

Inaugural-Dissertation zur Erlangung der Doktorwürde
der Tierärztlichen Fakultät der Ludwig-Maximilians-Universität
München

**Detection of clinical mastitis in dairy farms
operating with automatic milking systems in
Bavaria, Germany**

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München 2023

Aus dem Zentrum für Klinische Tiermedizin der Tierärztlichen
Fakultät der Ludwig-Maximilians-Universität München

Lehrstuhl für Physiologie und Pathologie der Fortpflanzung

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Angefertigt beim

Tiergesundheitsdienst Bayern e.V.

Fachabteilung Eutergesundheitsdienst

Poing-Grub

Mentor: Dr. Ulrike Sorge, Ph.D.

Gedruckt mit Genehmigung der Tierärztlichen Fakultät
der Ludwig-Maximilians-Universität München

Dekan: Univ.-Prof. Dr. Reinhard K. Straubinger, Ph.D.

Berichterstatter: Univ.-Prof. Dr. Rolf Mansfeld

Korreferent: Prof. Dr. Armin M. Scholz

Tag der Promotion: 22. Juli 2023

Die vorliegende Arbeit wurde gemäß §6 Abs. 2 der Promotionsordnung für die Tierärztliche Fakultät der Ludwig-Maximilians-Universität München in kumulativer Form verfasst.

Folgende wissenschaftliche Arbeiten sind in dieser Dissertationsschrift enthalten:

Bausewein M, Mansfeld R, Doherr MG, Harms J, Sorge US.

“Sensitivity and Specificity for the Detection of Clinical Mastitis by Automatic Milking Systems in Bavarian Dairy Herds”

Animals. 2022; 12(16):2131. <https://doi.org/10.3390/ani12162131>

received: 13th July 2022; accepted: 14th August 2022; published: 19th August 2022

Bausewein M, Mansfeld R, Doherr MG, Harms J, Sorge US.

“Survey on dairy farmers' management practices for and satisfaction with the detection of clinical mastitis by automatic milking systems in Bavaria, Germany.”

Milk Science International (76) 2023 P. 28-34. ISSN 2567-9538;

<https://doi.org/10.48435/MSI.2023.5>

received: 23th December 2022; accepted: 18th May 2023; published: 21th July 2023

Für meine Familie

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ABBREVIATIONS

4QCM	Four quarter conductivity measurement	SCC	Somatic cell count
AAS	Average answer score	SCM	Subclinical mastitis
AMS	Automatic milking system	S.	<i>Staphylococcus</i>
BTSCC	Bulk tank somatic cell count	SEM	Standard error of the mean
Ca.	Circa	SN	Sensitivity
CHM	Chronic mastitis	SP	Specificity
CI	Confidence interval	Str.	<i>Streptococcus</i>
CM	Clinical mastitis	TGD	Bavarian animal health services
CMT	California mastitis test	pH	Potential hydrogen
DHIA	Dairy Herd Improvement Association		
DIN	German Institute of Standardization		
DVG	German Veterinary Medical Society		
E.	<i>Escherichia</i>		
EC	Electrical conductivity		
e.g.	Example given		
et al.	Et aliter (et others)		
EU	European Union		
GLMM	Generalized linear mixed model		
GS	Gold standard		
i.e.	Id est (that is)		
IMI	Intramammary infection		
ISO	International Standardization Organization		
LDH	L-lactate dehydrogenase		
LED	Light emitting diode		
LKV	Landeskuratorium der Erzeugerringe für tierische Veredelung in Bayern e.V.		
MDi	Mastitis detection index		
MQC	Milk quality control		
MQC-C	Milk quality control cell count		
NIR	Near infra red		
OCC	Online cell count		
OR	Odds ratio		

