loss of their marking ( $n = 2$ ), 17 cows were excluded from the experiment. Therefore, the final data set included 131 cows, and between 20 and 24 cows per herd. The experimental cows were in average 124 DIM and DMY was  $33.7 \pm 9.3$  L/d. With regard to parity, 43 % of the cows were in their first lactation, 45 % in their second or third lactation, and 12 % in their fourth or higher. Detailed information on mean lactation number, DIM and DMY per farm are provided in Appendix Table A6.

### *Milking systems and technical settings*

The size of the milking parlours in which the investigations took place and the settings for operating vacuum, pulsator ratio and pulsator rate are listed in Appendix Table A6. All milking parlours were equipped with an ACR. In addition to this, the milking parlour of Herd 2 was equipped with a mechanical teat stimulation with a preset stimulation time of 30 s (300 cycles/min, 37 kPa, GEA Farm Technologies GmbH, Bönen, Germany) and an automatic stripping arm, starting when milk flow drops below 800 mL/min. The switch point settings for the ACR were between 250 mL/min and 480 mL/min with a delay-time of 25 s to 30 s for herds milked twice daily ( $n = 5$ ) and 750 mL/min with a delay time of 15 s for Herd 1 for which milking was performed three times daily.

### *Defined handgrip and handgrip interval*

In Experiments A and B, the procedure of hand-milking described in Meyer et al. (2020) was used by each of the four evaluators to collect the strip yields, and involved the following steps: (1) four finger behind the teat, the thump in front, (2) the four finger encircle the teat, (3) the thump is used to compress the teat on the upper end to prevent the milk in the teat canal and teat cistern from flowing up into the milk cistern of the udder, (4) the remaining four finger apply downward directed pressure on the teat by forming a fist, beginning with the index finger and end with the little finger. This milking handgrip was applied once every second (1 Hz). The four evaluators used a stopwatch to ensure a consistent rhythm for the hand-milking. If no milk was milked for at least five seconds, hand-milking was stopped. In case of another milk let down, the cows were excluded from further data analysis. Another milk let down was defined by the following criterions: the teat felt suddenly plumper and harder and the amount of milk that could be milked increased rapidly.

### *Experimental design Exp A*

Collection of post milking strip yield was carried out by one female evaluator during nine consecutive milking times in Herd 1, i.e. during three milking times per day and on three consecutive days. The evaluator was experienced in milking in different types of milking parlours and practiced milking at regular intervals. The previously defined handgrip and handgrip interval was used to sample the post milking strip yield of the experimental cows. Each quarter of the cows was hand-milked for 15 s. The chronological order in which the evaluator started to hand-milk the four quarters of a cow changed counter clockwise: starting with the right front (RF), left front (LF), left rear (LR) and right rear (RR) and continued at the

following cow with LF, LR, RR, RF and at the following cow with LR, RR, RF, LF and at the following cow with RR, RF, LF, LR. The gained strip yield per quarter per 15 s (SY15 A) was collected in four different containers, and weighed with a digital scale with a measurement resolution of 1 g (measuring accuracy:  $\pm 1$  g, KA7-DE, Amir, Shenzhen, China). The sum of all four SY15 A per cow was labelled as SUM15 A (Table 1). Afterwards every quarter was milked-out completely in order to record the amount of milk that still remained in the udder. This milk was collected and weighed in a fifth container with a maximum volume equivalent to a net weight of 540 g milk. Hence the records of the remaining strip yield per udder (RSY A) were limited to a maximum value of 540 g (Table 1). The amount of RSY A was added to SUM15 A and the resulting sum per udder was then defined as TOTAL A.

#### *Calibration record Exp A*

The calibration record (CALIBRATION A, Table 1) was used to investigate whether the evaluator decreased in strength concerning the hand-milking during a milking time and further, to investigate the maximal possible amount of milk that can be gained by the defined hand-milking in 15 s from cows that were prepared but not milked, yet. During each milking time, the evaluator selected randomly two cows to develop this calibration record. These cows were not included in the experimental milkings. The first cow was milked at the beginning (after the 10th experimental cow), and the second one close to the end of the milking time (after the 20th experimental cow). One randomly selected quarter of these cows were milked with the defined hand grip for 15 s. The hand-milking was carried out after stimulation of milk ejection and before the milking cluster was attached (about 120 s after start of teat stimulation, including approximately 11 s of tactile stimuli per cow). The maximum calibration record per milking time was set to 100% and a share was calculated for each record of SY15 A in relation to the calibration record of that milking time.

**Table 1** Experimental design and definition of collected strip yields in experiment A (Exp A, 1 herd, n = 28 German Holstein dairy cows) and B (Exp B, 6 herds, n= 131 German Holstein dairy cows)

	Exp A	Exp B
Number of herds	1	6
Number of experimental cows	28	131
Evaluators	A1	B1, B2, B3
Number of evaluated milkings per cow	9	3
Hand-milked quarters <sup>1</sup>	RR, LR, RF, LF	RR
Duration of hand-milking per quarter	1 x 15 s	4 x 15 s
Hand-milked remaining strip yield (RSY A) in the udder	Maximum of 540 g	
Types of collected strip yields <sup>2</sup>	1) SY15 A 2) SUM15 A 3) RSY A 4) TOTAL A 5) CALIBRATION A	1) SY15 B   2) TOTAL B

<sup>1</sup> right rear quarter (RR), left rear quarter (LR), right front quarter (RF), left front quarter (LR),

<sup>2</sup> strip yield per quarter per 15 s in Exp A (SY15 A) and Exp B (SY15 B); sum of strip yields per 15 s of all four quarters per cow (SUM15 A); remaining strip yield to a maximum of 540 g (RSY A); total strip yield per udder in Exp A (TOTAL A = SUM15 A + RSY A); total strip yield of the rear right quarter in Exp B (TOTAL B); strip yield of 15 s hand-milking of pre-stimulated and un milked cows in Exp A (CALIBRATION A)

*Experimental design Exp B*

Data collection was carried out by three female evaluators (B1, B2, B3). Each herd was visited once by each evaluator during the second milking of the day. Exp B was conducted in six consecutive weeks. The three evaluators visited the herds in a randomised order with a minimum time interval of two days between visits of different evaluators per farm. This was done to minimise effects of the preceding visit on the actual milk-out and the effects of potential exhausted muscles of the evaluator's wrist. Besides the strip yields the following data were recorded: teat length, teat width, udder and teat morphology, distance and position of the rear teats. All these data were collected once per herd before the first milking of the experimental cows started.

The teat length was measured with a scale (measuring resolution of 1 mm in length and 1 mm in width) from the teat base to tip; the teat width was measured on the midpoint of the teat length. The visual classification of the teat morphology was adopted from Grunert (1990): normal, fleshy, short, cone and bottle teat. The distance and the position of the rear teats was classified into four and three categories, respectively. The positioning of the milking cluster and of the rear teat cups were assessed during milking (Table 2).

The teat-liner interaction was evaluated indirectly by post milking teat scoring adopted and modified from Mein *et al.* (2001). Within the first minute after detachment of the cluster, the teat colour and the occurrence of a visible lip mark or palpable swelling at the base of the rear right teat were scored. A flashlight was used, if needed, to improve the data collection.

The data on technical settings of milking, milking routines and ACR were noted prior to the first visit, and were taken as one mutual farm influence ("herd") into account.

**Table 2** Classification of data records in 131 German Holstein dairy cows in Exp B concerning characteristics of udder and teats, and milking cluster position

Data record	Score				
	0	1	2	3	4
Udder morphology	all quarters have the same size (homogenous)	Front quarters higher positioned than rear quarters	Rear quarters higher positioned than front quarters	Right rear quarter is smallest of all four	Right rear quarter is biggest of all four
Teat shape	Normal teat	Fleshy teat	Short teat	Cone teat	Bottle teat
Distance of the rear teats	> 25 cm	5-25 cm	< 5 cm without touching each other	Teats touch or cross each other	
Position of rear teats	Teat ends point straight down on the footing	Teat ends point to inner thigh muscles of the cow	Teat ends facing each other		-
Milking cluster positioning	Cluster hangs parallel to the udder base; not twisted	Cluster hangs unevenly or is twisted	-	-	-
Position of the rear teat cups	Rear teat cups touch each other	Rear teat cups do not touch each other	-	-	-
Colour of the right rear teat	Normal coloured	Reddened	Blue coloured	-	-
Teat base of right rear teat	Normal, no lip mark or swelling	Visible mouth piece lip mark	Palpable swelling with a thickened ring	-	-

After assessing the teat condition, the evaluator began to hand milk the right rear quarter of the experimental cows for 15 s (SY15 B). Subsequently the right rear quarter was hand milked 15 s for another three times, adding up to a total hand-milking time of 60 s. Each of the four 15 s strip yields of the right rear quarter was collected separately in containers, weighed afterwards and was then summed up to TOTAL B. Thus, TOTAL B relates only to the rest milk of the right rear quarter while TOTAL A relates to the rest milk of the whole udder.

### *Statistical analyses*

Data handling, statistical analyses and graphics were performed in R (R-Core Team, 2018) using the packages `xlsx` (Dragulescu and Arndt, 2020), `lme4` (Bates et al., 2015) and `piecewiseSEM` (Lefcheck, 2018).

In order to analyse whether the location of the quarter (RR, LR, RF, LF) or the chronological order in which the quarter were hand-milked (1st, 2nd, 3rd, 4th) had an influence on the strip yield of the first 15 s of hand-milking (SY15 A), a linear model was fitted, based on a significance level of  $p < 0.05$ . The quarter location or the chronological order was included as independent variable and SY15 A as target variable.

To analyse whether the prediction of TOTAL A is improved if more than one quarter is hand-milked, three linear mixed-effect models were fitted. The data were assumed to be approximately normally distributed and to be heteroscedastic. These assumptions based on a graphical residual analysis. The first model included one randomly chosen quarter per cow as independent variable. Target variable in this model was TOTAL A minus SY15 A of the randomly chosen quarter. The second model contained two diagonal randomly chosen quarters, one front and one rear quarter, as independent variable. Target variable was TOTAL A minus SY15 A of the two diagonal randomly chosen quarters. The third model had SUM15 A of a cow as independent variable and RSY A as target variable. Thus, in each of the three models SY15 A of the quarters that were used for prediction of the rest milk were subtracted from TOTAL A. In order to compare the marginal and conditional  $R^2$  of the individual models, a Pseudo  $R^2$  was calculated (Nakagawa and Schielzeth, 2013). To analyse whether the calibration records of hand-milked milk decreased between the first and second hand-milked cow and to analyse whether the amount of CALIBRATION A, SY15 A, RSY A and TOTAL A differed among days or milking times, Kruskal Wallis tests were conducted.

The analysis of potential influencing factors on SY15 B started with the definition of an appropriate statistical mixed model (Laird and Ware, 1982; Verbeke and Molenberghs,

2000), including the herd (six levels) and the evaluator (three levels) as fixed factors. The cow was regarded as random factor. The data were assumed to be approximately normally distributed and to be heteroscedastic due to different levels of herd and evaluator. These assumptions were based on a graphical residual analysis. Based on the initial model, an analysis of variances (ANOVA) was conducted. In a next step a full mixed model was fitted with SY15 A as dependent variable and DIM, number of lactation, DMY, teat length, teat width, udder morphology, teat shape, milking cluster positioning, position of the rear teats, distance of the rear teats, position of the rear teats, teat colour, teat base as independent variables, the cow as random factor and herd and evaluator as fixed factors. A forward model selection based on AIC values (Akaike, 1973; Sakamoto et al., 1986) was conducted to decide which independent variables can be used for prediction. Independent variables in the minimum adequate model were selected, based on a significance level of  $p < 0.05$ . Afterwards, independent variables were checked for their variance inflation factor (VIF). Finally, non-significant independent variables were omitted, based on an analysis of covariances (ANCOVA) (Cochran, 1957), followed by multiple contrast tests (Bretz et al., 2011) in order to compare the several levels of herd and evaluator, respectively. For the final model, a Pseudo  $R^2$  was calculated (Nakagawa and Schielzeth, 2013). The same analysis was done to investigate potential influencing factors on TOTAL B.

## Results

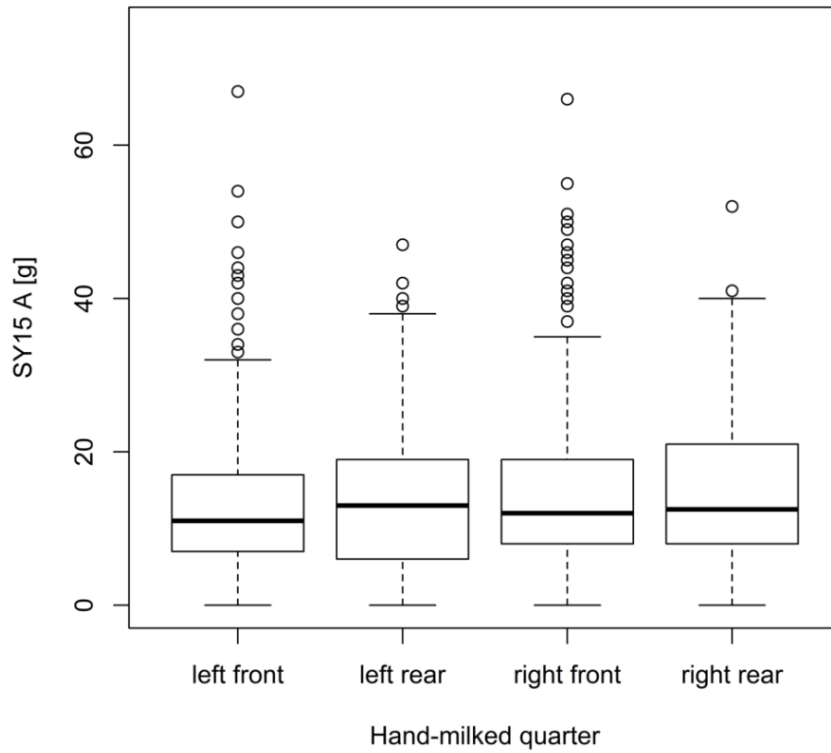
### *Exp A*

The main dataset of Exp A included the results of a total of 28 dairy cows, their identification number, the day and the milking time when the data were collected, the quantity of SY15 A hand milked in 15 s per four quarters, the sum of the 15 s strip yield of the four quarters (SUM15 A), the remaining strip yield (RSY A), and TOTAL A (Table 1). On average 14 g milk were collected during a standardized stripping of 15 s per quarter (range: 0-67 g) and in half of the milkings less than 12 g were gained. No significant differences were found among the three milking times per experimental day or among days for the means of SY15 A (df = 2, CI = 0.95), the means of RSY A (df = 2, CI = 0.95) and the means of TOTAL A (df = 2, CI = 0.95). The overall mean of SUM15 A in Exp A was 57 g (min 2 g; max 174 g), the overall mean of RSY A was 70 g (min 0 g; max 540 g) and overall mean of TOTAL A was 126 g (min 2 g; max 706 g). Two cows reached the limit of 540 g for RSY A in two different milking times each. As indicated in Table 3, highest SY15 A, RSY A and TOTAL A were measured in the second milking time of experimental day 3.

**Table 3** Mean strip yield in 15 s (SY15 A), remaining strip yield in the udder (RSY A) up to a maximum of 540 g, and total rest milk in the udder (TOTAL A), collected in 28 machine milked German Holstein dairy cows at three milking times (MT) per day during three days in Exp A

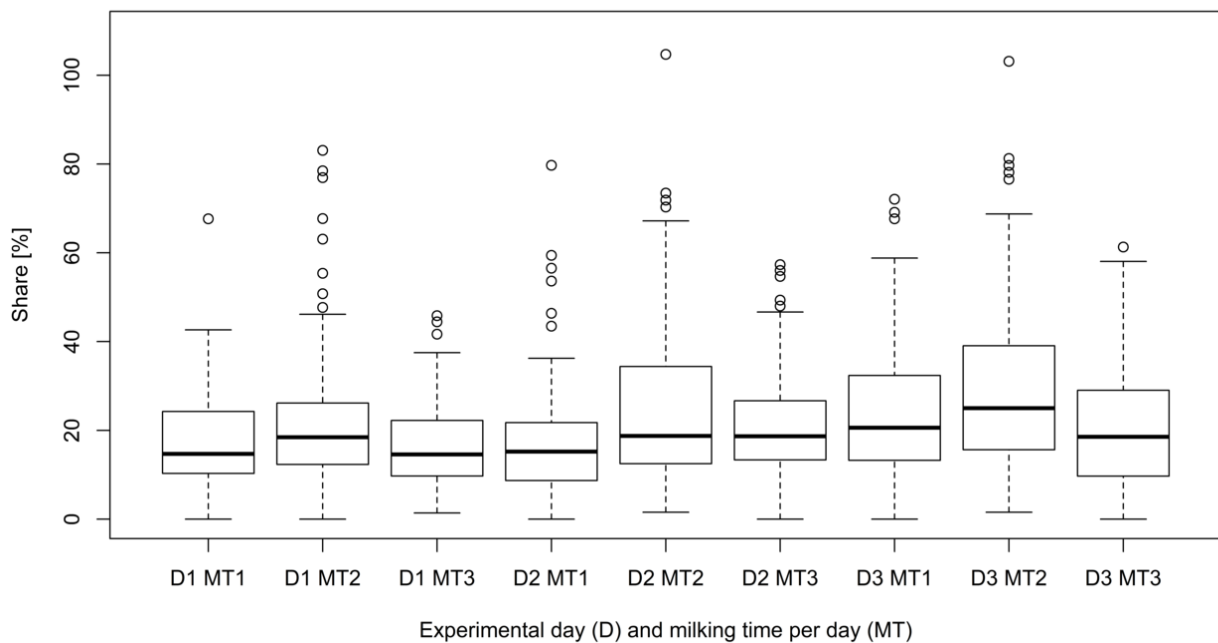
	Mean (median) SY15 A (g)			Mean (median) RSY A (g)			Mean (median) TOTAL A (g)		
	MT 1	MT 2	MT 3	MT 1	MT 2	MT 3	MT 1	MT 2	MT 3
Day 1	12 (12)	14 (12)	12 (12)	69 (44)	61 (45)	67 (44)	116 (98)	115 (100)	115 (98)
Day 2	12 (12)	16 (12)	16 (12)	54 (44)	84 (43)	68 (42)	103 (96)	148 (95)	130 (95)
Day 3	16 (12)	17 (12)	13 (12)	67 (42)	95 (43)	65 (44)	131 (95)	169 (95)	116 (98)

Neither the individual quarter's position, nor the chronological order in which the hand-milking was carried out, had an influence on the hand-milked quantity of SY15 A. The distribution of SY15 A was approximately the same in the four quarters (Figure 1).



**Figure 1** Boxplot and statistical outliers of the hand-milked strip yield in 15 s (SY15 A) of the four quarters of 28 German-Holstein dairy cows in Exp A, depending on quarter position

The second dataset of Exp A included the values of CALIBRATION A of 18 different cows (2 cows per milking time, 3 milkings per day, 3 days). The amount of CALIBRATION A during the nine milking times was in a range between 56 g and 75 g with an overall mean of 65 g. Neither any difference was found for the amount of milk from the first cow within the milking time and the second cow later on in the milking time, nor for the different experimental days or milking times ( $df = 17$ ,  $CI = 0.95$ ). With regard to the relation between SY15 A and the amount of milk collected in CALIBRATION A, the SY15 A exceeded only two times the amount of the maximum CALIBRATION A, once in the second milking time of day 2 and once in day 3 in milking time 2 (Figure 2). In most of the milking times the amount of SY15 A was less than 40 % of the maximum CALIBRATION A in the respective milking time (Figure 2).



**Figure 2** Boxplots and statistical outliers of the share of the hand-milked strip yields in 15 s per quarter (SY15 A) in relation to the maximum calibration record (CALIBRATION A) of that milking time (28 machine milked German Holstein dairy cows, three milking times (MT) per day within three experimental days (D) in Exp A)

The strip yield SY15 A of one randomly chosen quarter respectively two or four quarters per cow was used for prediction of the rest milk that remained in the udder. The best prediction of the rest milk was achieved by hand-milking all four quarters of a cow ( $R^2 = 0.71$ ; Table 4). Including the cow as random effect increased the coefficient of determination of the three different models into a range between 0.83 and 0.93. Thus, in the first two models, in which only one or two random quarters were chosen as independent variable, up to 59 % of the variation of the data was explained by the cow itself while in the third model, in which all four quarters per cow were included as independent variable, only 22 % of the variation of the data was explained by the cow.

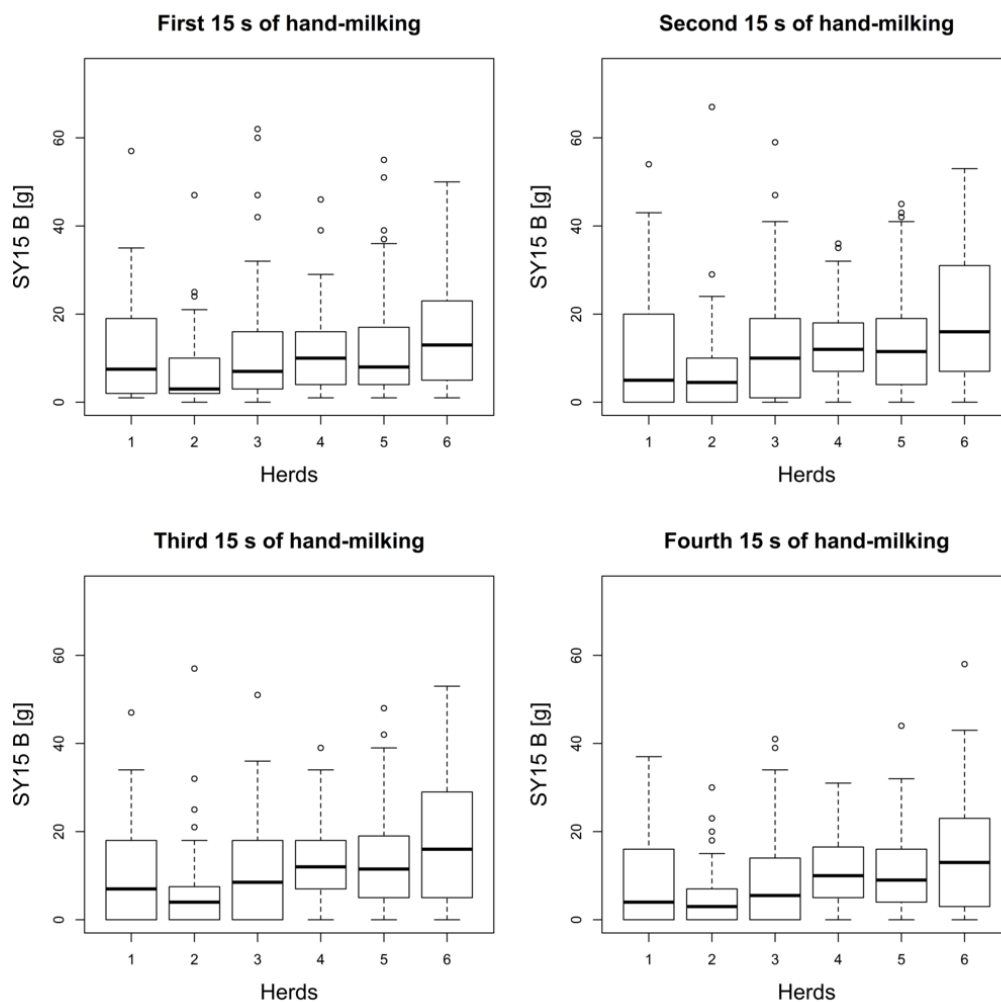
**Table 4** Accuracy of the prediction of the rest milk that remained in the udder by hand-milking one or two random selected quarters or all four quarters per cow (28 German Holstein dairy cows from one herd, 3 milking times, 3 days, 1 evaluator)

Model Number	Independent variable	Target variable	p-value of model	$R^2$ model without random effect <sup>1</sup>	$R^2$ model with random effect <sup>1</sup>
1	SY15 A of one random quarter	TOTAL minus randomly chosen SY15 A	0.001	0.25	0.84
2	Sum of SY15 A of two random diagonal quarters	TOTAL A minus sum of SY15 A of the two randomly chosen diagonal quarters	0.001	0.26	0.83
3	SUM15 A	RSY A	0.0001	0.71	0.93

<sup>1</sup>random effect: cow

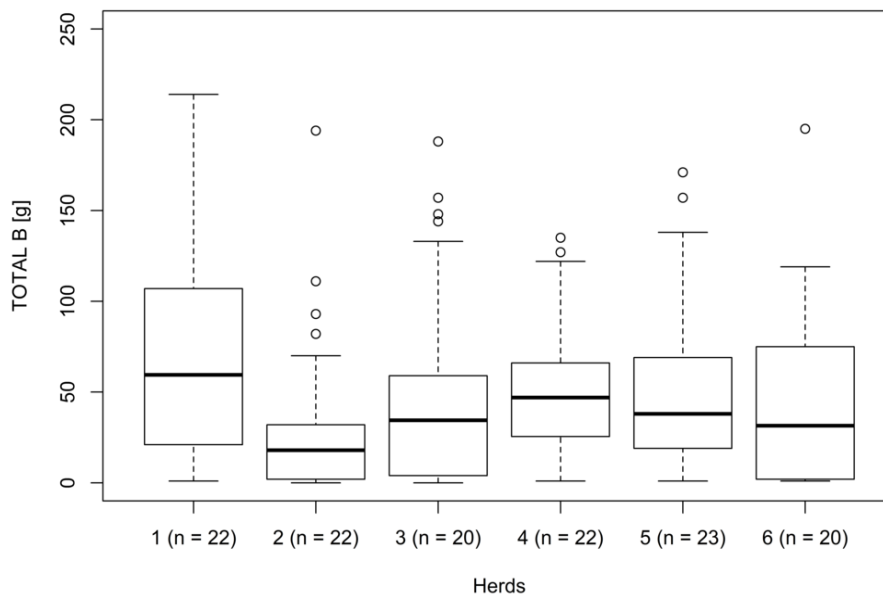
*Exp B*

The dataset of Exp B included the results of a total of 131 cows, their identification number, the herd (1 to 6) they belonged to, the quantities of SY15 B and TOTAL B, the day when the data were collected and the additional records. Maximum SY15 B was 62 g. Mean SY15 B in the first 15 s of hand-milking was 11 g, only 7 % of all quarters exceeded 30 g (Appendix Figure A7). The amounts of SY15 B in the second and third 15 s of hand-milking were in a similar range like those in the first 15 s, while a slight decrease was noticeable in the last 15 s of hand-milking (Figure 3). The percentage of cows without strip yield increased over the four 15 s time intervals of hand-milking. During the first 15 s of hand-milking, only for 0.7 % of the experimental cows no strip yield was hand-milked, while in the fourth 15 s of hand-milking the proportion of cows without milkable strip yield increased to 26 % of the cows.