I. INTRODUCTION

Technological progress has been deeply interfering with people's working lives and methods over the past 150 years, including the dairy industry. In the early 20th century, the invention of the pulsating milking machine provided the starting point for the introduction of mechanical milk extraction on dairy farms, progressively replacing laborious hand milking (Akam, 1980). However, it was the pressure of labor shortages in agriculture in the middle of the 20th century that forced the success of machine milking to accelerate in Germany (Settele, 2018). The further development of this technology took place under the constant influence of rationalization and increased productivity, from bucket milking systems to pipe milking systems and milking parlors (Jiang et al., 2017; Besier et al., 2016). Nevertheless, humans remained an irreplaceable part of the milking process: pre-cleaning, checking udder health by pre-stripping, attaching and, if necessary, removing the milking cluster are still carried out by the milker in conventionally milking farms. However, with the commercial introduction of automated milking systems (AMS) in the early 1990s, humans are no longer needed during the milking process (Hogenboom et al., 2019; John et al., 2016). The cows go to milking independently and the mentioned preparatory and accompanying actions during the milking process are automatically completed by the milking robots. Consequently, an AMS enables farmers to free themselves from the milking routines that determine their daily routine and to organize their working time more freely (Stræte et al., 2017; Hogeveen et al., 2004). This led to the fact that automation plays an increasingly important role in dairy farms, especially nowadays, as farmers expect it to secure the profitability of the farm as well as to improve the work-life balance (Koning, 2010). Thus, AMS can be considered one of the first developments in precision livestock farming with associated incisive changes in the dairy industry (John et al., 2016).

Ultimately, all these developments serve one purpose: the most efficient and safest production of milk. Clinical mastitis (CM) is a disease that has the greatest potential to cause pathological changes in milk and compromise animal welfare (Heikkilä et al., 2018). As a result, it also causes economic losses and poses a health risk to the end consumer (Pal et al., 2020; Cha et al., 2011). For this reason, farmers are legally obligated to deliver a flawless product. They must ensure that the health of their

herd and of the individual cow is under control (Nalon and Stevenson, 2019). Farmers are required to determine the animal's well-being during milking and to perform an organoleptic check on their milk. With the absence of a human on AMS farms during milking, the farmer must rely on the machine to detect udder health problems (Jacobs and Siegford, 2012). This is a task that has an impact on animal welfare, food quality assurance as well as profitability and is therefore essential in the dairy industry. For this purpose, AMS have various sensors available to determine changes in the milk and the cow's behavior. The data collected by the sensors are processed differently depending on the manufacturer and presented to the farmer on so-called warning lists on end devices. The final control of the animals is then again in the hands of the farmer while abnormal milk separation can be done automatically.

Automatic milking systems, especially their ability to detect health problems, have been the subject of many studies in the past 20 years (Cogato et al., 2021). The quality of CM detection by AMS in the field on Bavarian dairy farms has not yet been the subject of evaluation.

Therefore, the primary aim of this study was to assess the detection performance of the systems of four most widely represented AMS manufacturers in Bavaria. Moreover, AMS as well as non-AMS data, which might influence the detection performance, were further elaborated.

Additionally, a questionnaire was used to examine the farmers' interaction with the AMS by asking about management practices and their personal evaluation of their work with the AMS. The survey also included questions about their personal assessment of the detection CM by the AMS.

II. LITERATURE REVIEW

1. Bovine Mastitis

1.1. Definition, etiology and classification of mastitis

Bovine mastitis is defined as an inflammation of one or more quarters of the mammary gland and is almost always caused by infection with microorganisms (IDF, 2011).

The most common udder pathogens include bacteria, fungi, algae, and viruses (Bradley, 2002; Contreras and Rodríguez, 2011). However, the origin of mastitis cannot be attributed solely to colonization of pathogens of the milk ducts and tissues. External influences (e.g., incorrect milking settings, udder injuries), as well as internal influences (e.g., metabolic disorders, stress, immunological weakening), can be the cause or contribute to the development of mastitis (Tiwari et al., 2013; Holko et al., 2019). For this reason, mastitis should be considered as a multifactorial disease (Abebe et al., 2016).

Up to 95% of mammary infections are caused by bacteria (Zigo et al., 2021). These microorganisms are defined and divided into two groups (Eberhart et al., 1987): On the one hand contagious mastitis pathogens, which are adapted to the tissue of the mammary gland, survive mainly in the udder, and thus are most likely to be transmitted during the milking process. These include, among others, *Staphylococcus (S.) aureus*, *Streptococcus (Str.) agalactiae* or *Mycoplasma* spp. (Dufour et al., 2019). On the other hand, environmentally associated pathogens predominantly enter the udder through the teat canal from contaminated resting places primarily during resting periods after milking (Blowey and Edmondson, 2010) or as a result of insufficient milking hygiene (Svennesen et al., 2019; Hohmann et al., 2020; Breen et al., 2009). Representatives of this larger group are mainly *Escherichia (E.) coli*, *Streptococcus uberis*, coagulase-negative staphylococci, *Enterobacter* spp., *Corynebacterium* spp., and others (Hogan and Smith, 2012; Pyörälä and Taponen, 2009). Environmental pathogens are responsible for about 90% of intramammary infections (IMI; Neculai-Valeanu and Arifton, 2022). However, a purely binary pathogen classification into environmental and contagious should be used with caution, as for some pathogen species, e.g., *Str.*

dysgalactiae, both possible ways of transmission and reservoirs have been identified (Klaas and Zadoks, 2018; Wente and Krömker, 2020). Due to the different characteristic of gram stainability, the forms of mastitis can be distinguished into gram-positive mastitis and gram-negative mastitis form depending on the underlying bacteriological pathogen, which offer a strong influence on the therapeutical approach (Schukken et al., 2009; Pol and Ruegg, 2007; Nam et al., 2009)

Furthermore, pathogens are divided into two groups according to their pathogenicity, prevalence and the physical and economic damage they cause: major and minor pathogens (Heikkilä et al., 2018; Harmon, 1994). Major mastitis pathogens, which include *S. aureus*, *Str. uberis*, *Str. dysgalactiae*, *Str. agalactiae*, *Klebsiella* spp., and *Mycoplasma bovis* achieve a much greater impact in the inflammatory process and cause often CM (Dalanezi et al., 2020). Minor pathogens, are primarily physiological commensals of the udder skin and teat canal. Their pathogenicity is considered to have lower impacts on inflammation. This group includes *Enterobacter* spp., coagulase-negative staphylococci and *Corynebacteria* (Reyher et al., 2012).

However, the actual expression of an inflammatory event in response to bacterial invasion depends on certain factors and consequently varies. The pathogen, its virulence and quantity, as well as the counteractive immune response of the host animal and the environment, are crucial for the effects and manner in which the mastitis develops and its clinical expression (Hamann, 1991; Cheng and Han, 2020). Thus, bovine mastitis can be classified as subclinical mastitis (SCM), chronic mastitis (CHM), or clinical mastitis (CM), depending on the severity and duration of the inflammatory process, or in the case of chronic mastitis also treatment resistance.

Subclinical mastitis is the inflammation of the udder without visible changes in the milk and udder (IDF, 2011). Yet, this form of mastitis still results in changes in milk composition with increased somatic cell count (SCC). A threshold of 200,000 cells/mL was established by the International Dairy Federation (IDF) in 2013 to define IMI at cow level. However, quarters of healthy cows usually do not have more than 100,000 cells/mL, so this threshold is used especially in Germany as indicator of SCM, with or without the presence of isolated pathogens (DVG, 2002; IDF, 2013; Schwarz et al., 2010). The prevalence of SCM is much higher than that

of the clinical form (Seegers et al., 2003). It also leads to economic losses due to associated milk loss (Gonçalves et al., 2018). Subclinical as well as not fully recovered clinical mastitis due to e.g., therapy resistance can progress to the chronic form (Grönlund et al., 2003).

Chronic mastitis is generally defined as an elevated SCC in the last three to four monthly test days (Hiitiö et al., 2017; Rahman et al., 2012). A threshold SCC for CHM is set at >250,000 cells/mL, >400,000 cells/mL, or >700,000 cells/mL, depending on the literature (Bazzanella et al., 2020; Zecconi et al., 2018). However, a poor prognosis for udder recovery is expected (Linder et al., 2013; Zecconi et al., 2018). In addition, CHM carry the risk of pathogen transmission to other cows in the herd (Zadoks et al., 2003). Chronic mastitis exhibits prolonged udder infection with often no healing and may progress to periodic clinical symptom traits (Cheng and Han, 2020).