**Figure 3** Strip yield of the right rear quarter of 131 German Holstein dairy cows in six herds hand-milked in four consecutive 15 s-intervals by a defined hand-milking method

The range of TOTAL B in the six herds is shown in Figure 4. The lowest average strip yields were hand-milked in Herd 2 and the highest average strip yields were hand-milked in Herd 1.



**Figure 4** Distribution of the strip yield in 60 s of the right rear quarter (TOTAL B) of Herds 1 to 6. All herds, in total 131 German Holstein dairy cows, were visited once, each by Evaluator B1, B2 and B3

TOTAL B was influenced by the herd and the positioning of the milking cluster (Table 5). Herd 2 had significantly lower amounts of TOTAL B than Herd 4 ( $p < 0.001$ ,  $t = -3.7$ ,  $df = 126$ ) and Herd 1 ( $p < 0.001$ ,  $t = -3.7$ ,  $df = 126$ ) with a mean of 25 g, a minimum of 0 g and a maximum of 194 g (Figure 4). Incorrect cluster positioning led to slightly higher amounts of TOTAL B. The mean of TOTAL B for correctly positioned clusters was 40 ml (median 32 ml), and 61 ml (median 35 ml) if the cluster position was uneven or twisted. None of the other tested influencing factors, presented in Table 5, had a significant effect on the amount of TOTAL B. Some of them, however, had an influence on the amount of SY15 B.

The cows in Exp B had a mean teat length of 3.8 cm (min 1.8; max 6.6; right rear teats). Mean teat width at the middle of the teat was 2.3 cm (min 1.8; max 3.8). The cows in Herd 4 had slightly shorter and thinner right rear teats than the cows in the other herds (Table 5).

The udder morphology was classified as homogenous in the majority in all six herds. The lowest share of homogenous udder morphologies was recorded in Herd 2 with 64 %, the highest in Herd 4 amounting to 82 %. In Herd 2, 29 % of the rear quarters were lower

positioned than the front quarters. This udder morphology was noted second most in the whole sample. Rarely noted were other udder morphologies such as: the right rear quarter is the smallest (4.5 %) and the right rear quarter is the biggest (1.5 %).

The teat shapes of the cows were predominantly classified as normal. The only exception was Herd 3 with only 38 % normal teat shapes. In this herd, 45 % of the teats were classified as fleshy, 14 % as short and 3 % as cone shaped.

With the exception of Herd 3, the distance in the majority of the rear teats of the six herds was in the category between 5 cm and 25 cm. In Herd 3, a distance of less than 5 cm was predominant (47 %) and 8 % of the rear teat were classified as touching or crossing each other.

The position of the rear teats pointed straight downward to the footing in the majority of the cows, i.e. in between 84 % (Herd 1) and up to 100 % (Herd 4). Only in Herd 3, cows with rear teat ends facing each other (40 %) or pointing to inner thigh muscles of the cow (13 %) were recorded.

The positioning of the milking cluster was most often classified as correct in Herd 2 (92 %) and Herd 6 (91 %). The lowest proportion of correctly positioned milking clusters was found in Herds 4 and 1 (Table 5).

In the entire Exp B, the rear teat cups of 76 % of the cows were not touching each other during the milking process. In Herd 2 and Herd 5 this share was lower, only 67 % and 65 % of the cows had teat cups that were not touching each other during milking.

Teat colour scoring of the right rear teat was done after cluster detachment and was used as indicator for teat-liner interaction. The right rear teat of 65 % of the cows was recorded as normal coloured, 33 % of the right rear teats as reddened and 2 % as blue coloured. The highest proportion of teats that were classified as reddened was found in Herd 1 (54 %), and in Herd 3 reddened teats reached a share of 43 %. Due to normally black coloured skin of the right rear teat, 33 cows in the experiment were not assessed (Table 5). A visible lip mark at the right rear teat base was recorded in 51 % of the cows and a palpable swelling at the teat base in 18 % of the cows. Particularly often a visible lip mark at the teat base was recorded in Herd 1 (69 %), Herd 3 (66 %), Herd 5 (71 %) and Herd 6 (68 %).

The strip yield in the first 15 s of hand-milking (SY15 B) was significantly influenced by herd, evaluator, teat length, positioning of the milking cluster and palpable swellings at the teat base (Table 5). In Herd 2 significantly lower amounts of SY15 B were hand-milked than in Herd 1 ( $p < 0.01$ ,  $t = -3.2$ ,  $df = 127$ ). The amount of SY15 B differed significantly between Evaluator 1 and Evaluator 2 ( $p < 0.0001$ ,  $t = 3.7$ ,  $df = 243$ ) and Evaluator 1 and Evaluator 3 ( $p < 0.0001$ ,  $t = 3.74$ ,  $df = 243$ ) and also between Evaluator 3 and Evaluator 2 ( $p < 0.0001$ ,  $t = 6.20$ ,  $df = 243$ ). The longer the right rear teat was, the higher was the amount of post

milking strip yield in 15 s. Incorrect positioned milking clusters led to slightly higher amounts of SY15 B and a palpable swelling at the teat base led to slightly lower amounts of SY15 B.

**Table 5** Effects of herd characteristics, udder and teat morphologies, cluster positioning and teat scores in the six German Holstein dairy herds included in the Experiment B on the amount of strip yield in 15 s (SY15 B) and in 60 s (TOTAL B)

Independent variable							Target variable	
	Herd 1	Herd 2	Herd 3	Herd 4	Herd 5	Herd 6	SY15 B	TOTAL B
Evaluator							p < 0.0001	n.s.
Herd	herd, milking equipment and farm-specific settings of milking and ACR (cf. Supplementary Table S1)						p = 0.042	p = 0.002
Days in milk (min; mean; max)	51;114; 187	69;136; 193	87;117; 185	55; 110; 190	84; 125; 186	101; 111; 191	n.s.	n.s.
Parity (%) (1 <sup>st</sup> to 4 <sup>th</sup> Lactation; 2 <sup>nd</sup> or 3 <sup>rd</sup> Lactation; >= 4 <sup>th</sup> Lactation)	65; 33; 2	71; 23; 6	47; 45; 8	72; 25; 3	78; 21; 1	71; 22; 7	n.s.	n.s.
DMY (min; mean; max) <sup>1</sup>	30; 43; 64	16; 22; 32	25; 39; 51	21; 27; 35	24; 33; 46	28; 37; 52	n.s.	n.s.
Teat length (min; median; max)	2.8; 3.8; 5.0	2.4; 3.8; 6.6	2.8; 4.0 ;5.6	1.8; 3.4 ;5.0	3.0; 4.0 ;6.0	3.0; 4.1; 5.0	p = 0.026	n.s.
Teat width (min; median; max)	2.0; 2.4 ;3.0	1.8; 2.2 ;3.8	2.0; 2.4 ;3.2	1.8; 2.0 ;2.8	1.8; 2.0 ;2.6	2.0; 2.8; 3.1	n.s.	n.s.
Udder morphology (Score 0; Score 1-4) (%)	75; 25	64; 36	71; 29	82; 18	72; 28	73; 27	n.s.	n.s.
Teat shape (Score 0; Score 1-4) (%)	63; 37	55; 45	38; 62	60; 40	80; 20	89; 11	n.s.	n.s.
Distance of the rear teat (Score 0; Score 1-4) (%)	80; 20	78; 22	45; 55	62; 38	64; 63	90; 10	n.s.	n.s.
Position of rear teats (Score 0; Score 1-4) (%)	84; 16	92; 8	47; 53	100; 0	89; 11	95; 5	n.s.	n.s.
Milking cluster position (Score 0; Score 1)	53; 47	92; 8	79; 21	51; 49	87; 13	91; 9	p < 0.0001	p < 0.0001
Position of rear teat cups (Score 0; Score 1) (%)	97; 3	67; 33	80; 20	75; 25	65; 35	75; 25	n.s.	n.s.

Independent variable							Target variable	
	Herd 1	Herd 2	Herd 3	Herd 4	Herd 5	Herd 6	SY15 B	TOTAL B
Teat colour (%) (Score 0; Score1-2) (%)	40; 60	75; 25	55; 45	70; 30	62; 38	66; 34	n.s.	n.s.
Teat base (%) (Score 0; Score1-2) (%)	26; 74	78; 22	32; 68	50; 50	21; 79	30; 70	p = 0.032	n.s.

<sup>1</sup> DMY = Daily milk yield, from last official milk control

## Discussion

The present study aimed to evaluate whether the strip yield of one, two or four quarters, harvested during 15 s of hand-milking each with a predefined milking hand grip can be used to estimate the remaining strip yield and hence the total amount of rest milk in the udder cistern and the large milk ducts. In addition, influences of farm, lactation, udder morphology, quarter position and cluster positioning on the outcome of hand-milking were analysed in order to investigate whether the method is suitable for evaluation of the milking process.

### *Strip yields per quarter*

Compared to previous studies, the achieved post milking strip yields were considerably lower. In the investigation of Davis and Reinemann (2001), 71 % of the hand-milking strip yields per quarter per 15 s were between 0 and 40 ml, and 21% were higher than 100 ml. In contrast, 97 % of the strip yields per quarter per 15 s in both experiments in this study were below 40 g, and maximum post milking strip yields in Exp A and B were 67 g and 62 g, respectively. The low amounts of post milking strip yields in this study derive from the applied hand-milking method with a grip frequency of 1 Hz, while Davis and Reinemann (2001) applied the hand-milking without a defined grip frequency and stripping instead of a full-hand method as the hand-milking method presented in this paper.

### *Maximum amount of strip yield in 15 s per quarter hand-milked by defined hand-milking method*

The outcome of the pre-milking records in Exp A (CALIBRATION A) confirmed that the maximum amounts of milk that can be hand-milked with the applied method range between 56 g and 76 g. In exception of six quarter strip yields, SY15 A was less than 80 % of the maximum CALIBRATION A. The differences between the quantity of SY15 A and

CALIBRATION A are likely caused by changes in the intramammary pressure before and after machine milking. After teat preparation the udder and teat cistern of cows are filled up with milk and the teat cistern will be refilled with milk from the udder cistern much faster than after machine milking, when the fill level in the udder cistern is lower. Therefore, the amount of milk expected to be hand-milked with one handgrip will be much higher before than after machine milking. The difference supports the assumption that the amount of SY15 A is capable to reflect the quantity of rest milk in the udder.

*Statistical relationship between SY15 A and TOTAL A and choice of the quarter for the hand-milking*

Based on the findings of this study, the highest accuracy to predict the actual rest milk in the udder was reached when all four quarters were hand milked and the strip yields of the four quarters were added up. The sum of the four SY15 A of the experimental cow's quarters explained 71 % of the statistical variation in the data of RSY A. The random choice of two diagonal quarters, one of the front, one of the rear, allowed to explain far less of the variation of the rest milk in the udder, only 26 %. The random choice of one quarter for the hand-milking explained even less of the variation, only 25 %, of the rest milk in the udder. Most of the variety in the data of the rest milk in the udder, up to 60 % by choice of one random quarter, was explained by the cow itself, not by the hand-milked amount of milk in 15 s. This may reflect the fact, that the four quarter of a cow have individual milk flow characteristics (Tančin et al., 2003; Weiss et al., 2004) and milk flow rates (Tančin et al., 2006). This might lead to the problem that the amount of milk that can be hand-milked of each individual quarter of a cow may vary too much to draw a conclusion about the stored rest milk in the udder.

Influences on SY15 B and the TOTAL B

The major influence on the strip yields in 60 s (TOTAL B) in Exp B was exerted from the herd and the settings of the milking system in combination with the working routine procedures. The lowest strip yields were harvested in Herd 2, i.e. the herd that was equipped with an automatic stripping arm. The highest strip yields were recorded in Herd 1 in which the highest ACR settings were applied. Though, the strip yields in Herd 4 were not lower than those in Herd 6, although the ACR settings in the latter were lower. The hypothesis of this part of the study was that, in addition to the ACR settings, other factors such as udder and teat morphologies, lactation stage, parity and daily milk yield of the cows, the working routines of the milkers, e.g. positioning of the milking cluster, and potential liner and technical mismatches may have had an influence on the amount of strip yield. Post milking teat

condition scores, i.e. teat base condition and teat colour, were used as indicators for a failed interaction between teat, liner and technical settings. Coloured teats may indicate a pulsation failure caused either by mismatching teat and liner sizes or by inadequate vacuum or pulsator settings (Mein et al., 2001). Stiff mouthpiece lips, a mismatching mouthpiece volume or barrel shape, a high mouthpiece chamber vacuum and teat cup crawling are the main causes for visible lip marks or palpable swellings at the teat base (Mein et al., 2001). According to Mein et al. (1973), it is more likely that the teat is drawn deeper into the liner during the low milk flow period accompanied by a closure of the milk passageway between udder and teat cistern. This closure will occur earlier if the liner and technical settings do not fit the teat size well, leading to higher amounts of rest milk stored in the udder. Therefore, it was assumed that the teat colour and the visible lip mark or a palpable swelling at the teat base may have an influence on the milkable rest milk in the udder. This assumption was not proofed to be true, for teat condition indicators no influence on TOTAL B was found in this study. Though, palpable swellings at the teat base led to lower amounts of hand milked SY15 B in this experiment, indicating a lagged refill of the teat cistern during hand-milking. According to Hillerton et al. (2002) the frequency of palpable swellings at the teat base increased with the lengths of overmilking. Presumably the tissue at the teat base needs more time for recovery while in the current study hand-milking was performed immediately after the milking cluster was removed.

Further, the milking cluster positioning had an influence on SY15 B and on TOTAL B. It was assumed, that the amount of hand milked strip yield as an indicator for an incomplete milking might be negatively influenced by incorrect positioning of the milking cluster during the milkings as described in Hillerton et al., (1998) and by uneven udder morphologies. As described in Rogers and Spencer (1991) tilted udders increase liner slips. These interruptions of the milking process were assumed to lead to a higher incidence of incomplete milkings, associated with higher amounts of strip yield. These assumptions can be confirmed by this experiment with regard to the positioning of the milking cluster.

In previous studies, the collection of the strip yield was used to evaluate the milking process with regard to the ACR settings. In order to investigate whether the collection of the strip yield by the predefined hand-milking method is also suitable to evaluate the milking process with regard to the cluster positioning, further research is required. SY15 B was, additionally to the milking cluster positioning, influenced by the teat length of the right rear quarter. Longer teats led to a higher strip yield. This is also reported in Weiss et al. (2004), but it needs to be considered that Weiss et al. (2004) collected the strip yield by machine milking, while hand-milking was used in this study. However, it must be mentioned that this influence was not found on TOTAL B, i.e. the strip yield in 60 s. The influence of the teat length on

SY15 B in this study might be explained by two assumptions: at first, the teat cisterns of longer teats might be larger and therefore able to fill with higher amounts of post milking strip yield per milking hand grip than shorter teat cisterns. Thus, the amount of milk milked in the first 15 s of longer teats might be higher than in shorter teats. Correlations between cistern area and the cisternal milk volume is reported in Ayadi et al. (2003). And second, it can be assumed that longer teats might be easier to hand milk with a full hand method than shorter teats, because longer teats seem to be easier to encompass with all fingers and therefore the hand-milking grip might be more efficient in milking higher amounts of strip yield in a certain time of application.

Influences of individual milkers on hand-milking methods are already reported in previous studies (Davis et al., 2000; Davis & Reinemann, 2001; Mein et al., 2010). It was assumed that the definition of a defined milking hand grip and frequency could prevent the individual influences of the evaluators. This was not proofed to be true in this study. The hand-milked amount of strip yield in 15 s differed significantly among evaluators. It is conceivable that the procedure to record CALIBRATION A could possibly enhance the comparability between evaluators. The collected maximum amount of milk by this procedure could be used to compare and in order to harmonize a group of evaluators in their force of hand-milking. To determine if this procedure leads to the intended result and how many cows should be hand-milked during a milking time to achieve comparability between two or more evaluators, further research is requested.

None of the other variables tested in this study, i.e. parity, stage of lactation, daily milk yield, positioning of the rear teats, positioning of the rear teat cups, teat width, udder morphology, teat shape, distance of the rear teat and teat condition, affected the strip yields in 15 s and 60 s. This circumstance might be explained by the enormous influence of the individual cow on the amount of strip yield that can be hand-milked in 15 s (Exp A) or the fact, that the influencing factors had little variation. Only 16 % of the cows had inward or outward pointing teat ends, the teat cups of only 24 % of the cows were touching each other during the milking time and only 1.5 % of the cows had extreme distances of more than 25 cm between the rear teats. Probably these numbers of occurrences were too little to have a significant influence. In general, it might be concluded that the defined hand-milking for 15 s of all four quarter of a cow is an applicable method to evaluate for example the ACR settings. Whether it is also suitable to evaluate the positioning of milking clusters in a herd requires further research. Though, the predefined hand-milking method for 15 s per quarter enables to compare the degree of udder fillings after machine milking among herds under the condition that the hand-milking is performed by the same person.

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**Chapter Five:**

**Relationship between dairy cows' hind leg activity and  
vacuum records during milking**

Daniela Meyer, Angelika Häußermann, Eberhard Hartung

Institute of Agricultural Engineering, Kiel University, 24118 Kiel, Germany

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**Abstract**

In several milking parlours a restlessness of hind legs of cows during milking can be observed for no apparent reason. Previous studies have associated the hind leg activity of cows with mental or physical discomfort. Aim of this study was to investigate whether the observed hind leg activity is influenced by the milking process. The analysis was performed for the whole milking time and also separately for the following milking phases: let down phase, plateau phase and over-milking phase. The following variables were analysed for their influence on dairy cows' hind leg activity: the vacuum level in the mouthpiece chamber and short milk tube, the length of the milking phases, the vacuum curve type, bimodal curves, and the presence of teat-end hyperkeratosis. In turn it was also investigated whether the cows' hyperkeratosis is influenced by milking duration, length of milking phases or by the vacuum level in the short milk tube. Measurements and observations were done in ten milking parlours, each visited twice. Four milking units per parlour were equipped with vacuum loggers (VaDia 2.0, BioControl AS, Norway). One observer recorded hind leg activity per up to four cows, using the following categories: (1) stepping (claw raised < 15 cm) and (2) kicking (claw raised > 15 cm). A powerful, purposive kick to milking unit/milker was classified as p-kick initially. Due to a small number of such powerful, purposive p-kicks, they were added to the number of kickings for further analysis. The evaluation showed that none of the milking process related variables had a significant influence on the dairy cows' prevalence to show hind leg activity. The vacuum level in the mouthpiece chamber had an influence on the type of hind leg activity, however: the hind leg activity kicking was observed at significantly higher average mouthpiece chamber vacuum levels than steppings. Likewise, cows with teat-end hyperkeratosis had a higher prevalence to show kickings than steppings. The degree of teat-end hyperkeratosis was related to the milking duration.

**Keywords: milking phases, teat-end, mouthpiece chamber, stepping, kicking**

**Implications**

Often dairy cows are showing hind leg activity during the course of milking. Previous studies associated the dairy cows' hind leg activity during milking with physical discomfort and mental stress. The current study helps to understand whether the dairy cows' hind leg activity might be related to the vacuum level in the short milk tube and mouthpiece chamber during the milking process. It assists consultants and farmers to assess the gentleness of milking and to identify potential technical mismatches by observation of hind leg activity.

**Introduction**

The milking times are the repetitive times of the day when the dairy cow is in direct contact with the milking system and the milking operators. Technical settings and milking routines influence flow and hygiene of milk removal but also how gentle, stress-free and painless the milk removal is perceived by the animals. Thus, the milking process has a major impact on animal welfare. During the milking process, the milk flow can be basically divided into three phases, which are: let down, plateau and decline phase. The let down phase starts with the attachment of the milking cluster and is characterized by a rising milk flow rate. It turns into the plateau phase, which is characterized by a continuous milk flow rate (Borkhus and Rønningen, 2003). When the plateau phase ends, the decline phase begins, which is characterized by a declining milk flow rate or sudden interruption of milk flow of at least one quarter. Thus, individual quarter enter into the over-milking phase, while the udder as a whole is still in the decline phase (Tančin et al., 2006).

During the course of machine milking, the vacuum beneath the teat depends particularly on the milk flow. In periods without milk flow, the vacuum level beneath the teat equals approximately the operating vacuum. By presence of milk flow and transportation of milk through the milking cluster and milk tubes, the vacuum decreases (Besier and Bruckmaier, 2016). The plateau phase is generally characterized by a stable milk flow rate. In line with this, the vacuum beneath the teat, measured in the short milk tube (SMT), and the vacuum in the mouthpiece chamber (MPC) reach also a steady but low level, with small fluctuations caused by the pulsation. When the milk flow declines during the course of machine milking, the vacuum in the SMT, beneath the teat, increases to a higher level. At this point, the plateau phase turns into the decline phase leading to over-milking first at one quarter and later at all four quarters. The end of the plateau phase is characterized by a steep increase in the vacuum level in at least one MPC, caused by the teat at which milk flow dropped and which thus penetrates deeper into the teat cup (Borkhus and Rønningen, 2003). Additionally, the reduced or interrupted milk flow towards the end of the milking process leads to an increase of vacuum fluctuations in the MPC (Rasmussen, 2004).

In previous studies it was proven that the animal hind leg activity during milking times can be used as an indicator for mental stress (Rushen et al., 1999; Rushen et al., 2001) and physical discomfort (Rousing et al., 2004; Cerqueira et al., 2017). Reduced well-being of dairy cows during the milking times, induced by physical or mental stress can lead to a reduced milk production of the cow (Rushen et al., 1999) and should not be ignored in respect of the farms profitability. In addition to that, an increased hind leg activity during milking can cause serious injuries for the milker and the animal itself (Cavallina et al., 2008). According to DeRosa et al. (2002), the observations of cows' hind leg activity have a high test-retest rate. Therefore, observations during milking times could help to assess the welfare

of the cow and further, by using the behaviour as an early alert system, physical or mental stress and technical dysfunctions could be detected.

Earlier studies observed interrelations of the hind leg activity and different milking phases or MPC vacuum levels. Cerqueira et al. (2017) observed that the hind leg activity of dairy cows increased significantly in periods of over-milking, i.e. milking in absence of a milk flow. The authors assumed that the increased vacuum in the over-milking phase might be uncomfortable for the cows. Newman et al. (1991) reported that kicking of cows towards the end of milking was observed at MPC vacuum levels higher than 23 kPa. Though, no study known to us, had done observations on hind leg activity parallel to real-time SMT and MPC vacuum measurements during the whole course of a milking time.

The aim of this study was to relate some of the main milking records such as SMT and MPC vacuum level, vacuum curves, bimodality, milking duration and lengths of milking phases to the frequency and category of the cows' hind leg activity shown during milkings. Further aims were to clarify whether the frequency of hind leg activity differs among milking phases and to which extent teat morphology and teat scores contribute to the SMT or MPC vacuum level and hind leg activity, respectively.

The underlying hypothesis was that a high vacuum level at the teat-end or teat base will lead to physical discomfort and hence increases intensities or frequencies of dairy cows' hind leg activity.

## **Materials and Methods**

Data were collected in Northern Germany in the time period between November 2017 and February 2018. Vacuum records during milkings and observation of the cows' hind leg activity were done simultaneously. The dairy cows' teat length and width were measured before machine milking started and the teat condition was assessed afterwards.

### *Herds*

Ten German-Holstein dairy herds participated in this study. Herd size varied between 112 and 430 cows per farm (Table 1). Mean lactation number of the ten herds varied between 2.2 and 2.9 lactations. In three of the ten herds, less than 30 % of the cows were in their first lactation and in another three herds 38 % or more (Table 1). The average milk yield of the herds was between 8582 kg and 12509 kg per cow per year (Appendix Table A8).