Clinical mastitis is characterized by organoleptically detectable pathological changes in the udder and its secretions (Contreras and Rodríguez, 2011; Pinzón-Sánchez and Ruegg, 2011). Clinical mastitis can be divided into three degrees of progression or severity. These are also closely related to the underlying etiology (Oliveira and Ruegg, 2014; Oliveira et al., 2013). Low-grade, mild CM is characterized by abnormal milk without visible or palpatory changes in the udder or quarter. The milk may contain flakes, clots and pus, be odorous and could be altered in color. These changes range up to complete dissolution of the milk character. Moderate mastitis is characterized by abnormal milk and additional pathological findings of inflammation, i.e., pain, swelling, redness and warmth of the udder or quarter. Case of severe mastitis, include in addition to the pathological findings of the secretion and the mammary gland, systemic signs of disease, such as loss of appetite, fever, depression, or even coma up to death. (IDF, 2011; Hovinen et al., 2008; Oliveira et al., 2013).

Mastitis, especially CM, poses a risk to animal welfare, farm economics, and food quality due to the aforementioned inflammatory response of the tissues (Burvenich et al., 2003; Hertl et al., 2011).

1.2. Impact on animal welfare, economics and food quality

Mastitis, especially in its clinical form, affects the animal's well-being depending on its severity and leads to different changes in physiological behavior (Siivonen et al., 2011; Leslie and Petersson-Wolfe, 2012; Sepúlveda-Varas et al., 2016). Thus, the pain of mammary inflammation may be associated with high heart and respiratory rate, reduced lying periods, decreased feed intake, low ruminating activity, and restlessness (Fitzpatrick et al., 2013; Sepúlveda-Varas et al., 2016). During high-grade CM, the systemic infections can disrupt the general condition to the point where fever, lassitude, recumbency or death of the animal may occur (Burvenich et al., 2003; Hertl et al., 2011). For these reasons, mastitis is a serious threat to animal welfare and profitability of dairy farms.

The financial negative impact of mastitis goes so far as to make mastitis the most costly disease in the dairy industry worldwide (Halasa et al., 2007). An exact estimation of the costs is difficult due to different regional conditions, such as milk and treatment prices, but also the form of mastitis and lactation stage of the affected cow. Thus, the costs in different studies of a mastitis range from 140 - 570 EUR (Berry et al., 2004; Cha et al., 2011; Dahl et al., 2018; Rollin et al., 2015; Sørensen et al., 2010). Diagnosis and treatment costs of a cow account for only a small amount of the costs (Bar et al., 2008; Rollin et al., 2015). The main cost factors of CM are the loss of milk during the disease, the loss of milk during the prescribed waiting period for medication and the late lactation consequences due to the damaged secretory epithelium and the associated reduced fertility and the need of culling and restock (Rollin et al., 2015; Fuenzalida et al., 2015). It was estimated that in 2009 mastitis caused approximately 1.4 billion EUR losses to the German national economy (DVG, 2012). For this reason, preventive actions such as safety and hygiene practices during milking, high hygiene standards in the barn, vaccinations, and selective culling of chronic cows are crucial and of comparatively lower economic impact than high mastitis prevalence with treatment issue to prevent mastitis (Huijps et al., 2010; van Soest et al., 2016; Gussmann et al., 2019; Ismail, 2017). High hygiene standards are crucial not only for the prevention of new mastitis, but also for ensuring a safe food production.

Mastitis negatively affects the quality of milk and is therefore a threat to food safety. Inflammatory processes in the udder are associated with changes in secretory epithelium and vascular permeability, which lead to alterations in milk composition

(Kitchen, 1981). Thus, mastitis causes, for example, milk with abnormal ion concentrations (Batavani et al., 2007), higher protein but lower fat and lactose concentration (Forsbäck et al., 2010), high concentration of inflammatory enzymes (Larsen et al., 2010), as well as increased somatic cell content (Haas et al., 2004), and possibly contamination of pathogens and their toxins (Taponen et al., 2019; Fursova et al., 2018; Murinda et al., 2019). Especially in CM, some milk changes become visible due to the inflammatory process and appear in the form of abnormal milk, characterized by blood, cell debris, clots or even pus. All these milk abnormalities reduce the nutritional value of milk and pose risks both for safe processing into dairy products, like e.g. cheese, and for the health of the consumer, especially when consuming untreated raw milk (Bobbo et al., 2017; Johler et al., 2015; Jamali et al., 2015). The consequences are, for example, reduced shelf life of the products, defective further processing up to the danger of intoxication during consumption (van Asselt et al., 2017; Ding et al., 2016; Murphy et al., 2016). Due to the aforementioned risks for the consumer, all milk producers are legally obligated to comply with measures to ensure food quality.

2. Legal framework for milk production

In Germany, as in all European Union (EU) member states, dairy farms are obliged by European Commission regulations to ensure udder health, but above all consumer protection and food quality and must therefore comply with basic regulations on the production of foodstuffs (EC Regulation 178/2002). In addition, other regulations primarily address issues of risk analysis, precaution and traceability.

In 2006, the European Commission regulations replaced the previously national valid milk regulation "Regulation on hygiene and quality requirements for milk and milk-based products" of July 20th, 2000. In the following, the most important valid regulations in connection with udder health and milk production on farms are presented in more detail.

EU Regulation 852/2004 of the European Parliament and the Council of 29th April (2004b) establishes specific hygiene requirements for food imported into and exported from the EU, as well as hygiene rules for primary food production.

The EU Regulation 853/2004 of the European Parliament and the Council of 29th

April 2004a with specific hygiene requirements for food of animal origin supplements the above-mentioned EU Regulation. Important requirements for dairy farms are listed in Annex III, Section IX, Chapter I, Parts I and II, and hygiene regulations for the primary production of raw milk in dairy farms are set out. Thus, raw milk may only be obtained from cows that do not show any signs of infectious disease transmissible to humans, as well as any signs of disease that may cause contamination of the milk. Furthermore, cows suffering from an “udder wound likely to affect the milk” or “a recognizable inflammation of the udder” must be excluded from milk production. Under Part II, Section B, particular attention should be paid to the following:

“that milk from each animal is checked for organoleptic or physico-chemical abnormalities by the milker or a method achieving similar results and that milk presenting such abnormalities is not used for human consumption;”. The causes of abnormal milk are either damage to the teat tissue (blood-stained milk) or CM with secretion changes. Therefore it is specified that “(...) milk from animals showing clinical signs of udder disease is not used for human consumption (...)”.

In order to comply with this regulation, the milker is responsible for hygienic milking, assessing the health of the animal as well as the hygiene and health of the udder before each milking in conventional milking farms using direct sensory examination of the animal, the udder, and the milk.

By using an AMS, the entire milking process, including the evaluation of udder hygiene, the health of the animal, and especially the udder, is carried out by the machine due to the absence of a human during milking. The assessment of whether milk is abnormal and therefore not suitable for human consumption is so performed automatically. This assessment of the milk should achieve results comparable to those of a human milker. A standard for the hygiene requirements for milking with AMS was created to ensure in this regard food safety. This German Standardization, the DIN ISO 20966: 2008 "Automatic milking equipment-requirements and testing (ISO 20966:2007)" is equivalent to its origin, the ISO 20966:2007 (ISO, 2007). This standard gives in the informative annexes requirements for the performance of teat cleaning (Annex B), as well as for the detection of abnormal milk (Annex C). For this reason, the detection of blood in the milk must be carried out with a sensitivity (SN) of at least 80% and a simultaneous specificity (SP) of >99%. Abnormal milk must be detected with a minimum SN of 70% and a SP of at least

99% by the sensor system of AMS.

Automatic milking systems, however, may pose a risk with regard to hygiene requirements of EU Regulation 853/2004. No AMS manufacturer was able to provide certification according to DIN ISO 20966: 2008 for any of its systems on the market (KTBL, 2013a). Thus, it must be assumed that the equipment of the AMS for sufficient udder cleaning and detection of abnormal milk as well as automatic separation does not meet the minimum requirements in some areas. For this reason, a catalog of measures for dairy farms operating with AMS was published in the Federal Gazette, the “Bundesanzeiger”, which was revised and published in its current form on September 4, 2012 (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz, 2012). This serves to ensure food quality and animal health in AMS farms. Certain actions “should” be taken, if no expert assessment on the requirements of DIN ISO 20966 "Automatic milking equipment-requirements and testing (ISO 20966:2007)” can be presented for the used AMS. This includes measures such as registration with the responsible veterinary office, udder health pre-screening, daily tasks of warning list control and animal observation, monthly monitoring of SCC, and documentation. Twice a day, farmers should check out the udder health warning lists as well as the herd in the barn for health problems, especially the udder health.

Given the impact of CM on both animal welfare and food safety, in addition to the regulatory framework, it is essential to understand milking with AMS.