### *Housing system, milking parlours and technical settings*

All cows were kept in cubicle housings and milked in either parallel (n = 6 farms) or herringbone (n = 4 farms) parlours. In all herds, the dairy cows were milked twice a day. The operating vacuum for machine milking was set between 40 kPa (Herd F) and 46 kPa (Herd C and Herd H) (Table 1). At the time of data recording, the liners were in a maximum four months old, corresponding to approximately 240 milkings. All milking systems had a pulsator rate of 60 cycles per minute. The pulsator ratio was either 60:40 or 65:35 (Appendix Table A8). Four parlours were operated with simultaneous pulsation and milk line installed above the standing level of the cows. Six parlours were equipped with a milk line below the standing level of the cows, thereof one operated with simultaneous pulsation and five with alternating pulsation (Table 1).

**Table 1** Characteristics and technical settings of the milking parlours of the ten German-Holstein dairy herds participating in the observation of hind leg activity and simultaneous vacuum measurements during milkings

Herd	Herd size	Cows in first lactation (%)	Milking parlour type	Operating vacuum setting (kPa)	Level of the milk line <sup>2</sup>	Type of pulsation	ACR <sup>3</sup> settings (ml/min)	Age of the liner <sup>4</sup> (months)
A	210	33	Parallel	45	high	simultaneous	350	2
B	112	40	Herringbone <sup>1</sup>	45	high	simultaneous	400	2
C	248	40	Parallel <sup>1</sup>	46	high	simultaneous	200	2
D	190	38	Parallel	42	low	alternating	300	1
E	181	29	Parallel	43	low	alternating	200	3
F	140	29	Herringbone	40	low	alternating	300	3
G	247	35	Parallel	42	low	alternating	300	2
H	390	31	Parallel	46	high	simultaneous	480	4
I	194	32	Herringbone	42	low	simultaneous	300	1
J	430	28	Herringbone	44	low	alternating	300	1

<sup>1</sup> Swing-over milking system; <sup>2</sup> milk line located above (high) or below (low) standing surface of the cows; <sup>3</sup> ACR = automatic cluster remover; <sup>4</sup> at time of measurement

### *Experimental Design*

Each herd was visited in two consecutive milking times: once during morning milking and once during evening milking. In every milking parlour, the first four milking places either on the left or right-hand side from the operator pit exit point were chosen to be equipped with vacuum loggers and to carry out the observations of cows' hind leg activity. Cows with less than four intact udder quarters and cows which were milked separately into a can were excluded from the experiment.

### *Vacuum measurements*

Four vacuum loggers (VaDia 2.0, BioControl AS, Norway) were attached with adhesive tape on the right rear teat cup approximately a quarter of an hour before machine milking was started. The loggers recorded the vacuum in the MPC of the right rear and right front quarter and the vacuum in the SMT and short pulse tube (SPT) in the right rear quarter. Measurement accuracy was  $\pm 0.2$  kPa and measuring frequency was 200 Hz. Vacuum logging started automatically when vacuum was present. For the measurements in the MPC tiny metal tubes were punctured near the mouthpiece lip into the liners' mouthpiece. The measurements of the SMT and SPT vacuum were done with help of injection needles (Sterican; Size: 17, 0.55 mm x 25 mm; B. Braun Melsungen AG, Melsungen, Germany). They were inserted into the short milk and pulse tubes and fixed with individually designed plastic holders to ensure a stable measuring position with the opening of the needle located in the mid of the tube diameter and directed downstream. The metal tubes and the needles were connected to the vacuum loggers with vacuum-resistant silicon tubes with an inner diameter of 2 mm. Little filter elements and filter holders were integrated into the silicon tubes at measuring locations for SMT and MPC vacuum in order to protect the vacuum loggers from milk and humidity. The filters were replaced after each milking time. The vacuum loggers were initialized once before the start of the measurements and their clocks were synchronized with the tablet PC used for recording the hind leg activities. Synchronizing the clocks was a prerequisite to be able to match the animal hind leg behaviour and the vacuum records later on. The vacuum at the different measuring positions was logged continuously during the whole milking time. The records were automatically stored in an internal memory in the loggers and read out and saved on an external memory when back in office. Before the vacuum loggers were used in the next milking system, they were calibrated using the calibration tool and method described in the manual (VaDia Suite User Manual v2.2, 2017).

*Vacuum analysis*

The software “VaDia Suite” (BioControl AS, Norway) was used for analyzing the recorded vacuum measurements. In the software, the vacuum measurements per cow per milking time are visually displayed. The software provides an automatic tool for analyzing the milking phases. By using this option, the software sets markers automatically at milking phases transition points and provides information about start and end point of the appropriate milking phases, the minimum, maximum and mean vacuum values in the different measurement channels, i.e. MPC and SMT vacuum of the measured quarter, and lengths of the milking phases. This automatic function was only used to get start and end time of the different milking phases. The automatic setting of the markers was visually checked for all measurements and was adjusted manually in cases in which the markers were obviously not set correctly. Furthermore, the automatic function only calculates the start time of the over-milking phase for the right rear quarter. To get the start time of the over-milking phase of the front right quarter, the markers were switched and set manually.

The total milking time was subdivided into four milking phases in this study: let down (phase 1), plateau (phase 2) and over-milking phase (phases 3 and 4). Definitions for the different milking phases were as follows:

- (1) Let down phase: begins with the start of the milking, when the SMT vacuum rises above 25 kPa (VaDia Suite User Manual v2.2, 2017) and ends with the beginning of the plateau phase
- (2) Plateau phase: is derived by the vacuum measurements in the SMT and characterized by a stable and low vacuum. “VaDia Suite” monitors and averages the SMT vacuum in periods of 10 s from start of milking. When mean SMT vacuum declines less than 0.15 kPa between two periods, the midpoint of the first period is stated as the start of the plateau phase (VaDia Suite User Manual v2.2, 2017). The plateau phase ends with the beginning of the over-milking phase of either the front or rear quarter.
- (3) Over-milking phase of the first quarter (front or rear quarter, whichever is first): is derived by the vacuum measurements and based on an increase in MPC vacuum variation. Variation means the difference between maximum and minimum vacuum level per 2 s periods. “VaDia Suite” calculates the current and running MPC vacuum variation in periods of 2 s. In case that the current vacuum variation is equal to or above 1.3 times the preceding running average variation, the start of over-milking is noted. New running average is 0.7 times the old averaged running variation plus 0.3 times the current. (VaDia Suite User Manual v2.2, 2017). The over-milking phase of

the first quarter ends at take-off of the milking cluster, denoted by a SMT vacuum of 5 kPa below the maximum SMT vacuum.

- (4) Over-milking phase of the second quarter (front or rear quarter, whichever is second): is derived by the vacuum measurement and characterized by a rising MPC vacuum and ends at the milking cluster take-off (see (3)).

The vacuum curves were generally classified as vacuum curve type 1 (VCT 1) or vacuum curve type 2 (VCT 2). Vacuum curves with four clearly and at first sight distinguishable milking phases were classified as VCT 1 (Appendix Figure A13). The following criteria were used to classify a curve as VCT 1:

- (1) MPC vacuum level is substantially lower than SMT vacuum level in the plateau milking phase
- (2) approximately stable MPC and SMT vacuum records with only little cyclic vacuum fluctuations during the plateau milking phase
- (3) a steep increase of the MPC vacuum at the beginning of the over-milking phases

All other vacuum curves were classified as VCT 2 (Appendix Figure A14).

In addition, vacuum curves were noted as bimodal if the MPC vacuum reached a short-term peak during the let down phase or at the beginning of the plateau phase (Appendix Figure A15). The classification based on the assumption that the short-term peak of the MPC vacuum was caused by a drop in the milk flow in the let down phase and hence indicated a bimodal milk flow.

#### *Observation of cows' hind leg activity*

Observations of hind leg activity were done by the same person in all ten herds. The observer practiced in two milking times in Herd A before the experiment started. During the observations, the observer was positioned behind the fourth cow in herringbone milking parlours and in the mid of the four observed cows in side-by-side milking parlours. The observers' position was adjusted during the course of milking if necessary to maintain an unobstructed field of vision. Further, the observer was instructed to avoid rapid movements or conversations with the milker and to keep arms and hands close to the body. All cows on the four milking places equipped with vacuum loggers were included in the observation of their hind leg activity.

As soon as machine milking was started, the observation of the cows' hind leg activity began. The hind leg activity was categorized into three intensities: (1) stepping, (2) kicking, and (3) p-kick. Steppings were noted when the cow raised its hind claw less than 15 cm from the cows' standing surface and kickings when the cow raised its hind claw by about 15 cm or

higher from the standing surface. Powerful kicking towards the milking unit or the milker was classified as p-kick initially but was later added to the number of kickings. These definitions were adopted and modified from Kutzer et al. (2015). The observer used a software program for tablet computers to record the cow identification number, its milking place in the milking parlour and its hind leg activities with an exact time stamp of occurrence (hh:mm:ss.ms). Milliseconds were rounded down (hh:mm:ss). When observations of hind leg activities were matched with vacuum measurements, time windows of 3 s were created in order to allow for a reaction time of the observer of 1 s to 2 s.

Hind leg activities during over-milking were assigned to the first quarter if they occurred before the beginning of phase 4 (over-milking of second quarter). In the other cases, hind leg activities were assigned to over-milking of the second quarter.

#### *Teat morphology*

In a first step, after the cows' teat were cleaned by the milker and before machine milking was started, the observer measured the teat length from the teat base to tip and the teat width on the midpoint of the teat length with a scale (measuring resolution of 1 mm in length and 1 mm in width).

#### *Hyperkeratosis*

After machine milking ended and the milking cluster of all four observed cows was removed, the degree of hyperkeratosis of the right rear and right front teat was assessed. Adopted from Mein et al. (2001), the classification was the following: (1.1) no ring, (1.2) a raised ring with no roughness or only mild roughness and no keratin fronds, (2.1) a raised roughened ring with isolated fronds of old keratin extending 1 mm to 3 mm from the orifice, and (2.2) a raised ring with rough fronds of old keratin extending more than 4 mm from the orifice. For further analysis the degree of hyperkeratosis was summarized in two groups: teats with degree (1.1) and (1.2) to score 1 and teats with hyperkeratosis degree (2.1) and (2.2) resulting in score 2.

#### *Data handling and statistics*

Further data handling to match the recorded hind leg activity with the measured vacuum values, was performed in R (R-Core Team, 2019).

In the present study it was aimed to investigate the following scientific issues:

- (A) Frequency of hind leg activity: Influences of SMT and MPC vacuum level, hyperkeratosis, length of the milking phases, vacuum curve type, and presence of bimodality,
- (B) Category of observed hind leg activity: Influences of SMT and MPC vacuum level, hyperkeratosis, vacuum curve type and presence of bimodality,
- (C) Hyperkeratosis: Influence of SMT vacuum level, length of the milking phases, vacuum curve type, and
- (D) Teat morphology: influence on vacuum level and vacuum curve type

Different datasets were generated to answer these issues.

The statistical data analyses were done with help of generalized linear mixed models and performed in R (R-Core Team, 2019). Each of the described models below was calculated separately for each milking phase and the milking time as a whole.

All analysed models included only one independent variable, while the herd was set as fixed effect. Statistical analyses of the independent variables were based on a significance level of  $p < 0.05$ . To compare the different models the pseudo  $R^2$  was calculated.

#### *(A) Influences on the relative frequency of hind leg activity*

This dataset contained information of all observed cows with and without shown hind leg activities during the course of milking. The frequency of stepping and kicking per cow per milking phase was calculated by summing up all stepping and all kicking events of the milking phase and dividing them by the length of the corresponding milking phase (min) in which the hind leg activities occurred. In addition, the frequency of stepping and kicking per cow per milking phase was calculated only for cows which showed the corresponding behaviour. For the analysis, the SMT and MPC vacuum values of each milking phase were averaged.

The frequencies of stepping and kicking were set as target variable. In separate models it was analysed whether the independent variables: the mean SMT vacuum, the mean MPC vacuum of the rear or front quarter, both averaged over the different milking phases and the milking time as a whole, the length of the milking phases, the presence of bimodality, the vacuum curve type, or the presence of hyperkeratosis had an influence on the frequency of either stepping or kicking. In addition it was analysed whether the milking phases or herds differ significantly with regard to the frequencies of steppings or kickings per minute by Kruscal Wallis Dunn post-hoc tests.

*(B) Influences on category of hind leg activity*

In order to analyse the influence of the MPC and SMT vacuum level on the category of the observed hind leg activity, the category (stepping or kicking) was set as target variable. It was assumed, that the observer had a reaction time of up to 2 s between seeing the hind leg activity and recording the time stamp on the tablet. For this reason, the mean MPC and SMT vacuum was calculated for a time window of 3 s, including the moment at which the particular hind leg activity was recorded on the tablet and the 2 s preceding this record.

In separate models it was analysed whether the independent variables: the mean MPC vacuum levels of the right rear and right front quarter, the mean SMT of the right rear quarter, the hyperkeratosis of the right rear or left front quarter and the vacuum curve type had an influence on the shown category of hind leg activity - either stepping or kicking.

*(C) Milking process related influencing factors on the degree of hyperkeratosis and influence of teat morphology on vacuum level*

Additional data of the experiment were analysed to investigate main causes of hyperkeratosis and the influence of teat morphology on vacuum levels. Two datasets were built up:

The first dataset was used to analyse whether the cows' degree of hyperkeratosis (target variable) was influenced by the average SMT vacuum of the plateau phase, the average SMT vacuum of whole the milking time, the vacuum curve type, or the length of the individual milking phases (independent variables). Separate models were fitted.

The second dataset was used to investigate, whether the SMT of the right rear quarter or the MPC of the right front and right rear quarters (target variables), averaged over the plateau phase and the whole milking time, was influenced by the teat length or teat width (independent variables). Separate models were fitted.

All fitted models were calculated once over all ten herds and once separately for each herd. Both datasets considered all observed cows during the experiment, i.e. with and without recorded hind leg activity.

**Results**

Vacuum measurements and hind leg activity observations were analysed for 26 to 89 cows per herd (Table 2), in total 545 cows. Only 13 % of all observed cows showed no hind leg activity. The hind leg activity stepping amounted for 85% of all observed hind leg activities, kickings (without p-kicks) 14 % and p-kicks only 1 %. Between 65 % and 92 % of the observed dairy cows per herd showed at least one or more than one stepping event,

between 10 % and 54 % at least one or more than one kicking activity (without p-kicks), and up to 12 % at least one or more than one p-kick. In general, steppings (66 % to 94 %) were observed more frequently than kickings (6 % to 34 %, including p-kicks) in the ten herds. The major part of hind leg activities was observed in the plateau milking phase. Steppings and kickings were slightly more frequent in the over-milking phase of the first quarter than the second quarter (Table 2). In 62 % to 92 % of all herds' milkings, the over-milking phase started with the front quarter first.

**Table 2** Hind leg activity observations on steppings (claw raised < 15 cm), kickings (claw raised > 15 cm) and p-kicks (powerful, purposive kick to milking unit/milker) of 545 German-Holstein dairy cows during machine milking

Herd	Number of observed cows	Number of cows with hind leg activity	Number of steppings	Number of kickings (without p-kicks)	Number of p-kicks	Share of steppings, kickings <sup>1</sup> (%) on total hind leg activities per herd in different milking phases	Let down		Over-milking		Share of steppings on total hind leg activities (%)	Share of kickings <sup>1</sup> on total hind leg activities (%)	Share of cows with front quarters first in over-milking phase (%)
							Plateau	Over-milking first quarter	second quarter	Over-milking second quarter			
A	40	35	332	72	3	2:2	50:9	13:4	17:3	82	18	89	
B	26	19	87	42	3	0:0	45:22	10:7	11:5	66	34	79	
C	61	53	308	110	15	12:0	30:15	16:6	14:7	70	30	98	
D	51	44	310	30	7	3:0	58:5	15:3	14:2	90	10	80	
E	39	36	389	43	0	1:0	46:6	16:2	26:3	90	10	92	
F	55	48	302	15	5	1:0	50:2	28:2	15:2	94	6	81	
G	60	53	379	54	3	1:0	57:7	18:3	11:3	87	13	68	
H	66	53	173	40	5	2:1	50:9	15:7	11:5	79	21	85	
I	58	53	227	13	2	2:0	53:4	29:1	11:0	95	5	80	
J	89	76	424	36	4	3:0	47:6	22:2	19:1	91	9	62	

<sup>1</sup>kickings and p-kicks summarized

*Relative frequency of hind leg activity*

The mean frequencies of hind leg activity per minute in the different milking phases (let down; plateau; over-milking first quarter; over-milking second quarter) in the ten herds were: 0.1-1.7; 0.6-1.6; 0.2-0.6; 0.3-1.4 steppings and 0-0.6; 0-0.5; 0.01-0.2; 0.03-0.4 kickings (Appendix Table A9).

The frequency of stepping ( $p < 0.001$ ;  $df = 202$ ) and kicking per minute ( $p = 0.047$ ;  $df = 201$ ) differed significantly between milking phases. Stepping was shown with the highest frequency in plateau phase (mean: 0.90 steppings/min), significantly more often than in over-milking of the second quarter (mean 0.66 steppings/min) ( $p < 0.001$ ), let down (mean 0.50 steppings/min) ( $p < 0.001$ ) and over-milking of the first quarter (mean 0.43 steppings/min) ( $p < 0.001$ ). Stepping in over-milking of the second quarter was significantly shown more often than in let down phase ( $p < 0.001$ ) and in let down phase significantly more often than in over-milking of the first quarter ( $p < 0.001$ ).

The highest frequencies of kicking were shown in the let down phase (mean 0.21 kickings/min), significantly higher than in plateau (mean 0.16 kickings/min) ( $p < 0.001$ ) and over-milking of the second quarter (mean 0.14 kickings/min) ( $p < 0.001$ ) and first quarter (mean 0.11 kickings/min) ( $p < 0.001$ ). Frequencies of kickings in plateau phase were significantly higher than over-milking of the first quarter ( $p < 0.001$ ) and second quarter ( $p < 0.001$ ) (Appendix Table A9).

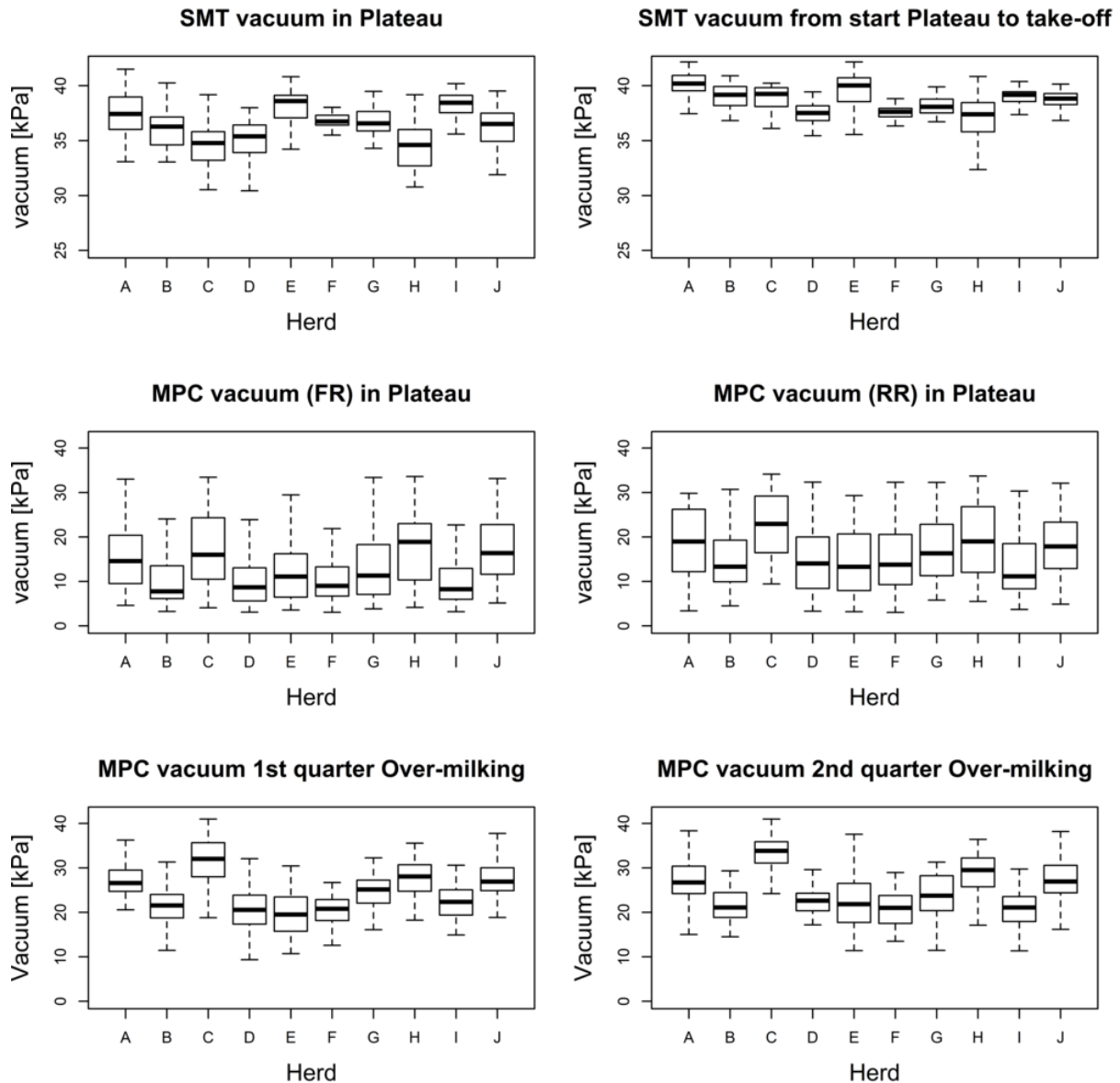
If only cows, which show the corresponding hind leg activity were considered, the mean frequencies of hind leg activity per milking phase (let down; plateau; over-milking first quarter; over-milking second quarter) were: 0-1.9; 0.7-1.9; 0.2-0.7; 0.3-1.5 steppings per minute and 0-2.7, 0.1-0.7; 0.1-1.0; 0.2-1.0 kickings per minute (Supplementary Material Table S3). Also here, the frequencies of steppings ( $p < 0.001$ ;  $df = 529$ ) and kickings per minute ( $p < 0.001$ ;  $df = 201$ ) differed significantly between milking phases. The differences with regard to the frequencies of stepping in milking phases were the same as mentioned above for all observed dairy cows. Highest frequencies were shown in plateau phase (mean 1.07 steppings/min), significantly more often than in over-milking of the second quarter (mean 0.79 steppings/min) ( $P < 0.001$ ), let down (mean 0.55 steppings/min) ( $p < 0.001$ ) and over-milking of the first quarter (mean 0.50 steppings/min) ( $p < 0.001$ ). Stepping in over-milking of the second quarter was significantly shown more often than in let down phase ( $p < 0.001$ ) and in let down phase significantly more often than in over-milking of the first quarter ( $p < 0.001$ ). With regard to kicking, the highest frequencies of kicking were shown in let down phase (mean 0.59 kickings/min), significantly more often than in over-milking of the second quarter (mean 0.48 kickings/min) ( $p < 0.001$ ), plateau phase (0.43 kickings/min) ( $p < 0.001$ )

and over-milking of the first quarter (0.32 kickings/min) ( $p < 0.001$ ). The frequencies in over-milking of the first quarter were significantly lower than in plateau phase ( $p < 0.001$ ) and the frequencies in over-milking of the second quarter significantly higher than in plateau phase ( $p < 0.001$ ) (Appendix Table A10). The frequencies of stepping and kicking per minute of cows showing the corresponding hind leg activity, varied slightly but not significantly among herds (Appendix Table A10).

#### *SMT and MPC vacuum level in milking phases and lengths of the milking phases*

Mean SMT vacuum per herd per milking phase (let down; plateau; over-milking first quarter; over-milking second quarter) varied between: 35.1-38.2 kPa; 34.6-39.1 kPa; 35.5-40.7 kPa; 35.9-41.6 kPa; 35.8-40.3 kPa. Mean MPC vacuum per herd per milking phase (let down; plateau; over-milking first quarter; over-milking second quarter) varied between: 22.4-29.6 kPa; 10.2-17.6 kPa; 20.2-31.2 kPa; 21.0-27.3 kPa at the front teat and between: 23.2-29.5 kPa; 13.3-22.4 kPa; 20.6-30.0 kPa; 20.8-34.1 kPa at the rear teat. The variation of the mean SMT and MPC vacuum records per cow per herd is shown in Figure 1. In 81 % of all vacuum curves, the front quarter entered the over-milking phase first.

The mean total milking time per herd varied between 05:06 minutes (Herd I) and 07:42 minutes (Herd G). While in eight of the ten herds the mean plateau milking phase had the largest share on total milking time, in two herds (Herd C, Herd J) the mean over-milking phase of the first quarter in over-milking was even longer than the mean plateau milking phase of these herds (Appendix Table A11).