3. Automatic milking system

The first commercial milking robot was installed in the Netherlands in 1992 (Simões Filho et al., 2020; Koning et al., 2002). After an initial rare use of AMS, the milking robot became more and more popular due to technological improvements, decreasing investment costs, and increasing workload of dairy farms (Harms and Wendl, 2012). Especially countries with family-run farms, which mainly milk high-yielding cows and obtain high milk prices, pushed the introduction of AMS over the last 20 years (Barkema et al., 2015; Svennersten-Sjaunja and Pettersson, 2008; Hansen, 2015). Today, estimates quantify over 50,000 AMS units in use around the world (Simões Filho et al., 2020).

This trend is also apparent in Bavaria - Germany's federal state with the largest milk

production (Frick and Sauer, 2018). Thus, at the beginning of the millennium, there were only just under 40 dairy farms operating with AMS in Bavaria. In 2021, approximately 2700 dairy farms in Bavaria were operating with AMS. This corresponds to about 16 % of all Bavarian dairy farms registered at the regional Dairy Herd Improvement Association (DHIA; LKV, 2022). The four dominant AMS manufacturers in Bavaria are - in alphabetical order - DeLaval, GEA Farm Technologies, Lely and Lemmer-Fullwood (LKV, 2022).

3.1. Structure and operation of automatic milking systems

Automatic milking systems are available on the market in various designs ranging from single box systems to multi-box systems to fully automatic milking carousels.

The most common systems in Bavaria are single and multi-box systems. The single box systems are made of modules: the milking box, mechanical arm including udder and teat cleaning system, teat cups, milk rack and sensor systems, containment system, systems for cleaning and disinfection, machine and control unit, and concentrate feed unit (Rossing and Hogewerf, 1997; Simões Filho et al., 2020; KTBL, 2013b). The structure of the system types differs in that the milking robot in multi-box systems can switch between the boxes and thus serve several milkings at the same time. A single box can serve about 65 cows with 2-3 milkings per day, while multi box systems with four boxes can reach up to 220 cows and 520 milkings per day (KTBL, 2013c).

Automatic milking systems of the respective manufacturers and types differ in their methodology, but they are the same in their tasks. These are based on conventional milking and include the following steps: cow identification, udder cleaning, check of udder health by pre-stripping, pre-stimulation, teat cup attachment, milking and teat cup removal, and teat post-milking teat disinfection, if necessary. These tasks of an AMS are illustrated in the following.

Depending on the system (free animal traffic or controlled animal traffic), the animal decides independently when to visit the AMS. Additional motivation is provided by a concentrated feed ration available there. Cows enter the boxes and are identified electronically by the machine through transponders. Depending on a given and adjustable milking permission - the milking interval can be set beforehand depending on lactation stage and expected milk yield - they are released from the box again or the milking process starts. The boxes are closed, and the

animal is given a concentrated feed ration. After sensor-assisted localization of the teats, the attachment arm starts cleaning the udder by combination of water and air jets or brushes and rollers. With the cleaning process also the pre-stimulation is done. Pre-dipping is optionally. This is followed by sensor supported attachment of the teat cups and the main milking process. The milk's fitness for human consumption is determined by means of sensor data. There is a possibility to separate abnormal milk. After detachment of teat cups there is a possibility of post-dipping the teats. The cow is released from the box after the milking process (Simões Filho et al., 2020; KTBL, 2013b). If necessary, the animals can be guided into parts of the barn depending on the type of cow traffic or the determined state of health. The milking robot cleans itself independently after each milking and, depending on the settings, performs an intermediate disinfection.

In addition, some AMS have other functionalities and diagnostic tools available, such as weight determination and heat detection of the cow. All collected data is displayed and stored for the farmer on electronic end devices, especially computers or smartphones, in the corresponding AMS management software.