**Figure 1** Mean vacuum levels, measured in short milk tube (SMT) at rear right (RR) and mouthpiece chamber (MPC) at rear right and front right (FR) quarter during plateau phase, from the beginning of the plateau phase to the milking clusters' take-off and in over-milking phases of the first and second quarter entering this phase in ten German-Holstein dairy herds (n= 545). The over-milking phase began in 81 % of the milkings first at the front right quarter.

*SMT and MPC vacuum level in a three seconds window around observed hind leg activity*

In the data set used for evaluation of the shown category of hind leg activity, in 79 % of all observed milkings, the cow's front quarter entered the over-milking phase first. Cows with no hind leg activity were not included in this data set.

Table 3 indicates that in all herds the herds' median of the MPC vacuum in the over-milking phases of the first and second quarter was slightly higher in case of kicking than in case of stepping. In over-milking of the first quarter, the median of the MPC vacuum ranged from 13.2 kPa to 21.8 kPa when steppings were observed and from 14.4 kPa to 29.2 kPa during kickings. In over-milking of the second quarter, the median of the MPC vacuum varied between 15.1 kPa and 25.5 kPa during steppings and between 17.6 and 29.5 kPa during kickings. In the plateau phase the herds' median of the MPC vacuum and the SMT vacuum was not consistently higher in case of kicking than in stepping.

**Table 3** Total number of observed steppings or kickings, median of the vacuum measurement values in the short milk tube (SMT) and in the mouthpiece chamber (MPC) of the rear right (RR) and front right (FR) quarter, averaged by a time period of three seconds around observed hind leg activity “stepping” (kicking) in ten German-Holstein dairy herds

Herd	Total number of cows for data analysis	Numbers of steppings (kickings <sup>1</sup> )	Median SMT vacuum (kPa)		Median MPC vacuum (kPa)		Median MPC vacuum while stepping (kPa)		Median MPC vacuum while stepping (kPa)	
			while stepping (kicking <sup>1</sup> )	Plateau Phase	while stepping (kicking <sup>1</sup> )	Plateau Phase	while stepping (kicking <sup>1</sup> )	Plateau Phase	while stepping (kicking <sup>1</sup> )	Plateau Phase
A	35	332 (75)	38.2 (38.3)	25.4 (25.4)	17.9 (19.8)	21.8 (23.2)	25.5 (26.4)	phase first quarter	second quarter	
B	16	68 (42)	36.4 (36.7)	18.4 (18.5)	13.4 (15.8)	13.2 (14.4)	16.1 (17.6)			
C	52	298 (122)	36.0 (35.9)	25.7 (26.3)	24.5 (22.9)	27.8 (29.2)	28.1 (29.5)			
D	44	310 (30)	36.0 (35.6)	13.6 (15.3)	6.1 (6.5)	19.0 (20.1)	20.9 (21.8)			
E	36	389 (43)	39.4 (39.5)	12.6 (11.6)	7.9 (8.0)	17.8 (19.2)	18.7 (20.8)			
F	46	289 (20)	37.4 (37.5)	13.9 (14.2)	12.4 (12.5)	13.9 (17.1)	15.1 (18.2)			
G	52	364 (55)	37.3 (37.2)	16.2 (16.1)	11.0 (11.8)	16.2 (17.9)	17.3 (18.3)			
H	53	173 (40)	35.5 (36.0)	24.0 (25.1)	20.7 (21.3)	20.9 (23.9)	24.1 (26.4)			
I	52	222 (13)	38.7 (39.0)	11.0 (11.2)	11.2 (11.8)	14.9 (19.5)	15.1 (21.0)			
J	73	418 (35)	37.8 (37.7)	20.0 (20.2)	22.8 (23.2)	19.9 (20.9)	22.6 (24.1)			

<sup>1</sup>kickings and p-kicks summarized

*Vacuum curve types, bimodality, teat condition and teat morphology*

The majority of the measured vacuum curves, between 65 % (Herd A) and 96 % (Herd B), fulfilled the criteria to be classified as VCT 1. Bimodal curves were found in 5 % of the milkings in Herd C and in up to 51 % of the milkings in Herd J (Appendix Table A12).

Most teats were scored with Hyperkeratosis score 1, in front teats 92 % and in rear teats 97 %. Hyperkeratosis score 2 at front teat was recorded most frequently in Herd I (14 %) and least frequently in Herd B (1 %). At rear teats, hyperkeratosis score 2 was most frequently recorded in Herd E (5 %) and least frequently in Herds A, B and G (0 %) (Appendix Table A12). The measured length of the rear teat was in a range from 2.6 cm to 6.8 cm; front teats ranged from 3.0 cm to 7.8 cm. The widths of the teats varied between a minimum of 2.0 cm and a maximum of 4.2 cm in front teats and between 2.0 cm and 3.8 cm in rear teats (Appendix Table A12).

*Influences on the frequency of hind leg activity*

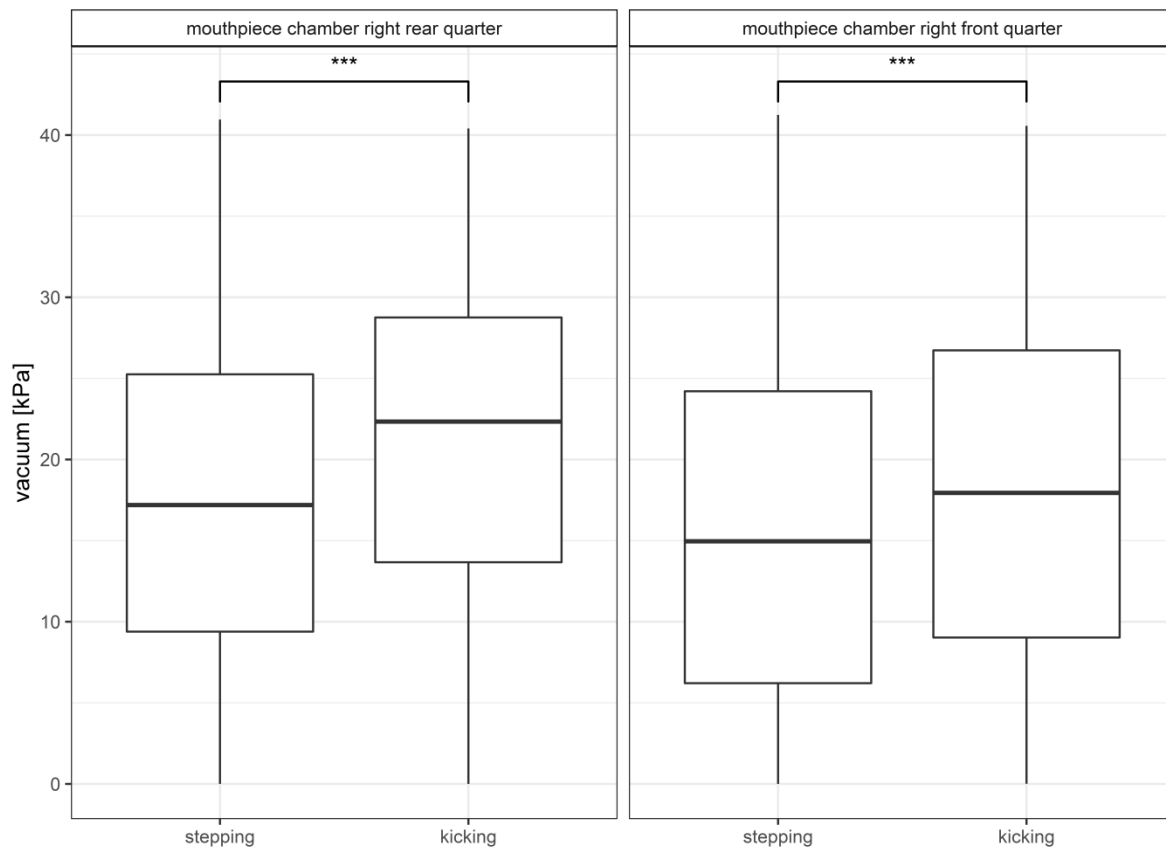
The length of the milking phases, the MPC vacuum level of the first and second quarter over-milking, the degree of hyperkeratosis, the presence of bimodality and the vacuum curve type were tested for their influence on the shown frequency of hind leg activities per minute. None of the above-mentioned variables had a significant influence.

*Influences on the category of hind leg activity*

In over-milking phases, the level of the MPC vacuum in the rear quarter ( $p < 0.01$ ) and the front quarter ( $p < 0.01$ ) was significantly higher when cows showed kickings. This effect was also found for the milking time as a whole ( $p < 0.001$ ;  $p < 0.001$ ) (Figure 2), but not in plateau phase.

Cows had significantly more often hyperkeratosis score 2, either at the front quarter ( $p < 0.05$ ) or the rear quarter ( $p = 0.067$ ), when hind leg activity kicking was observed.

Neither the presence of bimodality nor the vacuum curve type had an influence on the shown category of hind leg activity.



**Figure 2** Relationship between German-Holstein dairy cows' hind leg activity stepping or kicking and the mean vacuum level in the mouthpiece chamber of the right rear and right front quarter, averaged by a time period of three seconds around observed hind leg activity, during the course of milking

*Influence of the milking duration on the degree of hyperkeratosis and influence of teat morphology on SMT and MPC vacuum level and vacuum curve type*

The recorded degree of hyperkeratosis on front quarter ( $p = 0.02$ ) was significantly influenced by milking duration. Cows with a teat-end hyperkeratosis score 2 had a longer total milking time than cows with a teat-end hyperkeratosis score 1. Although not statistically significant, a trend was also seen for an influence of the milking duration on the hyperkeratosis on rear quarters ( $p = 0.064$ ).

Neither the SMT vacuum level in total milking time, nor the vacuum curve type had an influence on the recorded degree of hyperkeratosis on rear and front quarter.

The teat length, teat width and the vacuum curve type had no influence on the MPC vacuum level of the front right and rear right quarter, neither averaged over plateau phase nor averaged over the milking time as a whole.

## **Discussion**

The present study evaluated the influence of the machine milking process on the cows' hind leg activity. It was aimed to investigate whether the SMT and MPC vacuum level, vacuum curve types, bimodality, milking duration and lengths of milking phases have an influence on the frequency or category of the cows' shown hind leg activity during milking. Additional data were analysed in order to investigate the influence of the teat morphology on SMT and MPC vacuum level and vacuum curve type and whether the hyperkeratosis score is influenced by the length of the milking phases and the SMT vacuum level.

For visual observations it is essential to have an unrestricted view. Two methods are feasible: observations of hind leg activities by direct observations (Hemsworth et al., 1989; Breuer et al., 2000; Cerqueira et al., 2017) or by usage of video tape recording (Wenzel et al., 2003; Pastell et al., 2006). In this study direct observation was chosen, to be able to adjust the visual perspective when necessary during the course of milking. The use of written notices and a stopwatch have the disadvantage, that they may distract the observers view from the observed cows for a few seconds. Therefore a tablet was used, at which the observer had to press buttons to set time stamps for the observed hind leg activity stepping or kicking, in order to shorten the time the observer is distracted from observations. Direct observations in the milking parlour may influence the cows' hind leg behaviour. Munksgaard et al. (2001) have shown that the presence of an unknown person in the milking parlour while milking did not result in more steppings or kickings of the dairy cows, but the cows in the study were used to foreign persons visiting the farm. In the present study, the observer was foreign to the observed cows and the cows were not used to visitors. Nevertheless, attempts

were made to avoid influencing the cows' hind leg activity by the observer by behaving as silent and discreet as possible in the operator pit of the milking parlour.

The cows' hind leg activity in the milking parlour might be influenced in addition by other factors like stable flies or indoor temperature. According to Vitela-Mendoza et al. (2016), stable flies increase tail movements and leg stamps of beef cattle. As shown in Cerqueira et al. (2017), room temperatures above 27°C led to higher frequencies of kickings. Such effects of flies and high temperatures on the dairy cows' hind leg behaviour can be neglected in this study however. The experiment was carried out in the cold season of Northern Europe, between November and February, when flies and other insects are absent in milking parlours and temperature is low.

The definitions of the hind leg activities varied among studies, but most studies use a differentiation of steppings for low, vertical claw lifts and kickings for powerful leg lifts. The definitions are often based on centimeter dimensions for hoof liftings like in this study, and as done in Rushen et al. (2001) or Cavallina et al. (2008), or on anatomical characteristics of the observed cows as done in Breuer et al. (2000) or Rousing et al. (2006). In Cerqueira et al. (2017) the percentages of cows with at least one stepping or kicking activity ranged from 9.7 % to 90.6 %, respectively 0 % to 38 %. The rates of cows with a least one stepping or kicking activity in the present study were slightly higher. Also in Rousing et al. (2004), the percentages of cows showing at least one stepping and kicking activity were lower than in the present study. The differences regarding amounts of steppings and kickings in comparison to previous studies might be caused by the fact that the individual character of cows influences the dairy cows' behaviour during milkings (Van-Reenen et al., 2002). As reported in Rousing et al. (2004), also the herd affects the prevalence of shown steppings and kickings significantly. For this reason the herd was considered as fixed effect in the statistical analysis of the present study. The individual cow was not considered in the statistical models, due to the fact that the observed animals were chosen by their random choice of its milking places.

Previous studies reported a relationship between over-milking and the hind leg activity of cows. In Cerqueira et al., (2017), it was observed that the frequency of hind leg activity of dairy cows increased significantly in periods of over-milking, defined as milking in absence of milk flow. Likewise, Pastell et al. (2006) reported an increase in the hind leg activity of cows towards the end of the milking. In the present study, the frequency of stepping was highest in the plateau phase and kicking occurred most frequent in the let down phase. Second highest values for frequencies of stepping and kicking were found in the over-milking phase of the second quarter. High frequencies of kicking in the let down phase might be explainable by defensive behaviour of some cows at the beginning of the milking process. Although the

observation of hind leg activity started right after the milking cluster was attached and positioned by the milking operator, it cannot be excluded, that the kicking activity in the beginning of the milking could also be a reaction of the dairy cows to the milking clusters' attachment and the milker. The high frequency of stepping during the plateau phase could be an expression of general discomfort or unease during milking caused by the milking process, human-animal relationship, environment or neighboring cows (Rousing et al., 2004). As neither the vacuum curve type, i.e. the degree of vacuum level and vacuum fluctuations during the plateau phase, nor milking duration did influence the frequency of hind leg activity, stepping during the plateau phase might not be influenced solely by the milking process itself. The milking duration was assumed to have an influence on the relative frequency of hind leg activity per minute, since the probability to show hind leg activity was thought to increase parallel to machine-on time, but no influence was found. The hind leg activities stepping and kicking were second highest during over-milking of the second quarter. One reason particularly for the increased frequency of kickings in the over-milking phase of the second quarter could be that the rising vacuum during over-milking becomes more uncomfortable for the dairy cows as more udder quarters are affected.

The present study reports a significant relationship between MPC vacuum level and the shown category of hind leg activity. The category of shown hind leg activity was also related to the degree of dairy cows' hyperkeratosis. Hyperkeratosis in turn was influenced by the milking duration.

The underlying key assumption of the present study was that higher vacuum levels, particularly higher MPC vacuum levels, might lead to physical discomfort and in turn to mental stress for the animals which could result in more defensive behaviour in form of steppings and in particular kickings. This assumption was not proofed to be true with regard to the frequency of hind leg activity. Higher vacuum levels did not lead to significantly higher frequencies of hind leg activities. Low frequencies of hind leg activity were observed at low vacuum levels and also at high vacuum levels and vice versa. Though, the vacuum level influenced the shown category of hind leg activities. The dairy cows' kickings were observed at significant higher MPC vacuum levels at the right rear and right front quarter than steppings. This applies for the whole course of milking as well as for the over-milking phases of the first and second quarter.

As reported in Cerqueira et al. (2018), teat-end hyperkeratosis leads to more stepping behaviour due to physical discomfort of the dairy cows. Therefore it was assumed that the experimental cows show more intensive defensive behaviour and higher frequencies of hind leg activities at higher levels of hyperkeratosis. This assumption was proofed to be true for the category of the hind leg activity. Cows showing kickings had significantly more often a

marked hyperkeratosis at the front right teat. Whereas Cerqueira et al. (2018) observed more steppings at a higher degree of hyperkeratosis but no kickings. The relationship between hyperkeratosis and the hind leg activity was significant for the right front quarter, while for the right rear quarter only a statistical tendency was found. This might be explained by the low incidence of hyperkeratosis in rear quarters of 3 % only. The fact that front teats produce less milk than the rear teats (Weiss et al., 2004), resulting in an earlier completed milking, will lead to a longer period of over-milking in front teats and therefore to a higher incidence of hyperkeratosis (Edwards et al., 2013). In the present study, the degree of hyperkeratosis of the right rear and right front quarter was significantly influenced by the lengths of total milking time. An increasing risk for developing a higher degree of hyperkeratosis at a milking duration of longer than 5.3 minutes was also reported in Zucali et al. (2008).

Borkhus and Rønningen (2003) showed that long and wide teats had a lower MPC vacuum level during the plateau phase than smaller and narrower teats. In the present study no influence was found for the teat length and teat width on the MPC or SMT vacuum level. Probably the variety of teat morphologies was too little to identify differences.

As shown in the present study, the observed dairy cows' hind leg activity during milking was influenced, beside other parameters, by hyperkeratosis and the MPC vacuum level. Additional research is necessary to show whether observations of dairy cows' hind leg activity might be a relevant indicator to assess the animal welfare during milking with regard to the gentleness of the machine milking process.

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Not applicable.

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**Software and data repository resources**

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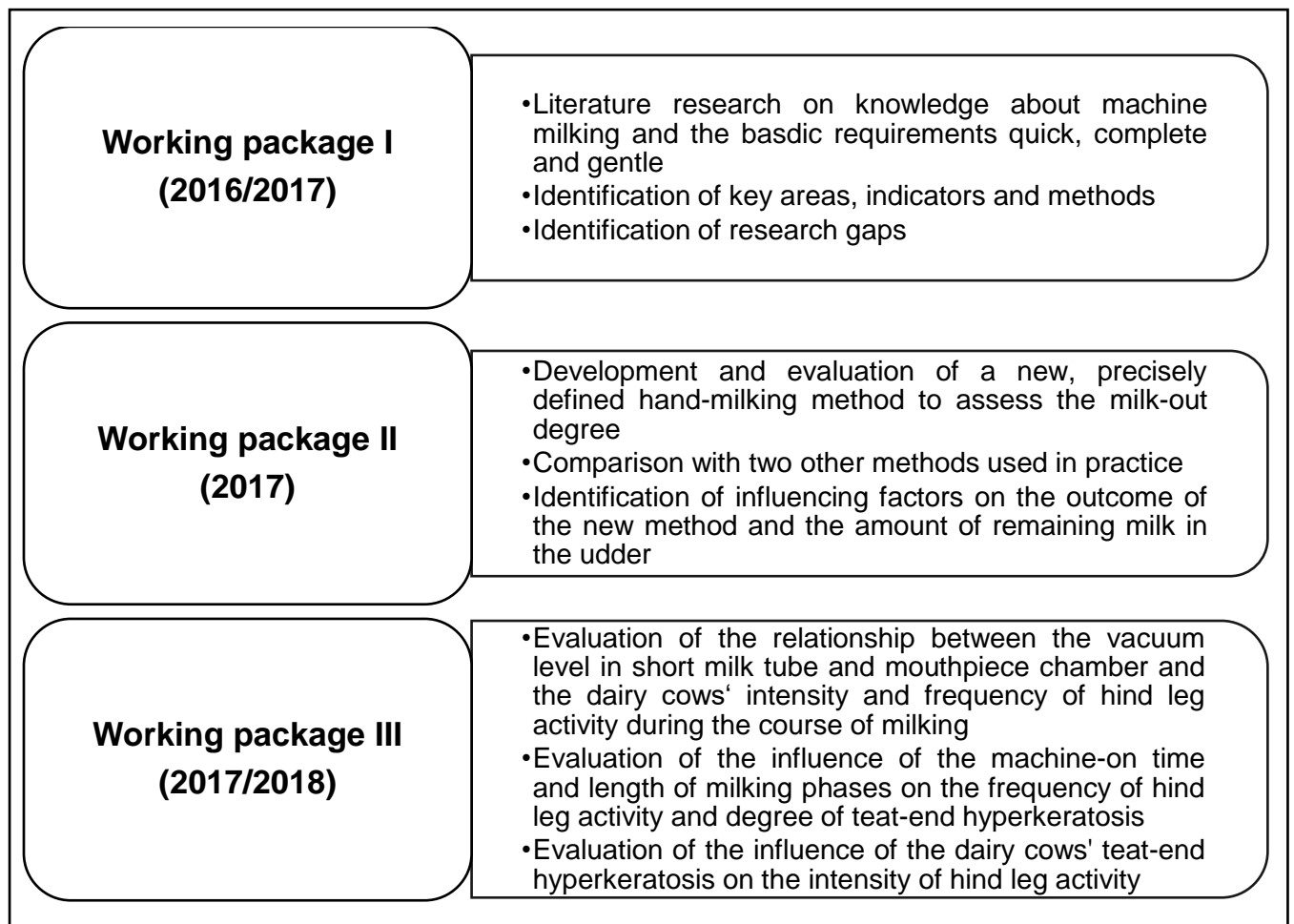
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## Chapter Six: General Discussion

It was aimed to simplify the assessment of the machine milking process for milking advisors with regard to its quickness, completeness and gentleness on a sound scientific basis. In the present chapter the chosen approach, achieved results and recommendations for future research will be discussed in general.

### Study design

The study design consisted of three logically coordinated working packages. The first working package was a literature research. As a result from this, a concept for milking advisors was developed, which identified key areas, indicators and methods which can be used to assess the machine milking process. Moreover, the literature review revealed research gaps with regard to methods enabling the assessment of machine milking (Figure 1).



**Figure 1** Temporal course of three working packages to support milking advisors in the assessment of the machine milking process

In consequence of this, two further working packages were conducted to develop and evaluate reliable and simply applicable methods which can be used for the evaluation of the machine milking process.

In the second working package, a new and precisely defined hand-milking method was developed and evaluated in order to assess the dairy cows' milk-out degree (Figure 1). This method was compared with two other methods already used in practice and described in literature (Chapter Three). Further, influencing factors on the outcome of the new precisely defined hand-milking method and the remaining milk in the udder were identified (Chapter Four).

In the third working package, vacuum measurements in the short milk tube and mouthpiece chamber were carried out during machine milking (Figure 1). The vacuum level in the short milk tube and mouthpiece chamber in different milking phases was evaluated for its influence on the dairy cows' intensity and frequency of hind leg activity during milking. Further, also the length of machine-on time and different milking phases was investigated for its influence on the frequency of the dairy cows' hind leg activity and the degree of teat-end hyperkeratosis. The degree of hyperkeratosis, in turn, was evaluated for its influence on the intensity of dairy cows' hind leg activity (Chapter Five).

### **Pros and cons of the developed concept and its usability for milking advisors**

Previous studies, which offer an overview on assessable indicators of the machine milking process focus only on segments of the milking process (Mein 1998; Jones 1999; Reinemann et al., 2001; Reinemann et al., 2000). The main focus in these publications was set on the vacuum system, the milking routines and/or cleaning and sanitation of the milking system. No previous investigation was found, which summarizes all conceivable, assessable indicators of the machine milking process in addition to examples of methods and thresholds for its assessment, as given in the developed concept in this study. Especially for milking advisors without prior experience in machine milking, the present concept might be advantageous to get an overall overview and a better understanding on the complexity of the machine milking process. Furthermore, the present concept illustrates connections and interdependencies between indicators. In previous investigations, which give an overview on indicators of the milking process, no information about interdependencies of indicators was found. By help of the illustration (Figure 2 in Chapter Two), milking advisor can see at first sight which indicators are linked to the existing problem of the farmer. In consequence, they could start the assessment goal-orientated. Moreover, the concept provides information about the point in time, when the assessment can be carried out. This could help milking advisors or future

researchers to organise the temporal course of the assessment. Thus, it is possible to either plan the assessment of several indicators parallel or to split the assessment in several milking times.

Due to the fact, that machine milking is an interaction of multiple factors during development of the concept it became obvious, that in best case milking advisor should have wide knowledge about dairy cows' physiology and a good technical understanding. Moreover, for the evaluation of some indicators technical equipment as vacuum loggers or a volume flow meter are mandatory. For this reason and the fact, that milking advisors have different prior educations (Gehrdau-Schröder, 2017), the development of a guiding digital tool working with illustrates and easy understandable instructions for the assessment is helpful and should be continued. The programming and consideration of interdependencies could be supported by the present concept.

Up to now, the present concept is not yet evaluated for its use in practice by milking advisors. This should be part of future investigations. The present concept is developed for the machine milking processes in milking parlours. It is conceivable to modify the present concept also for the assessment of automatic milking systems, due to the fact that automatic milking became more popular in the last years (Hunecke and Brümmer, 2018). The key area "Milking Parlour" could be replaced by the key area "Milking Box". The requirements on lighting, temperature, insects and flooring will be similar for the dairy cow to facilitate its entrance for milking. Further indicators might be the location and positioning of the milking box in the stable and the concentrated feed. The key area "Milking Operator" could be replaced by the characteristics of the robot arm and its settings and functions with regard to the milking routines and sensor technology to prevent and detect udder infections and other health issues.

### **Answered and still open research questions**

In order to address the issue of different prior educations and experiences of milking advisors, it was aimed to evaluate and develop methods for the present concept, which are easy to handle and need only basic equipment as a notebook and a stop watch. Further, it was aimed to put the dairy cow in the center of the investigations. Thus, the milking advisors could gather information about the milking process by observation of or investigation on the dairy cow itself without special equipment. There are already indicators available which proceed in this way and use the cow for evaluations of the milking process. Most of these cow related indicators assess the gentleness of the milking process. In Mein et al. (2001) relationships between the dairy cows' teat condition and technical settings of the milking

system, the choice of the liner and milking routines are described. The method to assess the teat condition is well explored and often chosen to evaluate the physical force acting upon the dairy cows' teat during the course of milking (Mein et al., 2001). Due to its high information value on technical mismatches it was chosen to be part of the present concept. The assessment of the effectiveness of teat cleaning and post milking teat disinfection and the udder cleanliness score are also valuable methods to evaluate the risk of bacterial transmissions and are carried out by direct investigations on the dairy cows' teat. Also the notice of hearable liner slips is carried out by observation of the cows during milking process. In order to evaluate the chosen liner size, the measurement of length and width of the dairy cows' teat are of great importance. As indicated in Chapter Two, the completeness of milkings can only be assessed by the dairy cows' milk-out degree. In order to determine the milk-out degree investigations directly on the dairy cows' udder are necessary. A poor milk-out degree indicates for incorrect or inconsistent settings of the automatic cluster remover settings in a parlour. Furthermore, the completeness of milking is influenced by the interaction between stimulation time and milk ejection reflex, a correct choice and condition of liner and technical settings of the milking system as pulsation, teat-end and mouthpiece chamber vacuum level, as indicated in Chapter Two. There are hand- and machine-milking methods with corresponding thresholds known in literature (Wehowsky, 1972; Davis et al., 2000). As explained in Chapter Two, especially for hand-milking methods, which need only little equipment, there was some doubt whether available thresholds can be applied in Europe. In addition to that, Davis et al. (2000) reported an influence of the evaluator of the hand-milking method on the amount of collected strip yield. Reasoned by that, a new and precisely hand-milking method was developed and evaluated in working package II (Chapter Three and Four).

The developed method enables the determination of milk-out degree by a full-hand milking method, as usual in Europe, which is carried out for 15 s per quarter. In order to avoid the influence of the evaluator it was aimed to standardize the method with help of a stop watch and a predefined handgrip-frequency. Despite these arrangements, the evaluators influenced the amount of hand-milked strip yield. It was assumed that the relation between teat length and the evaluators' hand size is decisive for influence. This seemed to be proven by the results in Chapter Four. It has been shown that longer teats led to higher amounts of strip yield. Longer teats were assumed to be easier to hand-milk, due to the larger surface which enables the application of the full-hand milking handgrip better as on short teats, in particular for milking operators with large hands. By a better hand-grip also the hand-force increases. Additionally, it is conceivable that longer teats could store more milk, due to larger teat cistern. Probably at this point two influences interdepend from each other.

The new precisely defined hand-milking method was compared to a subjective visual method and a further hand-milking method with regard to its relationship to the remaining milk in the udder. The visual method assesses the milk-out degree by quarter filling degree and the second hand-milking method, assesses the number of easy strips. In the present study no significant relationship between the visual method and the rest milk in the udder was found. Therefore, this method is not recommendable for milking advisors and farmers in order to assess the completeness of milking and not part of the present concept. No investigation was found in literature which had evaluated the relationship between the outcome of these two methods and the remaining milk in udder before. For the new precisely defined method and the method of counting the number of easy strips, a significant relation to the remaining milk in the udder was found. Also for the counting of easy strips, an influence of the evaluator on the outcome was found, though this method was also carried out by the precisely defined hand-milking. As a consequence of this knowledge, milking advisors can compare the milk-out degree among herds with the precisely defined method and the counting of easy strips under the permission that the evaluator stays the same.

During the milking process it must be highest priority for consultants not to disturb the ongoing process longer than necessary in order to do not decrease the milking parlour performance. It has been shown in Chapter Four that the new precisely defined method should be performed at all four quarter to be able to draw conclusions on the amount of rest milk on udder level. The milking characteristics vary too much among the four quarter of an udder (Tančin et al., 2006). That means a time exposure of 60 s per dairy cow. The method of counting the number of easy strips is probably more time-saving. The advantage of the new method in comparison to the counting of easy strips that milking advisor get informed about the quantity of rest milk in the udder. By help of this, it is possible to adjust the automatic cluster remover settings to a certain threshold.

In Chapter Four it was investigated that the rest milk in the udder and the amount of hand-milked milk in 15 s is influenced by the herd and the milking cluster positioning. The herd includes the milking machine equipment, settings and milking routines, which are the same for the whole specific herd. It was shown, that the herd with an automatic stripping-arm had the lowest amounts of rest milk and strip yield in 15 s and the herd with the highest automatic cluster remover settings had the highest amounts of rest milk and strip yield in 15 s. The influence of the milking cluster positioning underlines the importance of the milking operator with regard to the completeness of milking. Therefore, the milking cluster positioning is chosen as indicator to evaluate the work of the milking operator in the concept. Further, reported in Chapter Four, in some cows a palpable ring at the teat base was found. This indicates for a less gentle milking by long over-milking phases (Hillterton et al, 2002). The

ring at the teat base led to lower strip yields in 15 s, presumably due to constricted teat tissue (Chapter Four). The knowledge on the influence of the milking cluster position and palpable ring at teat base on the amount of hand-milked milk in 15 s is important for the application of this method in practice. In consequence, milking advisors know that palpable rings at teat base or incorrect cluster positions could distort the results.

Further indicators, which observe or investigate the dairy cow itself to draw conclusions about the machine milking process are observations on the animals' behaviour during milking. In order to evaluate the discomfort and stress of dairy cows during the milking process, previous studies chose the hind leg activity (Rousing et al., 2004), rumination (Bristow and Holmes, 2007) and rate of defecation and urination (Rushen et al., 2001). The relationship between the observed dairy cows' hind leg activity and actual concentrations of stress hormones in the cows' blood or elevated heart rate and discomfort is explored well in previous investigations (Gygax et al., 2006; Rushen et al., 1999; Rushen et al., 2001). The hind leg activity can be observed in all types of milking parlours without expensive equipment. For this reason, it was chosen for the working package III (Chapter Five). As described in Chapter Two, the quickness of the milking process can be increased by higher vacuum levels (Ambord and Bruckmaier, 2010) or shorter machine-on times (Clarke et al., 2004). The basic idea was to investigate whether the dairy cows' hind leg activity, as a sign of ungentle milking, is related to the vacuum level in short milk tube and mouthpiece chamber or machine-on time. It would simplify the work of milking advisors, when the observation of dairy cows' hind leg activity could be used to distinguish among gentle and ungentle vacuum levels and machine-on times. The hind leg activity could then be used as an early alert system for technical mismatches.

Definitions for the differentiation of the intensities of hind leg activities were given in centimeters in Chapter Five. The chosen definitions were also used in previous studies (Hemsworth et al., 1989; Rushen et al., 2001) and are easy in application without giving scope for interpretation. For this reason this method might be suitable for milking advisors without prior experience. The number of simultaneously observed cows can be varied – depending on the concentration ability of the observer. The hind leg activity was divided into three categories (Chapter Four), because it was thought that a more differentiated observation would be beneficial in the further analysis. Previous studies divided the hind leg activity only into two categories (Hemsworth et al., 1989; Rushen et al., 2001; Rousing et al., 2004). It has been shown that two categories were sufficient. The dairy cows show either little forms of weight shifting and small steps (steppings) or target-orientated powerful kicks (kickings). The shown category of hind leg activity, stepping or kicking, during the course of milking was related to the vacuum level in the mouthpiece chamber. Though, it was not

possible to define a certain vacuum level at which the dairy cows' switch from stepping to kicking. Both categories were observed at short milk tube and mouthpiece chamber vacuum levels from 0 kPa to 43 kPa.

Among milking phases the frequency of hind leg activity differed. Second highest frequencies of stepping and kicking were observed in the over-milking phase of the second quarter. The frequency of kicking was the highest in let down phase and over-milking of the second quarter. Both are milking phases in which changes of vacuum levels occur. A rising frequency of hind leg activity in over-milking phases was already observed in Cerqueira et al., (2017). Presumably, the instantly rising mouthpiece chamber vacuum level on the second quarter, caused by a low milk flow, lead to discomfort which in turn triggers more stepping and kicking. These observations could lead to the assumption, that the dairy cows' hind leg activity is related to changing vacuum levels. An argument against this assumption is that vacuum curve type 2 (Appendix A14) had no influence on the frequency of hind leg activity. The vacuum curve type is characterized by continuous variations of the vacuum level. Nevertheless, vacuum curve of curve type 2 has generally higher vacuum fluctuations (Appendix A14) and transitions between milking phases are not as clearly as in curve type 1 (Appendix A13). It might be conceivable that the changes of vacuum levels from one to another milking phase are lower in vacuum curve type 2. Nevertheless, the highest frequencies for stepping were observed in plateau phase. Generally speaking, the mouthpiece chamber and short milk tube vacuum level is the lowest in plateau phase during the course of milking. Probably the stepping behaviour is influenced by more factors as the vacuum level only. In previous research a relationship between stepping and the somatic cell count of the animals was observed (Cerqueira et al., 2017). Furthermore, cows in their first lactation seem to show less stepping behaviour than cows in higher lactations (Cerqueira et al., 2017). Also the individual character plays a major role on the dairy cows' behaviour (Van-Reenen et al., 2002), anxious cows show less stepping than confident cows. As reported in Chapter Five, the length of the machine-on time influenced the degree of hyperkeratosis. The degree of hyperkeratosis in turn influenced the intensity of hind leg activity. This is important knowledge for milking advisors when animal behaviour should be observed.

According to Mein and Reinemann (2014) longer milkings and higher vacuum levels lead to less gentleness of the milking process. Rasmussen (2004) reported that the presence of over-milking can be concluded by the kicking behaviour of dairy cows. In interpretation of the results that the machine-on time influenced the degree of hyperkeratosis and in turn the degree of the hyperkeratosis the dairy cows' hind leg activity and kickings were observed at higher mouthpiece chamber vacuum levels than steppings in over-milking phases, this seems to be proved true. Due to the investigations in Chapter Five, it is conceivable that the

hind leg activity of dairy cow' might be used in future to evaluate the gentleness and also the quickness of the machine milking process.

Though, some research questions have been answered with the working packages II and III, there are still missing methods and thresholds for the assessment of some indicators in the present concept. The usage of the animals' behaviour during machine milking as indicator for the gentleness of the milking process for milking advisors is still difficult, because thresholds are missing. Furthermore, the animal behaviour is likely to be influenced to a great part by the dairy cows' individual character and herd characteristics, as indicated in Chapter Five. For this reason, the identification of threshold might be a difficult task. The same problem can be reported with the observation of the human-animal-interaction. A threshold for the differentiation between good and poor interactions is missing. Furthermore, the differentiation could be difficult for milking advisor, because in order to assess a handling as friendly or adverse, the cows' reaction has to be observed and understood very well. This requires some training and experience of the milking advisor. There is a danger to misunderstand the animals' behaviour. The observation of antagonistic cow behaviour is described well in previous studies, but no thresholds are available and the observation is time consuming (Rousing and Wemelsfelder, 2006). The observation of the dairy cows' willingness to enter and exit the milking parlour is also not clearly defined or standardized in previous investigations. Though, Gómez et al. (2017) measured the time period which is needed from crossing a virtual line at the entrance of the milking parlour with the front legs until the cow stand with all four legs at its milking position. This approach could be added by the measurement of time the cow needs to cross the virtual line. Also the step length of dairy cows, in previous studies used to evaluate the slip-resistance of dairy cows (Phillips and Morris, 2001), could be one possible starting point to define the quickness or hesitancy to enter and leave the parlour. No previous studies were found on the observation of wounds and injuries caused by the milking parlour, which might be valuable indicators. Also thresholds for methods are missing for the visual evaluation of the cleanliness of the milking equipment. A conceivable solution for indicators, for which it is difficult to define thresholds is to collect data about machine milking processes of different farms with help of the digital tool, as soon as available, and to compare the results of the assessed milking process with the average of other farms.

**Quick, complete and gentle - a suitable framework for the concept or mutually exclusive?**

The earliest publication, which was found using quick, complete and gentle as requirements for the milking process, was published in 1965 (Van Sant, 1965). The assignment of the chosen indicators in the present concept to these three requirements resulted in an imbalance. Most indicators can be used to assess the gentleness (48 %) and quickness (32%), while considerably fewer indicators can be used to assess the completeness of milkings (20 %). Though, some indicators were assigned to more than one of the three requirements. The gentleness has the highest share of indicators, because it includes all indicators which could have an effect on the dairy cow and the cow is in the center of the milking process. In some studies, the cleanliness as a fourth basic requirement on the machine milking process can be found (Reinemann et al., 2003; Bobić et al., 2018; Gadhavi et al., 2020). In the present concept, the cleanliness of the milking system is assigned to the gentleness of the milking. Contaminations in the milking system, due to an insufficient cleaning and sanitation, could affect the bacterial transmission during milking and result in new intramammary infections and decreased gentleness of the milking for the cow, as explained in Chapter Two. Though, the cleanliness and sanitation of the milking system could not only affect the dairy cow, but also the milk quality by increased bacterial cell count in the bulk milk (Bava et al., 2011), which is an essential criteria for the payment by dairy companies. Also the cow cleanliness and milking routines have an influence on the bacterial cell count of the bulk milk (Zucali et al., 2011). From this point of view, the addition of “clean” as a basic requirement on the machine milking process would appear reasonable. However, a further sectioning of the requirements was not chosen for the present concept in order to keep the concept as simple as possible.

There are several previous studies which investigated how quickly, how completely and how gently the milking process can be without having negative impacts on other parts of the milking process, but no fixed targets are available (Rasmussen and Madsen, 2000; Jago et al., 2010; Mein et al., 2010). According to Mein and Reinemann (2014), to balance the three requirements quick, complete and gentle compromises must be made. They reported that the maximizing of the quickness will result in less complete or less gentle milking, due to more congestion in the teat apex and potentially teat cup crawls in periods of low milk flow. The maximizing of the completeness will lead to slower and less gentle milking, due to longer physical strain of the dairy cows' teat and longer periods of over-milking. The maximizing of the gentleness will result in slower and probably less complete milking, due to earlier cluster

remover settings and pulsation settings with higher ratios of massage phase than milking phases (Mein and Reinemann, 2014).

Quick milkings are aimed to reduce labour costs, electrical power and wear on equipment. It can be achieved by shorter machine-on times and higher milking parlour performances. If quicker milkings are achieved to the detriment of the completeness or gentleness of the machine milking process, the desired economic advantage might be not reached. The negative impacts on the udder health and milk production could outweigh the cost reductions. The economic losses of a subclinical and clinical mastitis, which could result from ungentle milkings, vary from 114 € and 182 € per cow (Huips et al., 2008). There are some indicators, which could be used to balance the three requirements.

One opportunity to shorten the machine-on time is to increase the peak milk flow rate by increasing the teat-end vacuum (Besier and Bruckmaier, 2016). Partly due to higher pressure differences across teat canal and partly because the teat canal is more stretched by higher vacuum levels (Mein and Reinemann, 2014). While in periods of high milk flow, the teat-end vacuum level is reduced, in periods of low milk flow, nearly the full operating vacuum acting upon the dairy cows' teat. Therefore, high teat-end vacuum level can be seen as disadvantageous with respect to the completeness and gentleness of the milking process in periods of low milk flow. Another method reported in literature, to increase the milk flow rate is to widen the pulsation ratio in order to expand the milking phases and shortened rest phases (Thomas et al., 1991). Though, the completeness and gentleness of milking can only be achieved when the congestion of the teat during milking is prevented. This contradicts a wide ratio between milking and rest phase according to Mein and Reinemann, 2014. The teat congestions by a widened pulsation ratio can be counteracted by a prolonged duration of the d-phase. This phase should be at least 150 ms (Reinemann et al., 2001). With respect to the pulsation settings it is also important to take the indicator liner into account. The liner is able to influence the quickness, completeness and gentleness of a milking process in a decisive way by its fit and compression. The compression prevents the congestion of teat apex and ensures a high milk flow (Mein and Reinemann, 2014). As reported in Hamann et al., 1993, the requirements on liner compression increase with increasing teat-end vacuum for an effective relieving of teat congestion and edema. Furthermore, liner with a high liner tension and increased wall thickness tend to milk faster (Mein and Reinemann, 2014) and liner with increased mid-barrel bore could lead to a more widened teat canal and increase the milk flow (Mein and Reinemann, 2014). Though, in concern of the gentleness, liner with high compression could increase teat-end hyperkeratosis (Neijenhuis et al., 2001). The risk for hyperkeratosis can be reduced by low compression liner and reduced teat-end vacuum (Mein and Reinemann, 2014).

An unnecessarily long duration of physical strain of the dairy cows' teat in phases of low milk flow could be avoided and prevented by increasing the threshold of the automatic cluster remover and lower the milk-out degree (Rasmussen, 1993). Alternatively, the machine-on time can be terminated by a predetermined maximum of milking duration, as investigated in Clarke et al. (2004) in cows in late lactation. According to Reid and Stewart (1997), the increase of the milking cluster remover from 0.32 kg/min to 0.82 kg/min, decreased the milking duration and milk quality and volume were unchanged. Rasmussen et al. (1993) reported also improvements of the dairy cows' behaviour due to increased cluster remover thresholds, indicating for more gentle milking. Most studies investigated the increase of the automatic cluster remover threshold in combination with best practice teat stimulation, consisting of fore-stripping and teat cleaning (Rasmussen et al., 1993; Reid and Stewart 1997). By help of longer durations of teat stimulation, the risk of bimodality can be reduced. According to Bruckmaier and Blum (1996), the machine-on time was longer and peak flow rate reduced without pre-stimulation and bimodal milk flow curves.

A high working efficiency of the milking operator and a calm and stress-free human-animal-interaction in order to avoid stress and fear of the dairy cows could be beneficial with regard to the quickness, completeness and gentleness of the milking process (Jones, 1999). The dairy cows should be able to enter and also leave the milking parlour as quick and stress-free as possible. The design of the milking parlour can support this by a high frictional flooring (Phillips and Morris, 2001), a good lighting, good climate and the avoidance of obstacles, steps or narrow curves. Insects should be controlled in milking parlours and waiting areas, due to its influence on hind leg activity (Vitela-Mendoza et al., 2016) and higher risk for cluster kick offs by the cows. Generally, stress should be avoided in order to ensure stress-free milking without disruptions. The willingness to enter the milking parlour and to leave it could be a valuable indicator for stress-free milking conditions and suitable milking parlour design. Under consideration of above mentioned indicators the three requirements could be balanced without compromising.

### **Quality and suitability of the chosen indicators for an initial troubleshooting and cause analysis**

As indicated in the section above, for some indicators, chosen to be part of the present concept, standardized methods and thresholds are missing for the assessment of the milking process. Though, the quality of the chosen indicators does not only depend on the availability of thresholds and methods but also on its suitability to be used for an initial troubleshooting or further analysis of causes, as mentioned in Chapter Two. All indicators of the concept and

its evaluation with regard to the availability of thresholds and methods for its assessment and possible suitability for an initial troubleshooting or cause analysis are listed in Table 1.

**Table 1** Assessment of the chosen indicators with regard to the availability of methods and thresholds and its suitability for an initial troubleshooting or further analysis of causes (“+” = available/suitable; “-” = not available/not suitable; “○” = no method/threshold available, but proposal made)

	Indicator	Method available	Target value/ Threshold available	Initial trouble-shooting	Cause analysis
<b>Milking System</b>					
Risk analysis for technical shortcomings	Basic information for a first risk analysis	-	-	+	-
Cleanliness	Cleanliness (visual)	-	-	+	-
	Cleaning-time test	+	+	-	+
	Bacterial cell count in bulk milk	+	+	+	-
Vacuum stability	- Dimensions and slope of milk line	+	+	-	+
	- Dimensions of milk tubes and milking claws	+	+	-	+
	- Unblocked vents of milking claw and vacuum capacity	+	+	-	+
	- Visible cracks	-	+	+	-
	- Hearable leakages	-	+	+	-
	- Vacuum measurements: <ul style="list-style-type: none"> <li>○ Operating vacuum level</li> <li>○ Fall-off test: vacuum pump reserve and regulator response</li> <li>○ Cyclic and irregular vacuum fluctuations</li> </ul>	+	+	-	+
- Liner slips	+	+	+	-	
Physical strain of teat	- Liners' choice	+	+	-	+
	- Liners' condition	+	+	+	-
	- Vacuum measurements <ul style="list-style-type: none"> <li>○ Teat-end vacuum</li> <li>○ Mouthpiece chamber vacuum</li> <li>○ Pulsation settings</li> <li>○ Length of over-milking phases</li> </ul>	+	+	-	+
	- Teat condition	+	+	+	-
	- Settings & thresholds	-	○	-	+
	- Milk-out degree <ul style="list-style-type: none"> <li>○ Hand-milking</li> <li>○ Machine-milking</li> </ul>	+	○	+	+
		+	+	+	+
<b>Dairy Cow</b>					
Herd characteristics	- Overview	-	-	+	-
Udder health status	- Somatic cell count	+	+	+	-
	- Visual inspection milk filter	-	○	+	-

	Indicator	Method available	Target value/ Threshold available	Initial trouble-shooting	Cause analysis
Sizes and morphologies	- Body length and width - Teat length and width - Udder and teat morphologies	+ + +	⊖ ⊖ ⊖	- - -	+ + +
Milking characteristics	- Peak milk flow - Milking speed - Milk yield	+ + +	+ + +	- - -	+ + +
Stress level	- Animal behaviour: ○ Hind leg behaviour ○ Rumination ○ Defecation and urination - Other: ○ Heart rate ○ Cortisol concentrations in milk or blood - Disturbed milk ejection	+ + + + + +	- - - + + +	+ + + - - -	- - - + + +
<b>Milking Operator</b>					
Bacterial transmission	- Milking order - Milking routines: ○ (Wearing gloves; Washing hands; Fore-stripping; Choice and concentration of pre teat dip; Choice and concentration of post teat dip; Intermediate disinfections) ○ Cleaning of udder and teats	+ ⊖ +	+ ⊖ +	+ + -	+ + +
Milk-out degree	- Milking routines: ○ Duration of stimulation ○ Duration of tactile appeal ○ Milking cluster positioning - Milk flow curves: ○ Bimodality	+ + + +	+ + + +	+ + + -	+ + + +
Vacuum stability	- Milking routines: ○ Unit attachment procedure ○ Number of simultaneously attached units	+ +	- +	+ +	- -
Interactions with the dairy cow	- Human-animal-interaction: ○ in milking parlour ○ while herding of the cows into the parlour	+ -	- -	+ +	+ +
Milking time management	- Milking intervals - Milking times per day	+ +	+ -	- -	+ +
Efficiency	- Cows per milker - Working-time measurements	+ +	- +	+ -	- +

	Indicator	Method available	Target value/ Threshold available	Initial trouble-shooting	Cause analysis
<b>Milking Parlour and Waiting Area</b>					
Milking parlour design	- Type of parlour	+	-	-	+
	- Number of milking positions	+	-	-	+
	- Size milking positions	+	-	-	+
	- One-end exit/ rapid exit	+	-	-	+
	- Entrance and exit design of the parlour:				
	o Ramps, steps and obstacles, narrow curves, sharp edges	-	-	-	+
	o Lighting; Temperature, Volume level	+	+	-	+
	o Flooring	+	-	-	+
	o Insects	-	-	-	+
	o Drinking troughs	+	-	-	+
	o Concentrated feed	+	-	-	+
o Electrical current	+	+	-	+	
- Willingness to enter and to leave the parlour	+	-	+	-	
- Injuries and wounds	+	⊖	+	-	
- Attendance time	+	-	-	+	
Waiting area design	- Size	+	-	-	+
	- Numbers of cows waiting at the same time	+	-	-	+
	- Attendance time	+	-	-	+
	- Antagonistic behaviour	+	-	+	+
	- Ramps, steps and obstacles, narrow curves, sharp edges	+	-	-	+
	- Lighting; Volume level; Temperature	⊖	+	-	+
	- Flooring	+	-	-	+
	- Insects	-	-	-	+
- Drinking troughs	+	-	-	+	
Work environment for milking operator	- (Variable) operating height	+	+	-	+
	- Body posture while attachment of milking cluster	+	+	+	+
	- Stairs; Flooring; Temperature; Lighting; Volume level	⊖	⊖	-	+
	- Insects	⊖	⊖	-	+
	- Walking distances	+	⊖	-	+
Milk-out degree	- Constructional possibilities for correct milking cluster positioning	⊖	-	+	+

Easy applicable indicators in practice are those, where a method and also threshold value for the assessment is known. For the assessment of some indicators no previous investigation was found with an exemplary description of a method. Though, in some cases no previous exemplary is needed, as for example for the measurement of the teat length. In other cases no thresholds are needed for the assessment as for example for unblocked vents, leakages or body sizes. For some indicators proposals were made, if no previous method or threshold was available. The milking routines, for example, could be compared to standard operating procedures, if available. For indicators as for example the over-milking or attendance time in the waiting area, it would be conceivable to set the target "as short as possible", but short is not clearly defined. The same problem is encountered for the vacuum fluctuations. There are good reasons why they should be as low as possible (Nyhan and Cowhin, 1967), but "low" is also not quantified. In consequence of this, no threshold or target value is proposed.