3.2. Benefits and risks of automatic milking systems for udder health

With regard to the advantages and disadvantages of the use of an AMS on udder health, the technical specifications should be mentioned in particular. Due to the design and operating principle of most attachment arms of AMS, each quarter is equipped with its own milk removal system. This reduces the risk of cross-contamination between quarters (Hogeveen et al., 2001). Similarly, the teat cups are removed quarter by quarter based on milk flow rate thresholds (Penry, 2018), reducing the risk of blind milking and therefore risk of teat end damage (Bava et al., 2005; Hillaerton et al., 2002). The shorter, non-regular milking times and the resulting more frequent milkings per day have to be seen in a conflicting way. On the one hand, this leads to an increased efflux of possible mastitis pathogens, on the other hand, however, it also leads to an increased stress on the tissue and the associated dilatation of the teat canal. This weakens the first physical barrier against invading pathogens (Hovinen and Pyörälä, 2011). Similarly, the procedure of a milking robot should be viewed from two points of view. The milking routine of an AMS is performed in a strict order without deviations, human errors or lack of concentration (Sandgren et al., 2009; Svennersten-Sjaunja and Pettersson, 2008). However, this "rigid" milking routine also poses dangers, as teats may be

insufficiently identified and pre-cleaning and attachment procedures may not be completed satisfactorily (Dohmen et al., 2010). This, in turn, may result in residual contamination of the teats due to insufficient cleaning of heavily soiled teats (Hovinen et al., 2005). Poor teat hygiene is associated with an increase in new IMI (Dohmen et al., 2010). However, the greatest risk to udder health is the use of one set of milking cups for the entire herd, which increases the transmission of cow-associated pathogens such as *S. aureus* (Baumgartner et al., 2013). To address this problem, AMS are equipped with intermediate disinfection technologies, but these do not always ensure complete pathogen elimination (Hovinen and Pyörälä, 2011). Another aspect worth mentioning is functionality of the used sensor technology and the management decisions and practices of the farmer. Modern AMS models are equipped with a variety of sensors, providing the farmer with a mass of health data every day at each milking and thus an overview of each animal in the herd. Nevertheless, the farmer makes himself more or less dependent on these data because of his absence during milking. Faulty sensors or their processing are thus a risk to udder health.

There are conflicting views in the literature on the effects of using AMS on udder health. While there is an increase in average SCC during the transition phase for several months compared to conventional milking farms (Hovinen et al., 2009; Kruip et al., 2002; Koning et al., 2003), other studies have not found lasting strong negative effects of the AMS on SCC (van den Borne et al., 2021; Tousova et al., 2014; Castro et al., 2018). This could also be observed on Bavarian farms. Conventional milking farms and AMS farms hardly differ. They have similar farm sizes, and the average herd milk yield (7,934 kg milk) as well as average herd cell count (205,000 cells/mL) of AMS farms are only slightly above the group of conventional milking farms (Endres, 2017).

Overall, it can be said that through the continuous development of AMS and the improved management of farmers with their AMS, udder health in AMS farms has improved and can, with the right management, be held at a comparable level like conventional milking farms (Hogenboom et al., 2019; Castro et al., 2018; van den Borne et al., 2021; Svennersten-Sjaunja and Pettersson, 2008).

3.3. Overview of mastitis detection by automatic milking systems

The detection of udder health disorders by an AMS is based on the principles of data collection, processing, interpretation and final presentation for farmer decisions and, if activated, an autonomous action like separation of abnormal milk (Penry, 2018). Changes in milk composition during the development and occurrence of mastitis as well as behavioral changes of the animal are detected by sensors of the AMS. This data is stored and then processed by different algorithms and models and finally presented to the farmer via reports or attention lists on an end device. These reports can be based on single calculated parameters from individual sensor data, or based on a combination of different sensor data (King and DeVries, 2018). The processing and presentation varies by manufacturer and are proprietary knowledge of the manufacturer (Hovinen and Pyörälä, 2011). However, attention or warning lists for udder health disorders currently used in the field are not always based on scientifically and transparently validated sensors or processing algorithms (King and DeVries, 2018).

Overall, the objectives of udder health monitoring are, firstly, in its function as an early warning system, the early detection of IMI (Brandt et al., 2010) and secondly, the detection of CM with associated milk changes in order to meet the legal requirements for food safety. This is essential to fulfill an adequate independent elimination of abnormal milk.

Udder health is indirectly determined by AMS through a variety of changing parameters in milk. This requires methods based on chemical or physical milk analyses and can be performed in two ways: via inline sensors and online sensors. Inline sensors are monitoring the continuously flowing milk during the milking process. Online sensors are analyzing a separated specific amount of milk (Penry, 2018).