Indicators for an initial troubleshooting offer easy available information about the machine milking process by its assessment. The assessment could be performed visual or audible. Alternatively, the information is offered by herd management programs or external sources. These indicators offer first obvious hints on potential malfunctions or disruptions of the machine milking process. In case that these indicators indicate for a problem a further analysis of causes can be performed.

Good examples are the dairy cows' teat condition and liner slips during milking. They give hints for mismatches between the milking system, its settings and its effects on the dairy cows' teat. These two indicators are useful start points for an assessment. In case of poor conditioned teats or high amounts of liner slips, a further analysis of causes can be started. The milking advisors can use Figure 2 in Chapter Two to get to know which indicators should be considered for a further analysis. Also the observation of milking routines can give valuable hints for the risk of bacterial transmission and bimodality during machine milking. The assessment of the milking cluster positioning could give hints for the milk-out degree. The observation of the animals' willingness to enter and leave the parlour, wounds and injuries, the animal behaviour in parlour and waiting area and the human-animal-interaction could give hints for stressful milkings due to the milker or the milking parlour and waiting area design. The problem encountered with these indicators is already mentioned above. When milking advisors focus in a first step on all indicators for the initial troubleshooting they get already obvious hints for the physical strain of the teat, the vacuum stability, the milk-out degree, the stress level and udder health status of the dairy cows', the risk of bacterial transmission and unfavourable conditions for the dairy cows in the milking parlour and waiting area, which can be proofed in a further analysis (Table 1).

Indicators for a further analysis of causes can be assessed, if indicators of the initial troubleshooting gave hints for mismatches. The analyzing indicators search for the reasons of the obvious issues. Some indicators can be used for both, the initial troubleshooting and the analysis of causes as well. One good example here, are the milking routines. They could give valuable first hints or, in case of udder health issues of the herd, could be used for a further cause analysis for potential bacterial transmissions. In addition to that, also the human-animal-interaction could be used for both. If the dairy cows enter the milking parlour only hesitantly, the human-animal-interaction could be causing. As well as the milking parlour design and its environmental conditions inside. Also the milk-out degree could either indicate for issues with the automatic cluster remover settings or mismatches between pulsation settings, liner choice and stimulation time or it can be used to investigate the impacts of the milking cluster position or adaptations of the milking cluster remover settings in a further analysis. The differentiation between indicators for an initial troubleshooting and those for a cause analysis are not yet tested in practice use. Future investigation will show whether this approach is time-saving and how reliable the indicators for an initial troubleshooting indicate for causing issues.

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## Chapter Seven: General Conclusion

This study developed and introduced a concept for milking advisors based on a literature research. In the developed concept key areas and indicators were defined, which need be taken into account to enable the assessment of the quickness, completeness and gentleness of the milking process. The interrelations and interdependencies of the chosen indicators were illustrated. In order to close research gaps with regard to the assessment of some indicators, simple applicable methods were developed and evaluated. For the assessment of the completeness of milkings, a new precisely defined hand-milking method to collect strip yield was developed and compared with two other methods, which are already applied in practice. Subsequently influencing factors on this method and the remaining milk in the udder was investigated. For the assessment of the gentleness and quickness, the vacuum level in the short milk tube and mouthpiece chamber and machine-on time was chosen to investigate for its influence on the dairy cows' hind leg activity and hyperkeratosis. Results of the developed concept and investigations on methods for the assessment of the machine milking process led to the following conclusions:

- The developed concept identified four key areas: Milking System, Dairy Cow, Milking Operator and Milking Parlour and Waiting Area
- Interdependencies between indicators are complex and for milking advisors it is of great importance to get informed which indicators need to be considered for the assessment of a certain issue
- Under consideration of the following indicators: pulsation settings, liner choice, teat condition, automatic cluster remover settings and milk-out degree, milking routines, working efficiency of the milking operator, parlour design, human-animal-interaction, animal behaviour and willingness to enter the parlour, the milking process can be quick, complete and gentle at the same time
- Indicators can be used for an initial troubleshooting or analysis of causes
- The assessment of the cleanliness, leakages, teat and liner condition, liner slips, somatic and bacterial cell count, milk-out degree, milking cluster positioning, milking routines, teat stimulation, unit attachment, human-animal-interaction, animal behaviour, wounds and injuries and the dairy cows' willingness to enter the parlour are suitable indicators for an initial troubleshooting
- The assessment of milk flow and vacuum measurements, body and teat sizes, milking routines, milk-out degree, milking parlour and waiting area design, its environmental conditions and working-time measurements are suitable indicators for a further analysis of causes

- The new precisely defined hand-milking method, developed in this study, and the counting of easy strips can be recommended to predict the rest milk in the dairy cows' udder by milking advisors
- The precisely defined hand-milking method might be easier in application without prior experience, due to clear guidelines and a quantifiable amount of collected strip yield
- Evaluators can compare the milk-out degree of dairy herds with the precisely defined hand-milking method and method of counting the number of easy strips under the premise that the evaluator stays the same
- All four quarter of a cows' udder should be hand-milked for 15 s per quarter to enable the best prediction of the amount of remaining milk in the udder
- Milking advisor should not choose cows with incorrect milking cluster positions and palpable rings at the teat base for the hand-milking of strip yield to avoid distorted results
- Further research is needed to evaluate whether the cluster positioning can also be assessed by the milk-out degree.
- Thresholds of 100 ml per quarter, as reported in previous literature, are too high for the new developed, precisely defined hand-milking method
- Kicking is related to higher vacuum levels in mouthpiece chamber and higher degrees of teat-end hyperkeratosis than stepping
- The machine-on time does not affect the frequency of hind leg activities, but the degree of teat-end hyperkeratosis
- Kicking seem to present an avoidance behaviour, while stepping seem to be caused by multiple factors

In summary it can be said, that the developed concept is able to support the work of milking advisors by giving an overview on assessable indicators, methods and interdependencies. In future, the present concept should be evaluated by milking advisors in practice. A simple applicable method for the evaluation of the milk-out degree was developed. Thresholds for this method need to be determined in future investigations. The dairy cows' hind leg activity seem to indicate for ungentle milkings due to high vacuum levels and degrees of teat-end hyperkeratosis, but further research is needed to establish the hind leg activity as indicator. The development of a digital tool, which supports the milking advisors in the assessment of the machine milking process, could help to standardize the assessment and should be continued in future.

## Chapter Eight: General Summary

The worldwide demand for dairy products increases. Simultaneously, politicians and consumers ask for a more sustainable production and stricter requirements to guarantee animal welfare. Farmers must comply with these requirements also during the machine milking process. In order to perform the dairy cows' milking resource-saving, economic effective and as dairy cow-friendly as possible to maintain and improve the animals' health, the milking process should be quick, complete and gentle. Often the milking process is not balanced and not all three attributes are fulfilled at the same time. In this case farmers ask for help from milking advisory services. Currently there is no official apprenticeship provided for milking advisors, therefore advisors have different levels of prior education and no gold standard for the approach of advisory service is available in machine milking.

In consequence of this, the aim of the present study was to develop a helping concept for milking advisors, which simplifies the assessment of the machine milking process in milking parlours on a sound scientific basis. Research gaps with regard to methods and thresholds for the assessment were identified and began to close with the present study. Methods, which are easy to handle and put the dairy cow into the center of the investigation, were developed and evaluated.

The concept was developed by an intensive literature research and introduced in Chapter Two. The Milking System, the Dairy Cow, the Milking Operator and the Milking Parlour and Waiting Area were identified as key areas of the milking process. The Milking System has a major role on the quickness, completeness and gentleness of the machine milking process. For each key area indicators were presented, which provide further information about the machine milking process and enable the assessment. Some indicators can be used for an initial troubleshooting and other for an analysis of causes. Influences and interdependencies of indicators were highlighted and illustrated by the present concept. The concept provides an overview on methodologies and thresholds from literature and help milking advisor to organise the temporal course of the assessment process.

In Chapter Three, a new, precisely defined hand-milking method to assess the completeness of the milking process was developed and compared to two other methods, which are already applied in practice. The first method was a visual method, which assesses the degree of the quarter filling. This method was not related to the actual rest milk in the quarter. The second method, a quantitative assessment of the number of easy strips, and the new precisely defined hand-milking method for 15 s per quarter, were suitable to predict the rest milk in the udder. Three evaluators visited six farms, each farm once by each evaluator. Despite to the strict definitions of the hand-milking handgrip and handgrip frequency it was

not possible to avoid the influence of the evaluator on the amount of collected strip yield in 15 s and the numbers of easy strips. In consequence of this, the amount of strip yield among herds can only be compared under the premise that the hand-milking is performed by the same person. The influence of the evaluator results presumably from the ratio of his/her hand size and the teat length. Further research is needed to define thresholds for the precisely defined hand-milking method. Previous thresholds were set at 100 ml. Reasoned by a slower milking by the precisely defined hand-milking in comparison with a stripping hand-milking method, the threshold must be lower for the new developed method.

In Chapter Four, the precisely defined hand-milking method was investigated for cow and milking system related influencing factors on the outcome of the method and the rest milk in the udder. The investigation was conducted in one farm, with one evaluator, which performed the hand-milking in three milking times per day on three consecutive days. It was outlined, that the rest milk in the udder is influenced by the positioning of the milking cluster and herd specific settings of the milking technique, the milking routine and the herds' individual characteristics. The outcome of the full-hand hand-milking of 15 s per quarter was influenced by the herd, evaluator, milking cluster positioning, teat length and palpable rings at teat base. Longer teat and incorrect milking cluster positions led to higher amounts of strip yield in 15 s per quarter. Palpable rings at the teat base led to less amounts of strip yield in 15 s. Best prediction results for the rest milk in the udder were achieved when all four quarter of a dairy cow was hand-milked for 15 s. The individual milking characteristics of the quarter varied too much to predict the amount of rest milk in the udder by only hand-milking one or two quarter. In Chapter Five, the intensity of the dairy cows' hind leg activity during the course of milking was observed in ten farms and two milking times per farm and day for its relation to the vacuum level in the short milk tube and mouthpiece chamber. Additionally, the machine-on-time and lengths of milking phases was investigated for its influence on the frequency of observed hind leg activity and its influence on the degree of hyperkeratosis. Finally, also the influence of hyperkeratosis on the shown intensity of hind leg activity was evaluated. The dairy cows' hind leg activity was divided into stepping (claw raised < 15 cm) and kicking (claw raised > 15 cm). Kicking was shown at significant higher mouthpiece chamber vacuum levels than stepping. A certain vacuum level, when stepping turns into kicking, cannot be derived from the results. Cows, which have shown kicking, had significant higher degrees of teat-end hyperkeratosis than cows which have shown stepping. The length of machine-on time did not affect the frequency of hind leg activity, but the degree of hyperkeratosis. Longer machine-on times led to higher degrees of hyperkeratosis. Highest frequencies for steppings were found in plateau phase and over-milking of the second quarter. Highest frequencies for kicking

were found in let down phase and also the over-milking of the second quarter. The kicking was assumed to be avoidance behaviour, while stepping is influenced by various factors.

In summary it can be said, that the developed concept gives milking advisors a first overview on key areas of the milking process and indicators which can be inspected to assess the milking process. The new precisely defined hand-milking method is a suitable method to collect the strip yield, but further research is needed to determine a threshold for the assessment of completed milk-outs. The dairy cows' hind leg activity seem to indicate for discomfort during machine milking due to mouthpiece chamber vacuum and hyperkeratosis. Though, the hind leg activity seems to have several influencing factors. Further research is needed to validate the hind leg activity as indicator for gentle milkings.

Due to the variety of milking advisors prior experience and education, the development of a digital tool, which guides the milking advisor through data collection and offers recommended actions for the farmer, is seen as useful. The work on this digital tool was already started and should be continued in future.

## Chapter Nine: Zusammenfassung

Die Nachfrage nach Milchprodukten steigt weltweit an. Gleichzeitig wächst auch der Wunsch von Politik und Verbrauchern nach mehr Nachhaltigkeit und einer größeren Berücksichtigung des Tierwohls in der Milchproduktion. Landwirte müssen diesen steigenden Anforderungen auch während des Melkvorgangs gerecht werden. Den Melkvorgang gilt es dabei so ressourcenschonend, wirtschaftlich effizient, artgerecht und gesundheitserhaltend wie möglich zu gestalten. Um dies zu gewährleisten sollte der Melkvorgang daher möglichst drei wesentliche Kriterien erfüllen und zügig, vollständig und schonend zugleich sein. Häufig erfüllen Melkvorgänge in der Praxis nicht diese Kriterien oder nicht alle drei zu gleichen Teilen. In diesem Fall kommt es zu Auswirkungen auf die Wirtschaftlichkeit, die Effizienz oder die Gesundheit der Tiere, sodass Landwirte professionelle Hilfe von Melkberatern in Anspruch nehmen. Es gibt in Deutschland keine anerkannte, standardisierte Ausbildung für Melkberater, sodass die Berater unterschiedliche Vorerfahrung auszeichnet. In Folge dessen gibt es auch keinen wissenschaftlich basierten Goldstandard für das Vorgehen der Berater.

Das Ziel war es aus diesem Grund ein Konzept für die Melkberatung zu entwickeln, dass Melkberatern als Hilfestellung für die Bewertung des Melkvorganges auf wissenschaftlich fundierter Basis dienen kann. Zudem sollten Forschungslücken in Bezug auf verfügbare Methoden und Grenzwerte zur Bewertung des Melkvorganges mit Hilfe dieser Arbeit geschlossen werden. Aufgrund der unterschiedlichen Vorerfahrung der Melkberater war das Ziel einfach anwendbare Methoden zu entwickeln und evaluieren, die kein teures Equipment benötigen und die Kuh in das Zentrum der Untersuchung stellen.

In Kapitel Zwei konnten als Resultat einer Literaturrecherche vier Haupteinflussfaktoren auf den Melkvorgang identifiziert werden: die Melktechnik, die Kuh, der Melker und der Melkstand und Vorwartebereich. Vor allem die Melktechnik hat einen enormen Einfluss darauf, wie schnell, vollständig und schonend der Milchentzug vollzogen wird. Für jeden dieser Haupteinflussfaktoren konnten Indikatoren herausgearbeitet werden, die den Melkvorgang wesentlich bestimmen und die von Melkberatern für die Überprüfung und Bewertung herangezogen werden können. Einige der präsentierten Indikatoren eignen sich für eine erste Fehlersuche, während andere für eine weiterführende Ursachensuche genutzt werden können. Einige Indikatoren stehen zueinander in Beziehung und beeinflussen sich gegenseitig. Diese Einflüsse wurden in dieser Arbeit aufgezeigt und dargestellt. Hinzukommend bietet das Konzept Informationen über mögliche Methoden und Zeitpunkte für die Bewertung der Indikatoren, die von den Melkberatern genutzt werden können den zeitlichen Ablauf ihrer Bewertung im Voraus zu planen.

In Kapitel Drei wurde eine neue, definierte Handmelkmethode mit einer definierten Melkgeschwindigkeit zur Erfassung des Ausmelkgrades entwickelt und mit zwei bereits in der Praxis angewandten Methoden hinsichtlich ihrer Aussagekraft über die verbliebene Nachgemelksmenge im Euter verglichen. Das Handmelken erfolgte hierbei für 15 s pro Euterviertel. Eine der zu vergleichenden Methoden bedient sich der visuellen Bewertung des Füllungsgrads des Euterviertels während eine weitere den Ausmelkgrad über das Zählen der Anzahl satter Milchstrahlen bestimmt. Für die Untersuchung wurden sechs Betriebe jeweils einmal von insgesamt drei Nachmelkern besucht. Für die visuelle Bewertung des Füllungsgrads des Euterviertels konnte kein Zusammenhang zu dem im Viertel verbleibenden Nachgemelk nachgewiesen werden. Für die neue, definierte Handmelkmethode und das Zählen der satten Milchstrahlen war ein signifikanter Zusammenhang zur verbleibenden Nachgemelksmenge im Viertel nachweisbar. Trotz der genauen Definition der Handmelkmethode und der Melkgeschwindigkeit konnte der Einfluss des Nachmelkers auf die gewonnene Milchmenge bzw. die Anzahl satter Milchstrahlen nicht verhindert werden. Dieser Einfluss ist vermutlich auf das Verhältnis zwischen Handgröße der Nachmelker und der Länge der Zitzen zurückzuführen. Weitere Forschung ist notwendig, um Grenzwerte für die Bewertung von vollständigen und unvollständigen Ausmelkgraden für die neue Handmelkmethode festzulegen. Es zeigte sich jedoch, dass bisher in der Literatur beschriebene Grenzwerte von 100 ml pro Euterviertel zu hoch angesetzt sind und in dieser Untersuchung mit der vorgestellten definierten Handmelkmethode nicht erreicht werden konnten.

In Kapitel Vier wurde die neue, definierte Handmelkmethode und das Nachgemelk im Euter der Kuh hinsichtlich ihrer kuh- und melksystembezogenen Einflussfaktoren überprüft. Die Untersuchung wurde in einem Betrieb mit einem Nachmelker an drei Melkzeiten pro Tag an drei aufeinanderfolgenden Tagen durchgeführt. Es konnte gezeigt werden, dass die Nachgemelksmenge im Euter von inkorrekten unter dem Euter hängenden Melkzeugen und betriebsspezifischen Einstellungen des Melksystems, der Melkroutine sowie der Charakteristika der Milchkuhherde beeinflusst wird. Die ermolene Milchmenge in 15 s pro Euterviertel wurde beeinflusst von den spezifischen Einstellungen, Melkroutinen und den Charakteristika der Herde des Betriebes, dem Nachmelker, einer inkorrekten Melkzeugposition, der Zitzenlänge und dem Auftreten eines fühlbaren verhärteten Ringes an der Zitzenbasis. Die ermolene Milchmenge in 15 s war höher bei einer inkorrekten Melkzeugposition und längeren Zitzen an und niedriger bei Auftreten eines fühlbaren, verhärteten Ringes an der Zitzenbasis während des Melkens. Die besten Ergebnisse zur Voraussage der tatsächlichen Nachgemelksmenge im Euter wurden erzielt, wenn alle vier Viertel einer Kuh mit der neuen Handmelkmethode für 15 s nachgemolken wurden. Zu

erklären ist dies vermutlich durch die sehr individuellen Melkcharakteristika der einzelnen Viertel einer Kuh.

In Kapitel Fünf wurde die Intensität und Frequenz der Hinterbeinaktivität (Trippeln/Treten) von Milchkühen während des Melkens beobachtet. Mit Hilfe einer gleichzeitigen Messung der Vakuumbhöhe im Zitzengummikopf sowie im kurzen Milchschauch unter der Zitze wurde der Einfluss der Vakuumbhöhe auf die Intensität und Frequenz der Hinterbeinaktivität untersucht. Des Weiteren wurde der Einfluss der Melkzeitdauer, als auch der einzelnen Melkphasendauern auf die Frequenz der Hinterbeinaktivität sowie die Ausprägung von Hyperkeratosen untersucht. Die Untersuchung des Einflusses der Ausprägung von Hyperkeratosen auf die Intensität und Frequenz der Hinterbeinaktivität war ebenfalls Gegenstand des Experiments. Es wurden zehn Betriebe an jeweils zwei Melkzeiten desselben Tages besucht. Es zeigte sich, dass Treten bei signifikant höheren Vakuumwerten stattfand als Trippeln. Es konnte keine Vakuumhöhe abgeleitet werden ab der die Tiere eher Treten statt Trippeln. Tiere, die stark ausgeprägte Hyperkeratosen an der Zitzenspitze aufwiesen, zeigten zudem signifikant häufiger die Hinterbeinaktivität Treten. Die Melkdauer und Dauer der Melkphasen hatte keinen Einfluss auf die Häufigkeit von Hinterbeinaktivitäten. Jedoch auf die Ausprägung von Hyperkeratosen. Tiere mit ausgeprägten Hyperkeratosen hatten eine signifikant längere Melkdauer. Die Häufigkeiten für Trippeln waren in der Hauptmilchflussphase und der Phase des Blindmelkens des zweiten Euterviertels am größten. Die Häufigkeiten für Treten in der Anstiegsphase und ebenfalls der Phase des Blindmelkens des zweiten Viertels. Während Treten als Abwehrverhalten interpretiert wird, scheint Trippeln multikausal zu sein.

Zusammenfassend lässt sich festhalten, dass das in dieser Arbeit präsentierte Konzept Melkberatern hilft einen Überblick über die Haupteinflussfaktoren und Indikatoren des Melkvorganges zu erhalten. Die neue, definierte Handmelkmethode ist geeignet das Nachgemelk zu erfassen. Weitere Forschung ist notwendig, um Grenzwerte für die Bewertung der Nachgemelksmenge festzulegen. Die Hinterbeinaktivität von Kühen während des Melkens lässt ein Unwohlsein der Tiere durch die Vakuumbhöhe im Zitzengummikopf und das Auftreten von ausgeprägten Hyperkeratosen vermuten. Weitere Forschung ist notwendig, um die Eignung der Hinterbeinaktivität als Indikator für unangenehme Vakuumbhöhen und Hyperkeratosen zu evaluieren. Bedingt durch die unterschiedliche Vorbildung und Vorerfahrung von Melkberatern erscheint die Weiterentwicklung eines digitalen Tools, das den Berater bei der Datenaufnahme und Auswertung unterstützt als sinnvoll und sollte in Zukunft fortgeführt werden.

## Chapter Ten: Appendix

### Appendix Table A1

Overview on the assessable indicators of a machine milking process

Milking System							
Objective of assessment		Indicators	Timing for data collection	Methodology for data collection	Example for simple methodology in literature	Threshold/target available values in literature	Impact on
Risk analysis for technical shortcomings	Quickness Completeness Gentleness	<ul style="list-style-type: none"> <li>Milking equipment</li> <li>Threshold and settings of equipment</li> <li>Regularity and last date of technical services</li> <li>Regularity and last date of liner changes</li> <li>ISO 6690 certification</li> </ul>	Before After	Personal Interview	-	-	<ul style="list-style-type: none"> <li>Potential sources of disturbances in milking system</li> </ul>
Cleanliness	Gentleness	Visual grade of cleanliness: <ul style="list-style-type: none"> <li>Organic residues</li> <li>Biofilms</li> </ul>	Before	Direct Observation	Reinemann et al. (2000)	-	<ul style="list-style-type: none"> <li>Bacterial transmission<sup>1</sup></li> <li>Bacterial cell count in bulk milk<sup>2</sup></li> </ul>
		Bacterial cell count in bulk milk	Before After	Personal Interview	-	EU Regulation 1662 (2006)	<ul style="list-style-type: none"> <li>Milk quality</li> <li>Payment</li> </ul>
		Type and recommended settings for Cleaning and Sanitation: <ul style="list-style-type: none"> <li>Time</li> <li>Temperature</li> <li>Chemicals &amp; Concentration</li> <li>Operating vacuum</li> <li>Air injector timing</li> <li>Cycles for cleaning</li> <li>Water flow rate</li> <li>Water hardness</li> </ul>	After	Measurement	Reinemann et al. (2000)	Manufacturers' recommendations; Reinemann et al., (2000)	<ul style="list-style-type: none"> <li>Bacterial transmission<sup>1</sup></li> <li>Bacterial cell count in bulk milk<sup>2</sup></li> <li>Material wear of rubber/silicone parts</li> </ul>

Objective of assessment		Indicators	Timing for data collection	Methodology for data collection	Example for simple methodology in literature	Threshold/target available values in literature	Impact on	
Cleaning	Gentleness	Cleaning-time test: <ul style="list-style-type: none"> <li>• Time</li> <li>• Temperature</li> <li>• Operating vacuum</li> </ul>	After	Measurement	Reinemann et al. (2000); Rasmussen et al. (2003)	Manufacturers' recommendations; Reinemann et al. (2000)	<ul style="list-style-type: none"> <li>• Bacterial transmission<sup>1</sup></li> <li>• Bacterial cell count in bulk milk<sup>2</sup></li> </ul>	
Vacuum stability	Quickness Gentleness	Milk line: <ul style="list-style-type: none"> <li>• Length</li> <li>• Height</li> <li>• Diameter</li> <li>• Slope</li> </ul>	Before After	Measurement	ISO 6690 (2007)	ISO 6690 (2007)	<ul style="list-style-type: none"> <li>• Milk transportation<sup>3</sup></li> <li>• Vacuum drops<sup>4</sup></li> <li>• Machine-on time<sup>5</sup></li> <li>• Udder infections<sup>6</sup></li> </ul>	
		Milk tubes: <ul style="list-style-type: none"> <li>• Diameter</li> <li>• Length</li> </ul>	Before After	Measurement; Direct Observation	ISO 6690 (2007)	ISO 6690 (2007)		
		Claws: <ul style="list-style-type: none"> <li>• Type</li> <li>• Weight</li> <li>• Dimensions</li> <li>• Unblocked air vents</li> </ul>	Before After During	Direct Observation	O'Callaghan (2002); ISO 6690 (2007)	ISO 6690 (2007)		
		Visible cracks	Before	Direct Observation	-	Absent (Jones, 1999)		
		Audible leakages	During	Direct Observation	-			
		Unit Fall-off test: <ul style="list-style-type: none"> <li>• Vacuum pump reserve</li> <li>• Regulator response</li> </ul>	Before	Measurement	Reinemann et al. (2001); ISO 6690 (2007)	Reinemann et al. (2001); ISO 6690 (2007)		
		Operating vacuum: <ul style="list-style-type: none"> <li>• Vacuum level</li> <li>• Matching manufacturers recommendations</li> <li>• Comparison with vacuum gauge in milking parlour</li> </ul>	Before	Measurement	ISO 6690 (2007)	ISO 6690; Manufacturers' recommendations		<ul style="list-style-type: none"> <li>• Teat-end vacuum<sup>7</sup></li> <li>• Cleaning and disinfection<sup>8</sup></li> <li>• Liner slips<sup>9</sup></li> </ul>

Objective of assessment		Indicators	Timing for data collection	Methodology for data collection	Example for simple methodology in literature	Threshold/target available values in literature	Impact on
Vacuum stability	Quickness Gentleness	Vacuum fluctuations: <ul style="list-style-type: none"> <li>• Cyclic</li> <li>• Irregular</li> </ul>	During	Measurement	Rønningen (2017)	-	<ul style="list-style-type: none"> <li>• Udder infections<sup>6</sup></li> </ul>
		Liner slips and unit fall-offs: <ul style="list-style-type: none"> <li>• Amount of audible infiltrations (irregular vacuum fluctuations)</li> </ul>	During	Direct Observation	Mein and Reid (1996)	(Mein and Reid (1996)	<ul style="list-style-type: none"> <li>• Udder infections<sup>6</sup></li> <li>• Workload for milking-operator</li> <li>• Milking parlor performance</li> </ul>
Physical strain of dairy cows' teat	Quickness Completeness Gentleness	Liner choice: <ul style="list-style-type: none"> <li>• Type</li> <li>• Size</li> <li>• Fit into teat cup shell</li> </ul>	Before After	Direct Observation	Reinemann et al. (2001)	Reinemann et al. (2001); Jones (1999)	<ul style="list-style-type: none"> <li>• Teat tissue changes<sup>10,15, 17</sup></li> <li>• Risk of udder infections<sup>11</sup></li> <li>• Friction between teat and barrel<sup>12</sup></li> <li>• Liner slips<sup>13</sup></li> <li>• Milk-out degree<sup>14,22</sup></li> <li>• Milking speed<sup>15</sup></li> <li>• Vacuum height acting upon the teat<sup>7</sup></li> <li>• Hind leg activity<sup>16</sup></li> <li>• Duration of physical strain of the teat<sup>17, 7</sup></li> </ul>
		Liner condition: <ul style="list-style-type: none"> <li>• Material</li> <li>• Damages</li> <li>• Twisted liners</li> <li>• Age</li> </ul>	Before	Direct Observation	-	Not aged, no damages and not twisted (Jones, 1999; Davis et al., 2000)	
		Pulsation: <ul style="list-style-type: none"> <li>• Rate</li> <li>• Ratio</li> <li>• Length of a,b,c and d phase</li> </ul>	Before During	Measurement	ISO 6690 (2007)	Reinemann et al. (2001); ISO 6690 (2007)	
		Teat-end vacuum level: <ul style="list-style-type: none"> <li>• In different milking phases</li> </ul>	During	Measurement	ISO 6690 (2007)	ISO 6690 (2007)	

Objective of assessment		Indicators	Timing for data collection	Methodology for data collection	Example for simple methodology in literature	Threshold/target available values in literature	Impact on
Physical strain of dairy cows' teat	Quickness Completeness Gentleness	Mouthpiece chamber vacuum: <ul style="list-style-type: none"> <li>In different milking phases</li> </ul>	During	Measurement	Rønningen (2011); Rønningen (2017); Meyer et al. (2020, unpublished)	Rønningen (2011)	<ul style="list-style-type: none"> <li>Teat tissue changes<sup>10,15, 17</sup></li> <li>Risk of udder infections<sup>11</sup></li> <li>Friction between teat and barrel<sup>12</sup></li> <li>Liner slips<sup>13</sup></li> <li>Milk-out degree<sup>14,22</sup></li> <li>Milking speed<sup>15</sup></li> <li>Vacuum height acting upon the teat<sup>7</sup></li> <li>Hind leg activity<sup>16</sup></li> <li>Duration of physical strain of the teat<sup>17,7</sup></li> </ul>
		Length of milking phases: <ul style="list-style-type: none"> <li>Over-milking</li> </ul>	During	Measurement	Rønningen (2017); Meyer et al. (2020, unpublished)	-	
		Teat condition: <ul style="list-style-type: none"> <li>Colour changes</li> <li>Swelling at or near teat base</li> <li>Swelling/firmness at or near teat-end</li> <li>Teat skin condition</li> <li>Teat-end Hyperkeratosis</li> </ul>	After detachment	Direct Observation	Mein et al. (2001)	Mein et al. (2001)	<ul style="list-style-type: none"> <li>Risk for udder infections<sup>18</sup></li> <li>Cleanliness<sup>19</sup></li> <li>Animal behaviour<sup>16</sup></li> <li>Somatic cell count<sup>20</sup></li> <li>Milk-out degree<sup>21, 22</sup></li> <li>Milk flow<sup>21</sup></li> </ul>
Automatic cluster remover and automatic stripping arm	Completeness Gentleness	<ul style="list-style-type: none"> <li>Settings</li> <li>Thresholds</li> </ul>	Before After	Personal Interview, Measurement	-	<i>Measured values should meet the settings</i>	<ul style="list-style-type: none"> <li>Milk-out degree<sup>23</sup></li> <li>Machine-on time<sup>24</sup></li> <li>Milking parlor performance<sup>24</sup></li> <li>Length of over-milking periods<sup>24</sup></li> <li>Teat condition<sup>24</sup></li> </ul>

Objective of assessment		Indicators	Timing for data collection	Methodology for data collection	Example for simple methodology in literature	Threshold/target available values in literature	Impact on
Automatic cluster remover and automatic stripping arm	Completeness Gentleness	Milk-out degree: <ul style="list-style-type: none"> <li>Hand-milking</li> <li>Machine-milking</li> </ul>	After	Measurement	Joe et al. (2010); Davis and Reinemann, (2001); Reinemann et al. (2001); Meyer et al. (2020); Chapter Three and Four	Joe et al. (2010); Davis and Reinemann, (2001); Reinemann et al. (2001); Meyer et al. (2020)	<ul style="list-style-type: none"> <li>Milk production<sup>25</sup></li> <li>Milk composition<sup>25</sup></li> </ul>
<b>Dairy Cow</b>							
Herd characteristics	Quickness Gentleness	<ul style="list-style-type: none"> <li>Breed</li> <li>Parity</li> <li>Days in milk</li> </ul>	Before After	Personal Interview, Herd Management Software, Direct observation	-	-	<ul style="list-style-type: none"> <li>Milk production</li> <li>Milk composition</li> <li>Body size</li> <li>Somatic cell count<sup>26</sup></li> <li>Teat and udder morphology<sup>27</sup></li> </ul>
Udder health status	Gentleness	<ul style="list-style-type: none"> <li>Somatic cell count on herd level or cow level</li> </ul>	Before After	Direct Observation Measurement Herd Management Software External sources	Official milk control report; Dairy company; California Mastitis Test (Paduch et al., 2013)	Official milk control report; EU Regulation 1662 (2006); DVG (2012); Paduch et al. (2013)	<ul style="list-style-type: none"> <li>Milk quality</li> <li>Payment</li> </ul>
		Inspection of milk filter: <ul style="list-style-type: none"> <li>Flakes</li> <li>Clots</li> </ul>	After	Direct Observation	-	<i>No flakes or clots wanted</i>	

Objective of assessment		Indicators	Timing for data collection	Methodology for data collection	Example for simple methodology in literature	Threshold/target available values in literature	Impact on
Sizes and morphologies	Completeness Gentleness	Body size: <ul style="list-style-type: none"> <li>• Height</li> <li>• Length</li> <li>• Width</li> </ul>	Before After	Measurement  Direct Observation	-	<i>Homogeneous sizes are preferred</i>	<ul style="list-style-type: none"> <li>• Size of milking positions, entrance and exit</li> </ul>
		Teat: <ul style="list-style-type: none"> <li>• Length</li> <li>• Width</li> <li>• Form</li> <li>• Distance</li> <li>• Teat-end shape</li> </ul>	Before attachment	Measurement  Direct Observation	Bakken (1981); Grunert et al. (1990); Chrystal et a. (2001)	<i>Homogeneous sizes are preferred</i>	<ul style="list-style-type: none"> <li>• Liner choice<sup>30</sup></li> <li>• Hyperkeratosis<sup>20</sup></li> <li>• Milk flow characteristics<sup>28, 29</sup></li> </ul>
		Udder: <ul style="list-style-type: none"> <li>• Form</li> <li>• Height</li> </ul>	Before attachment	Direct Observation	Habermehl et al. (2005)	Evenly udders are preferred (Jones, 1999)	<ul style="list-style-type: none"> <li>• Milking cluster positioning<sup>31</sup></li> <li>• Liner slips<sup>32</sup></li> </ul>
Milking characteristics		<ul style="list-style-type: none"> <li>• Peak milk flow</li> <li>• Average milk flow</li> <li>• Milk yield</li> </ul>	Before After During	Measurement, Herd Management Software	Reinemann et al. (2001); Marony et al. (2004)	Mein (1998)	<ul style="list-style-type: none"> <li>• Dimensions of milk transporting parts<sup>35</sup></li> <li>• Milking parlour performance</li> </ul>
Stress level		Animal behaviour: <ul style="list-style-type: none"> <li>• Hind leg activity</li> <li>• Rumination</li> <li>• Defecation and urination</li> </ul> Other: <ul style="list-style-type: none"> <li>• Heart rate</li> <li>• Cortisol concentrations in milk or blood</li> </ul>	During	Direct Observation; Measurement	Rushen et al. (2001); Rousing et al. (2004); Bristow and Holmes (2007); Rushen et al., (1999); Hopster et al., (2002); Gygax et al., (2006)	-	<ul style="list-style-type: none"> <li>• Animal comfort<sup>34</sup></li> <li>• Milk ejection<sup>35</sup></li> <li>• Milk production<sup>41</sup></li> <li>• Work safety<sup>41</sup></li> </ul>

Objective of assessment		Indicators	Timing for data collection	Methodology for data collection	Example for simple methodology in literature	Threshold/target available values in literature	Impact on
	Completeness	Disturbed milk ejections	During	Direct Observation Measurement	Marony et al. (2004)	Should be avoided (Bruckmaier and Blum, 1996)	<ul style="list-style-type: none"> <li>• Milk-out degree</li> <li>• Physical strain of teat<sup>7</sup></li> </ul>
<b>Milking Operator</b>							
Bacterial transmission	Quickness Gentleness	Milking order: Grouped	During	Direct Observation	-	Cows with udder infections milked last (Ruegg et al., 2002)	<ul style="list-style-type: none"> <li>• Bacterial transmission<sup>1</sup></li> <li>• Udder health<sup>1</sup></li> </ul>
		Milking routines: <ul style="list-style-type: none"> <li>• Wearing gloves</li> <li>• Washing hands</li> <li>• Fore-stripping</li> <li>• Choice and concentration of pre teat dip</li> <li>• Cleaning of udder and teats</li> <li>• Choice and concentration of post teat dip</li> <li>• Intermediate disinfections</li> </ul>	During	Direct Observation	Schreiner and Ruegg (2002); DIN ISO 20966 Appendix B (2008); Ostendorf (2017); Drieling (2017);	Schreiner and Ruegg (2002); Ostendorf (2017); Drieling (2017); <i>Performance &amp; consistency of full milking routines; Comparison with standard operations procedures</i>	<ul style="list-style-type: none"> <li>• Bacterial transmission<sup>36</sup></li> <li>• Udder health<sup>36</sup></li> <li>• Milk ejection reflex<sup>37</sup></li> <li>• Milking parlour performance</li> <li>• Somatic cell count<sup>38</sup></li> <li>• Bacterial cell count<sup>38</sup></li> </ul>
Milk-out degree	Completeness Gentleness	Milking routines: <ul style="list-style-type: none"> <li>• Duration of stimulation</li> <li>• Duration of tactile appeal</li> <li>• Milking cluster positioning</li> </ul>	During	Direct Observation; Measurement	Jones (1999); Chapter Four	Bruckmaier (2005); Meyer et al. (2020, unpublished)	<ul style="list-style-type: none"> <li>• Milk ejection reflex<sup>37</sup></li> <li>• Milking in absence of milk flow<sup>7</sup></li> <li>• Risk of teat cup crawls/liner slips<sup>12</sup></li> <li>• Milk-out degree</li> </ul>

Objective of assessment		Indicators	Timing for data collection	Methodology for data collection	Example for simple methodology in literature	Threshold/target available values in literature	Impact on
Milk-out degree	Completeness Gentleness	Milk flow curves: Bimodality	During	Measurement	Marony et al. (2004)	<i>Absent</i> (Bruckmaier and Blum, 1996)	<ul style="list-style-type: none"> <li>• Milk ejection reflex<sup>37</sup></li> <li>• Milking in absence of milk flow<sup>7</sup></li> <li>• Risk of teat cup crawls/liner slips<sup>12</sup></li> </ul> Milk-out degree
Vacuum stability	Quickness Gentleness	Milking routines: <ul style="list-style-type: none"> <li>• Unit attachment process</li> <li>• Number of simultaneously attached units</li> </ul>	During	Direct Observation	Jones (1999)	ISO 6690, 2007)	<ul style="list-style-type: none"> <li>• Vacuum reserve<sup>30</sup></li> <li>• Vacuum fluctuations</li> <li>• Udder health<sup>6</sup></li> </ul>
Interactions with the dairy cow	Gentleness	Human-animal-interaction: <ul style="list-style-type: none"> <li>• In milking parlour</li> <li>• While herding the cows into the waiting area or the parlour</li> </ul>	During	Direct Observation	Hemsworth et al. (2000); Prüser (2017)	-	<ul style="list-style-type: none"> <li>• Stress level of dairy cows<sup>39</sup></li> <li>• Dairy cows' immune system and milk production<sup>39,41</sup></li> </ul>
Milking time management	Quickness Gentleness	<ul style="list-style-type: none"> <li>• Milking intervals</li> <li>• Milking times per day</li> </ul>	Before During After	Direct Observation; Personal Interview; Herd Management Software	-	<i>As constant as possible</i> (Weiß et al, 2002)	<ul style="list-style-type: none"> <li>• Milk production<sup>40</sup></li> <li>• Milk composition<sup>40</sup></li> <li>• Lying behaviour and feed intake</li> <li>• Milking parlour performance</li> </ul>
Efficiency	Quickness	<ul style="list-style-type: none"> <li>• Cows per milker</li> <li>• Working-time measurements</li> </ul>	During	Direct Observation; Measurement	Armstrong and Quick (1986)	<i>Comparison with other farms</i>	<ul style="list-style-type: none"> <li>• Milking parlour performance</li> <li>• Work load of milking operators</li> <li>• Labour costs</li> </ul>

<b>Milking Parlour and Waiting Area</b>							
<b>Objective of assessment</b>		<b>Indicators</b>	<b>Timing for data collection</b>	<b>Methodology for data collection</b>	<b>Example for simple methodology in literature</b>	<b>Threshold/target available values in literature</b>	<b>Impact on</b>
Milking parlour design	Quickness Gentleness	Size: <ul style="list-style-type: none"> <li>Type of parlour</li> <li>Number of milking positions</li> <li>Size of milking positions</li> <li>One-end exit/rapid exit</li> </ul>	Before During	Direct Observation; Measurement	Gómez et al. (2017)	Adapted to average body size of the herd (Gómez et al., 2017)	<ul style="list-style-type: none"> <li>Milking parlour performance</li> <li>Injuries and wounds</li> <li>Willingness to enter and leave the parlour</li> </ul>
Milking parlour design	Quickness Gentleness	Injuries or wounds <ul style="list-style-type: none"> <li>Wounds</li> <li>Bald spots</li> <li>Skin abrasions</li> </ul>	During	Direct Observation	-	<i>No injuries wanted</i>	<ul style="list-style-type: none"> <li>Animal welfare</li> <li>Milking parlour performance</li> <li>Willingness to enter the parlour</li> </ul>
		Entrance and exit: <ul style="list-style-type: none"> <li>Ramps, steps and obstacles, narrow curves, sharp edges</li> <li>Drinking troughs</li> <li>Concentrated feed</li> <li>Lighting</li> <li>Temperature</li> <li>Volume level</li> <li>Flooring</li> <li>Insects</li> </ul>	Before After During	Direct Observation; Measurement	Telenzhenko and Bergsten (2005);  <i>Comparison with preferred conditions by dairy cows</i>	<i>Comparison to target values</i> (McDowell, 1972; ASABE, 2006; Reinemann et al., 2002; Phillips, 2009)	<ul style="list-style-type: none"> <li>Milking parlour performance</li> <li>Willingness to enter and leave the parlour</li> <li>Animal welfare</li> </ul>

Objective of assessment		Indicators	Timing for data collection	Methodology for data collection	Example for simple methodology in literature	Threshold/ target available values in literature	Impact on
Milking parlour design	Quickness Gentleness	Into the parlour: <ul style="list-style-type: none"> <li>• Sharp equipment</li> <li>• Lighting</li> <li>• Volume level</li> <li>• Flooring</li> <li>• Insects</li> <li>• Temperature</li> <li>• Drinking troughs</li> <li>• Feeding stations for concentrated feed</li> <li>• Electrical current</li> </ul>	Before After During	Direct Observation; Measurement	<i>Comparison with preferred conditions by dairy cows</i>	<i>Comparison to target values</i> (McDowell, 1972; ASABE, 2006; Reinemann et al., 2002; Phillips, 2009)	<ul style="list-style-type: none"> <li>• Milking parlour performance</li> <li>• Willingness to enter the parlour</li> </ul> Animal behaviour
Milking parlour design	Quickness Gentleness	Willingness to enter the parlour	During	Direct Observation; Measurement	Gómez et al. (2017)	-	Milking parlour performance
		Willingness to leave the parlour	During	Direct Observation; Measurement	-		
Attendance time	Quickness Gentleness	Waiting Area Milking Parlour	Before After During	Measurement; Herd Management Software	-	-	<ul style="list-style-type: none"> <li>• Milking parlour performance</li> <li>• Lying behaviour and feed intake</li> <li>• Animal welfare</li> </ul>

Objective of assessment		Indicators	Timing for data collection	Methodology for data collection	Example for simple methodology in literature	Threshold/target available values in literature	Impact on
Waiting area design	Quickness Gentleness	Waiting area design and design of passageway from stable to waiting area: <ul style="list-style-type: none"> <li>• Size</li> <li>• Highest number of cows waiting simultaneously in the area</li> <li>• Ramps, steps and obstacles, narrow curves, sharp edges</li> <li>• Lighting; Volume level; Temperature</li> <li>• Insects</li> <li>• Antagonistic behaviour</li> <li>• Drinking troughs in waiting area</li> </ul>	Before After During	Direct Observation; Measurement	<i>Comparison with preferred conditions by dairy cows</i>  Antagonistic behaviour (Rousing and Wemelsfelder, 2006)	<i>Comparison to target values</i> (McDowell, 1972; ASABE, 2006; Reinemann et al., 2002; Phillips, 2009)	<ul style="list-style-type: none"> <li>• Milking parlour performance</li> <li>• Animal behaviour</li> <li>• Risk for injuries</li> <li>• Willingness to enter the parlour</li> </ul>
Working environment for milking operator	Quickness Gentleness	Operating height	Before After	Direct Observation	Jakob et al. (2012)	Jakob et al. (2012)	<ul style="list-style-type: none"> <li>• Health</li> <li>• Job satisfaction</li> <li>• Safety at work</li> </ul>
		Body posture while milking cluster attachment	During	Direct Observation			
		Design parlour: <ul style="list-style-type: none"> <li>• Stairs; Flooring; Temperature; Lighting; Volume level</li> <li>• Insects</li> <li>• Walking distances</li> </ul>	During	Personal Interview; Direct Observation; Measurement	<i>Interview with Milking Operator</i>	-	
Milk-out degree	Completeness	<ul style="list-style-type: none"> <li>• Constructional possibilities for correct milking cluster positioning</li> </ul>	During	Direct Observation	<i>Observation of correct/incorrect cluster positioning (cf. Chapter Four)</i>	<i>Not influenced by the constructional design</i>	<ul style="list-style-type: none"> <li>• Milk production<sup>25</sup></li> <li>• Milk composition<sup>25</sup></li> </ul>

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**References Appendix Table A1**

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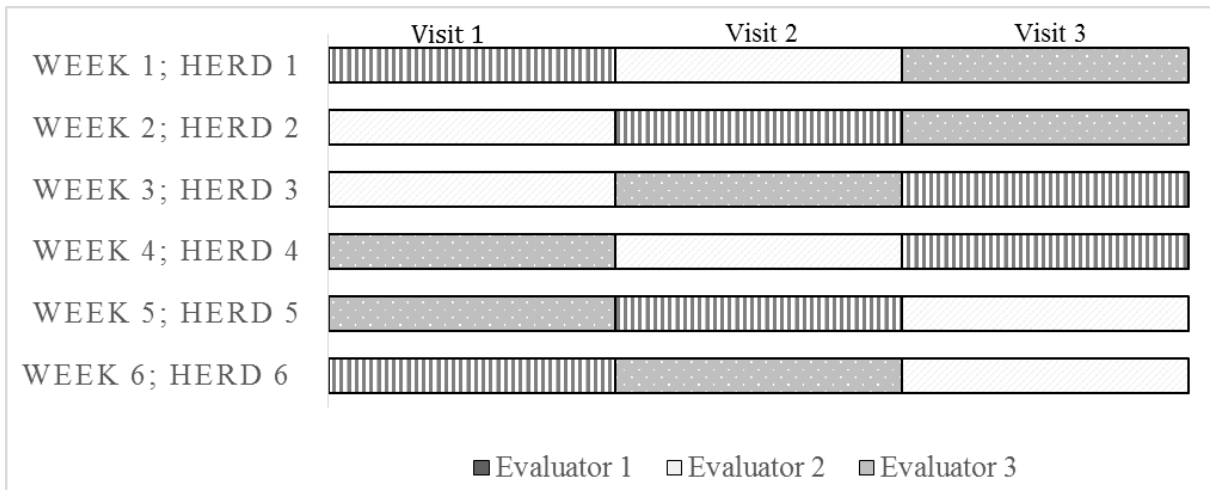
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**Appendix Table A2**

Characteristics of herds, parlours and parlour settings at the six dairy farms included in the evaluation of completeness of milk-out

	Farm					
	A	B	C	D	E	F
Herd characteristics						
Herd size	198	81	390	194	140	303
Mean lactation number	2.3	2.2	2.6	1.3	1.6	2.1
Parlour types and technical settings						
Milking parlour type	parallel	auto-tandem	parallel	herring-bone	herring-bone	herring-bone
Size of milking parlour	2x12	2x4	2x20	2x12	2x8	2x8
Operating vacuum setting (kPa)	42	37	44	43	37	43
Pulsation ratio	60:40	60:40	60:40	65:35	60:40	60:40
Pulsation rate (cycles/min)	60	60	60	60	60	58
Automatic cluster remover setting (ml/min)	300	300	480	300	250	750
Delay times (s)	25		25	30	30	20
Mean SMT vacuum during the milk flow plateau phase <sup>1</sup>	-	34.4 (n = 32)	35.1 (n = 44)	37.6 (n = 32)	36.7 (n = 28)	-

<sup>1</sup> Vacuum measurements carried out in July and August 2017 with VaDia (BioControl, Rakkestad, Norway) (SMT: short milk tube; n = number of milkings)

**Appendix Figure A3**

Randomised temporal organisation of the three evaluators when assessing completeness of milk-out in herd 1 to 6. All dairy herds were visited once by each evaluator

## Appendix Material A4

Logistic and linear regression model description

A logistic regression model was fitted to analyse the relationship between the outcome of the VISUAL method (target) and the herd, the evaluators and the strip yield in 60 s (SY60) (predictors). A linear regression model was introduced to analyse the relationship between the outcome of the DEFINED and the EASYSTRIPS method (target) and the herd, the evaluators and the strip yield in 60 s (predictors). The models were defined as

$$Y_i = \beta_0 + \beta_1 x_i + \beta_2 x_i + \beta_3 x_i + \varepsilon_i$$

Where

$Y_i$  is the outcome of the assessment of the cow's quarters with the VISUAL, the DEFINED or the EASYSTRIP method

$\beta_0$  is the intercept

$\beta_1 x_i$  is the effect of the herd

$\beta_2 x_i$  is the effect of the evaluator

$\beta_3 x_i$  is the effect of the strip yield in 60 s

$\varepsilon_i$  is the random error for the model

## Appendix Material A5

Exemplary R Code of the linear regression model for the method EASYSTRIPS

```
lmEASYSTRIPS <- lme(EASYSTRIPS ~ SY60 + herd + evaluator, data=data1,
random=~1|ID, na.action=na.exclude, weights=varIdent(form=~1|herd*evaluator),
method="ML", control=list(maxIter=255,msMaxIter=255,opt="nlminb"))
```

```
anova(lmEASYSTRIPS)
```

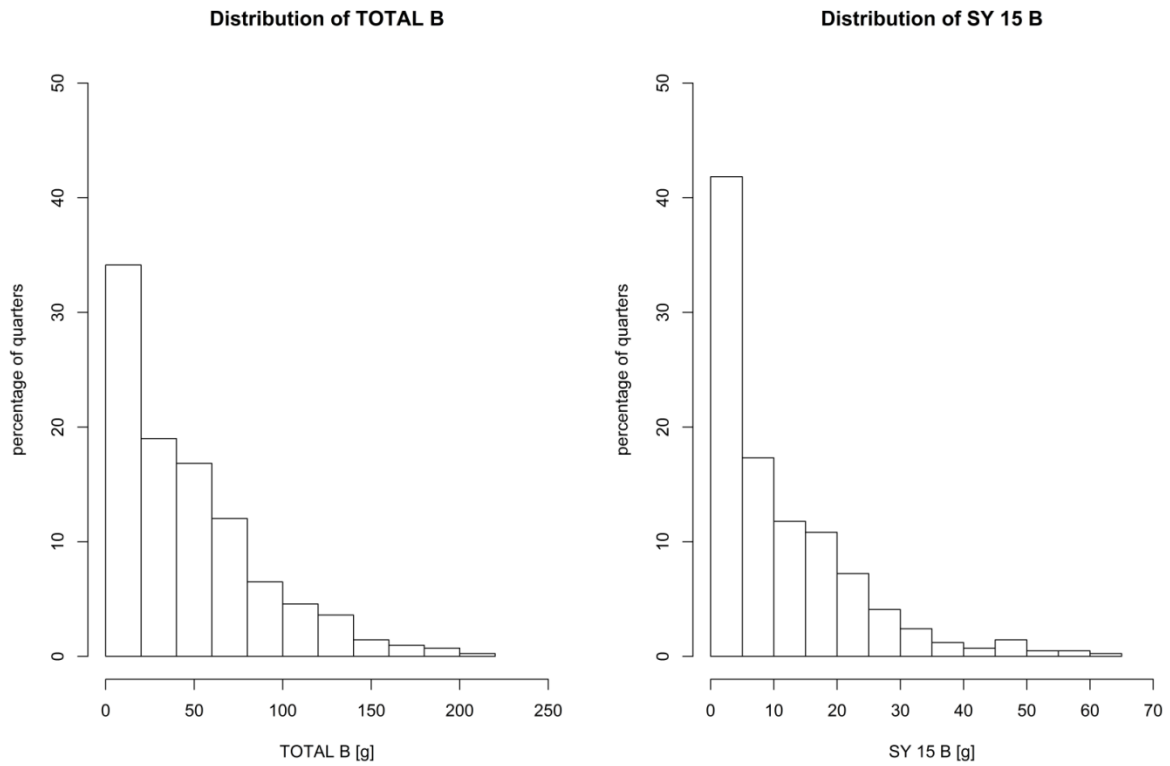
```
summary(lmEASYSTRIPS)
```

## Appendix Table A6

Characteristics of herds, parlours and parlour settings in the six German Holstein dairy herds included in the analyses of Exp A (Herd 1A) and Exp B (Herds 1B to 6B)

Herd	1A	1B	2B	3B	4B	5B	6B
Herd size	303	303	81	390	194	140	198
Experimental cows characteristics							
Mean lactation number	28	22	20	22	23	24	20
Mean days in milk	2.6	2.1	2.2	2.6	1.3	1.6	2.3
Mean daily milk yield (L/d)	142	111	136	117	110	125	152
Mean daily milk yield (L/d)	38.6	43.1	22.7	38.9	27.2	32.9	37.4
Parlour types and technical settings							
Milking parlour type	Herringbone	Herringbone	Auto-tandem	Parallel	Herringbone	Herringbone	Parallel
Size of milking parlour	2x8	2x8	2x4	2x20	2x12	2x8	2x12
Milking line system	Low-line	Low-line	Low-line	High-line	Low-line	Low-line	Low-line
Operating vacuum setting (kPa)	43	43	37	44	43	37	42
Pulsator ratio	60:40	60:40	60:40	60:40	65:35	60:40	60:40
Pulsator rate (cycles/min)	58	58	60	60	60	60	60
Automatic cluster remover (ACR) setting (ml/min)	750	750	300	480	300	250	300
Delay times (s) of ACR	20	20	15	25	30	30	25
Mean SMT vacuum during the milk flow plateau phase <sup>1</sup>	-	-	34.4 (n = 32)	35.1 (n = 44)	37.6 (n = 32)	36.7 (n = 28)	-

<sup>1</sup> Vacuum measurements carried out in July and August 2017 with VaDia (BioControl, Rakkestad, Norway) (SMT: short milk tube; n = number of milkings)

**Appendix Figure A7**

Distribution of the strip yield in 60 s (TOTAL B) and the strip yield in the first 15 s (SY15 B) of hand-milking of the right rear quarter of 131 experimental German Holstein dairy cows in six dairy herds. The hand-milking in Exp B was performed by three different Evaluators (B1, B2, B3) with a strip frequency of 1 HZ

**Appendix Table A8**

Herd and parlour characteristics of ten German-Holstein dairy herds included in the observation of hind leg activity and concurrent vacuum measurements

Herd	Herd size	Mean milk yield per cow per year (kg) <sup>1</sup>	Mean lactation number	Manufacturer of the milking system	Milking parlour size	Pulsation rate (cycles/min)	Pulsation ratio	Numbers of milkings per day
A	210	8582	2.6	Fullwood	2x16	60	60:40	2
B	112	8670	2.2	GEA	2x16	60	60:40	2
C	248	10238	2.2	Dairy Master	2x20	60	60:40	2
D	190	8000	2.2	DeLaval	2x12	60	65:35	2
E	181	10160	2.7	DeLaval	2x12	60	60:40	2
F	140	9152	2.6	GEA	2x8	60	60:40	2
G	247	9002	2.4	DeLaval	2x12	60	65:35	2
H	390	10735	2.4	Fullwood	2x20	60	60:40	2
I	194	8967	2.3	Boumatic	2x12	60	60:40	2
J	430	12509	2.9	DeLaval	2x12	60	60:40	2

<sup>1</sup> 305 days in milk

**Appendix Table A9**

Mean frequencies of hind leg activity (stepping; kicking) per minute of 545 observed German-Holstein dairy cows in ten herds

Herd	Total milking time	Let down	Plateau phase	Over milking first quarter <sup>1</sup>	Over milking second quarter
A	0.9; 0.1	1.7; 0.4	0.9; 0.1	0.6; 0.2	1.3; 0.4
B	0.8; 0.1	0.8; 0.5	0.9; 0	0.2; 0.2	0.4; 0.2
C	0.9; 0.2	0.7; 0.4	1.1; 0.1	0.3; 0.1	0.5; 0.2
D	0.6; 0.1	1.1; 0.2	0.6; 0.1	0.5; 0.2	0.7; 0.1
E	0.8; 0.1	1.5; 0.6	0.8; 0.1	0.5; 0.03	1.4; 0.2
F	0.8; 0.5	0.1; 0	1.1; 0.5	0.5; 0.04	0.8; 0.1
G	1.7; 0.4	0.3; 0.2	1.8; 0.3	0.5; 0.08	0.4; 0.1
H	0.7; 0.4	0.4; 0.1	0.8; 0.4	0.2; 0.06	0.3; 0.1
I	1.6; 0.2	0.2; 0	1.6; 0.2	0.5; 0.01	0.7; 0.03
J	1.1; 0.2	0.2; 0.1	1.1; 0.1	0.5; 0.07	0.5; 0.03

<sup>1</sup> in 81 % of the milkings, the front right quarter entered the over-milking phase first

**Appendix Table A10**

Mean frequencies of hind leg activities (stepping; kicking) per minute of German-Holstein dairy cows showing the corresponding hind leg activity at least once or more than once per milking time (n = 470 cows from 10 herds)

Herd	Total milking time	Let down	Plateau phase	Over-milking first quarter <sup>1</sup>	Over-milking second quarter
A	1.8; 0.6	1.9; 2.7	1.9; 0.6	0.7; 0.5	1.5; 1.0
B	0.8; 0.5	0; 0	1.2; 0.7	0.3; 0.3	0.6; 0.5
C	0.7; 0.5	0; 0.4	0.9; 0.6	0.3; 0.2	0.6; 0.4
D	1.0; 0.4	1.5; 0.6	1.1; 0.3	0.6; 1.0	0.8; 0.5
E	1.4; 0.4	1.5; 1.6	1.6; 0.5	0.6; 0.1	1.5; 0.5
F	0.9; 0.2	0.1; 0	0.9; 0.1	0.6; 0.3	0.8; 0.7
G	0.9; 0.2	0.3; 0:3	1.1; 0.2	0.5; 0.1	0.5; 0.2
H	0.6; 0.3	0.4; 0.3	0.7; 0.4	0.2; 0.3	0.3; 0.6
I	0.8; 0.3	0.2; 0	0.8; 0.3	0.5; 0.1	0.8; 0.2
J	0.9; 0.4	0.2; 0.2	1.0; 0.4	0.5; 0.4	0.6; 0.2

<sup>1</sup>in 80 % of the milkings, the front quarter entered the over-milking phase first

## Appendix Table A11

Duration (s) of the total milking time and duration (s) and share (%) of the milking phases (n = 545 observed German-Holstein dairy cows in ten herds)

Herd	Mean total milking time (s)	Mean let down phase (s; %)	Mean plateau phase (s; %)	Mean over-milking phase	
				first quarter <sup>1</sup> (s; %)	second quarter (s; %)
A	318	8; 3	181; 57	122; 39	71; 22
B	320	6; 2	175; 55	135; 42	68; 21
C	377	8; 2	180; 48	185; 49	118; 31
D	371	15; 4	225; 61	122; 33	71; 19
E	410	8; 2	200; 49	193; 47	114; 28
F	413	9; 2	220; 53	172; 42	81; 20
G	460	15; 3	266; 58	172; 37	100; 22
H	369	17; 5	192; 52	152; 41	85; 23
I	314	8; 3	175; 56	129; 41	50; 16
J	337	18; 5	126; 37	187; 55	100; 29

<sup>1</sup>in 81 % of the milkings, the front quarter entered the over-milking phase first

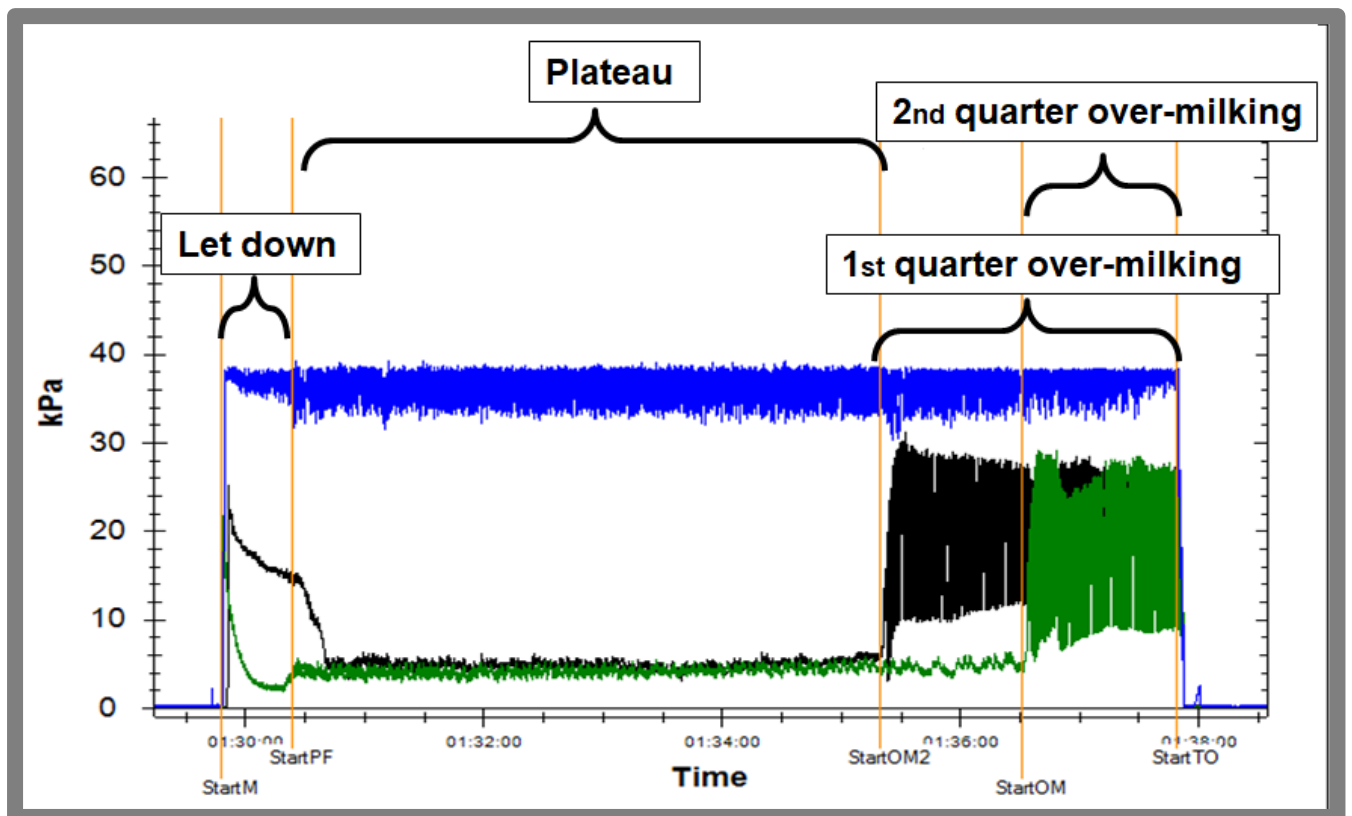
**Appendix Table A12**

Frequencies of hyperkeratosis at rear right (RR) and front right (FR) quarter, vacuum curve types and bimodality in ten German-Holstein dairy herds with and without observed hind leg activities stepping or kicking (n = 545 cows)

Herd	Frequency of hyperkeratosis score 2 <sup>1</sup> in FR quarters (%)	Frequency of hyperkeratosis score 2 <sup>1</sup> in RR quarters (%)	Mean teat length (cm) FR quarter (min; max)	Mean teat width (cm) FR quarter (min; max)	Mean teat length (cm) RR quarter (min; max)	Mean teat width (cm) RR quarter (min; max)	Frequency of vacuum curve type 2 <sup>2</sup> (%)	Frequency of bimodal curves (%)
A	3	0	4.3 (3.0; 6.0)	2.5 (2.2; 2.8)	3.8 (2.6; 6.3)	2.4 (2.0; 2.8)	35	10
B	1	0	4.7 (3.8; 6.0)	2.5 (2.0; 3.2)	3.8 (3.0; 5.0)	2.4 (2.0; 3.2)	4	8
C	3	2	5.0 (4.0; 7.8)	2.7 (2.2; 3.2)	4.3 (3.0; 6.0)	2.6 (2.0; 3.2)	34	5
D	12	4	4.7 (3.0; 6.4)	2.5 (2.0; 3.4)	4.0 (2.8; 6.4)	2.5 (2.0; 3.2)	24	22
E	5	5	4.6 (3.6; 6.4)	2.5 (2.0; 3.6)	3.8 (3.0; 5.0)	2.3 (2.0; 2.8)	21	8
F	9	2	4.7 (3.2; 6.8)	2.6 (2.2; 4.2)	4.1 (2.8; 6.8)	2.5 (2.0; 3.8)	13	33
G	7	0	4.3 (3.0; 5.4)	2.6 (2.0; 3.4)	3.7 (2.8; 5.0)	2.4 (2.0; 3.2)	18	33
H	3	3	4.2 (3.0; 6.0)	2.6 (2.0; 3.2)	3.6 (2.6; 5.2)	2.4 (2.0; 3.0)	29	45
I	14	4	4.4 (3.0; 6.2)	2.4 (2.0; 3.2)	3.8 (3.0; 5.4)	2.4 (2.0; 3.0)	16	21
J	10	1	4.3 (3.0; 7.0)	2.5 (2.0; 3.0)	3.6 (2.8; 5.2)	2.4 (2.0; 2.8)	31	51

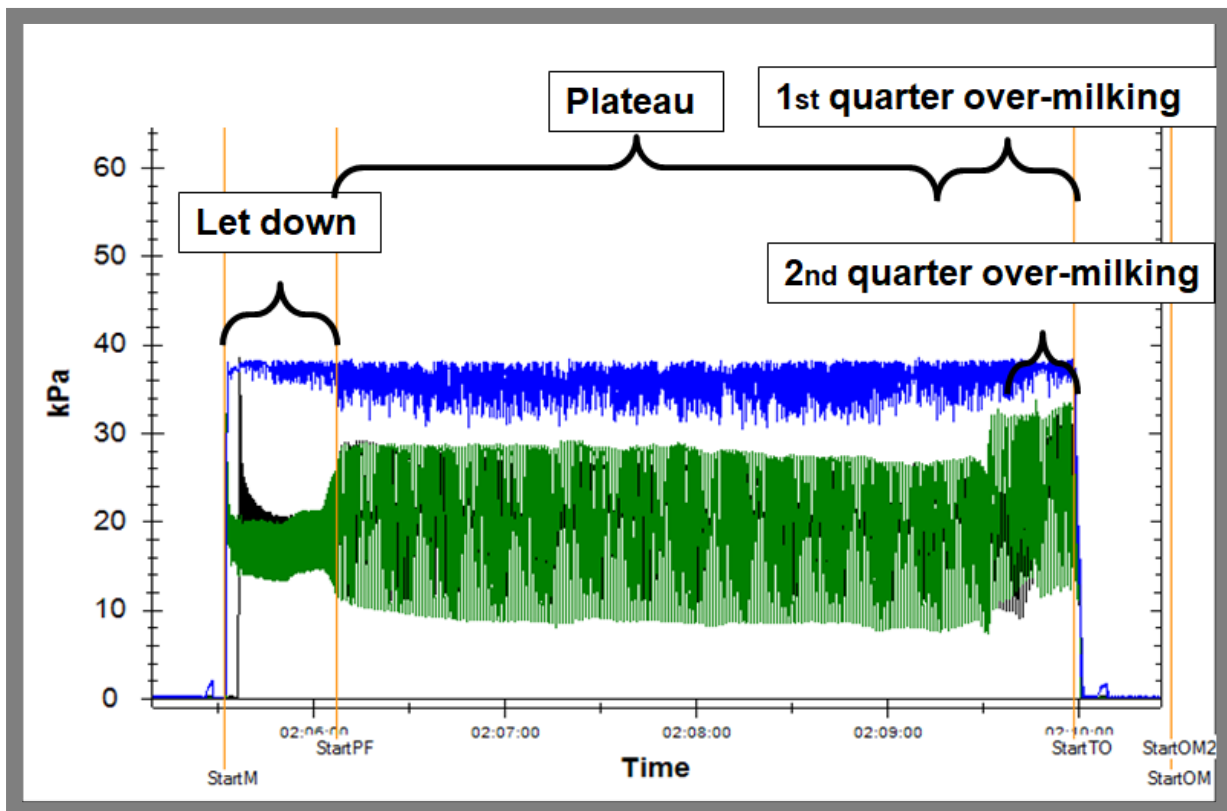
<sup>1</sup> Hyperkeratosis score 2: a raised roughened ring with isolated fronds of old keratin extending 1 mm to 3 mm from the orifice or a raised ring with rough fronds of old keratin extending > 4 mm from the orifice; <sup>2</sup> Vacuum curve type 2: unstable vacuum with unclearly distinguishable milking phases

Appendix Figure A13



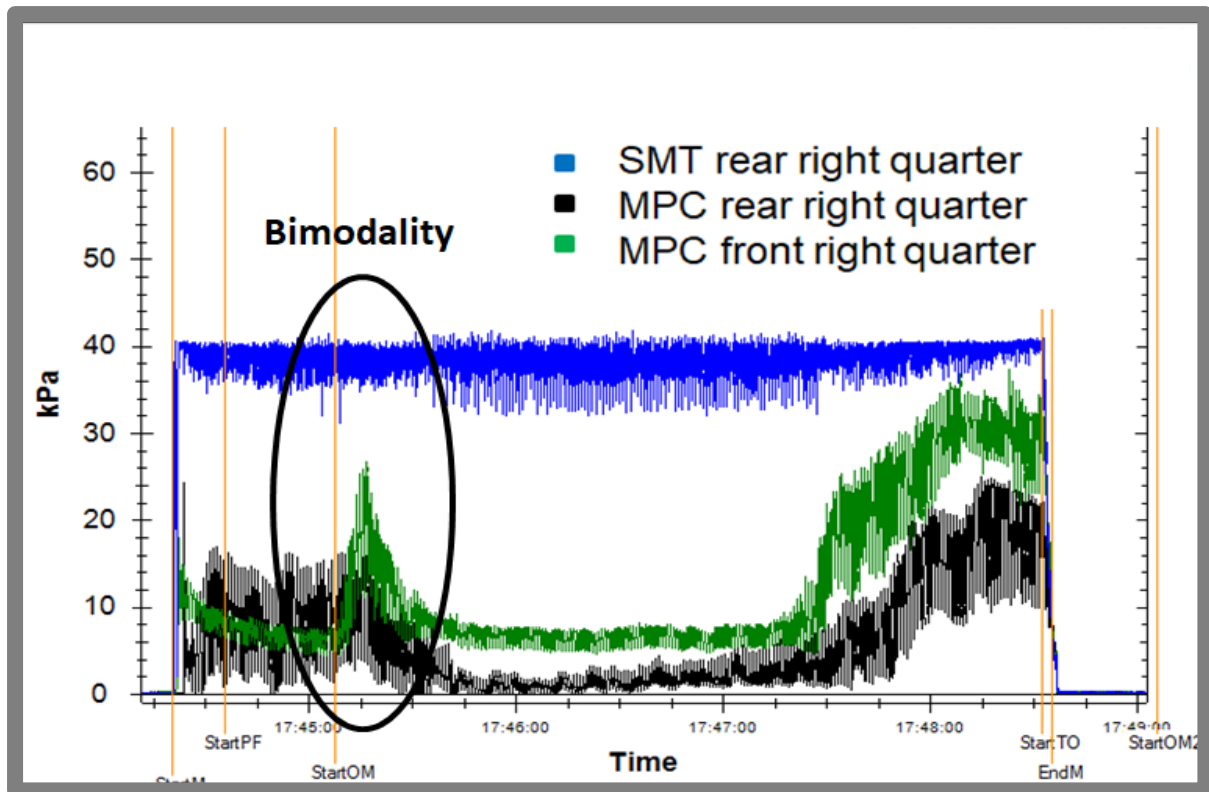
Differentiation between milking phases: let down, plateau and over-milking of first and second quarter in curves of vacuum curve type 1 (VCT 1) (created in "VaDia Suite", Biocontrol AS, Norway)

Appendix Figure A14



Example for vacuum curve type 2 (VCT 2) and differentiation between milking phases: let down, plateau and over milking of first and second quarter. Vacuum curve type 2 have an unsteady vacuum level in the short milk tube and mouthpiece chamber (MPC) with high cyclic vacuum fluctuations in the plateau phase and only a slightly increase of the MPC vacuum in the beginning of the over-milking phases (figure created in “VaDia Suite”, Biocontrol AS, Norway)

Appendix Figure A15



Example for bimodal curve with a short-term peak of the vacuum measured in the mouthpiece chamber (MPC) during the let down phase or at the beginning of the plateau phase (figure created in "VaDia Suite", Biocontrol AS, Norway)

## **Curriculum Vitae**

### ***Persönliche Daten***

Name: Daniela Meyer  
Geburtstag und Geburtsort: 10.09.1989 in Rotenburg/Wümme  
Staatsangehörigkeit: Deutsch

### ***Schulbildung***

08/1996-08/2000 Grundschole Stemmen, Deutschland  
08/2000-06/2009 Eichenschule Scheeßel, Deutschland  
Abschluss: Allgemeine Hochschulreife

### ***Studium***

10/2009-06/2013 Christian-Albrechts-Universität zu Kiel, Deutschland  
Bachelor Agrarwissenschaften (Nutztierwissenschaften)  
Abschluss: Bachelor of Science  
10/2013-08/2014 Christian-Albrechts-Universität zu Kiel, Deutschland  
Master Agrarwissenschaften (Nutztierwissenschaften)  
08/2014-05/2015 Wageningen University, Niederlande  
Master Animal Science  
05/2015-08/2016 Christian-Albrechts-Universität zu Kiel, Deutschland  
Master Agrarwissenschaften (Nutztierwissenschaften)  
Abschluss: Master of Science

### ***Berufliche Tätigkeiten***

04/2016-09/2019 Wissenschaftliche Mitarbeiterin am Institut für  
Landwirtschaftliche Verfahrenstechnik der Christian-  
Albrechts-Universität zu Kiel, Deutschland  
09/2019-heute Farm Management Support des Lely Center  
Westerstede, Niederlassung Oyten, Deutschland