The most widely used sensors for monitoring udder health detect electrical conductivity (EC), somatic cell content (SCC), milk color, milk temperature, milk yield, as well as milk constituents and enzymes (Hogenboom et al., 2019). In the following subsections, the different diagnostic tools of AMS are briefly described. Further, Table 1 gives a short overview of sensors for udder health monitoring used in AMS of the four main AMS manufactures in Bavaria.

Sensor-based parameters	AMS manufacturers / latest AMS version			
	DeLaval / VMS 300/310	GEA / DairyRobot R9500	Lely / A5	Lemmer-Fullwood / M2erlin
Milk yield	X	X	X	X
Milk flow	X	X	X	X
Electrical conductivity	X	X	X	X
Blood	X	-	X	X
Milk color	-	X	X	X
Milk temperature	-	X	X	-
Somatic cell count	Optional	X	Optional	-
Milk fat	-	-	X	X
Milk protein	-	-	X	X
Lactose	-	-	X	X
Urea	Optional	-	-	-
Progesteron	Optional	-	-	-

Table 1: Overview of sensors used in AMS for udder health monitoring by the four major AMS manufacturers in Bavaria according to manufacturer information (status 2021).

3.3.1. Electrical conductivity

Detection of EC of milk is the most widely used inline sensor system and a standard sensor in all AMS to detect udder health disorders (Wethal et al., 2020; Brandt et al., 2010). This is mainly due to the fact that the sensor does not require reagents, is comparatively cheap, and can be cleaned in situ (Mottram, 2016; Brandt et al., 2010). The operating principle of this sensor system is the determination of the conductivity of a liquid, in this case milk, for electric current. For this purpose, an electric voltage field is generated between two electrodes in an electrolyte solution. The conductivity depends primarily on the concentration of the salts dissolved in the solution and the temperature of the solution and is measured in units of Siemens per metre (S/m, McCarthy, 2002). The anions and cations present in the milk allow the determination of EC of the milk (Mucchetti et al., 1994; Kandeel et al., 2019). A healthy udder secretes milk with sodium- and chloridions concentrations below the blood serum level (Hogeveen, 2002). Different thresholds for physiological EC of the milk can be found in the literature: Norberg et al. (2004) named a value below 4.87 mS/cm for healthy milk, whereas Hamann and Zecconi (1998) recorded a

healthy foremilk with a EC value of 4.9-6.4 mS/cm. Other researchers set EC of health cows 3.8-5.5 mS/cm (Hillerton and Walton, 1991; Juozaitienė et al., 2015). Therefore it is difficult to determine a suitable EC threshold for “healthy” milk, because the EC value of the milk is subject to physiological fluctuations, e.g. fat content of the milk, measured milk fraction, temperature, lactation stage, as well as measurement-related fluctuations, e.g. air pockets in the milking line or sensor calibration (Norberg et al., 2004; Bruckmaier et al., 2004b; Nielen et al., 1992; Khatun et al., 2017; Bruckmaier et al., 2004a). Beside these influences, there are pathological changes, which affect EC of the milk. Inflammation of the udder tissue causes loosening of the tight junctions between the epithelial cells, destruction of the ion pumping systems and increases the permeability of the blood vessels. Consequently anions and cations, like sodium-, potassium-, and calciumions, increasingly flow into the milk, while the viscosity of the milk decreases due to low secreting ability of the damaged tissue (Mabrook and Petty, 2003; Bansal et al., 2005). The hereby, besides the increased pH, increased EC of the milk altered by inflammation can be used as a diagnostic tool for the detection of udder health problems (Bansal et al., 2005; Neculai-Valeanu and Ariton, 2022). For example, a study found a slight increase in EC for low-grade infections in one quarter, and an increase in EC of up to 8 mS/cm in one quarter for severe infections (Hamann and Zecconi, 1998).

However, using EC alone is less suitable for detecting CM due to the above mentioned influences (Khatun et al., 2017). Another factor that complicates the measurement of EC during mastitis is the susceptibility of the sensors to interference from flakes in the milk (Norberg et al., 2004). In order to increase the detection performance of EC for CM and thus counteract the influences, the individual measurement and comparison of EC at quarter level is to be preferred to the overall measurement at udder level (Hogeveen et al., 2010). Furthermore, it is recommended to include the milking interval and use the measurement of EC of the foremilk (Khatun et al., 2017; Khatun et al., 2019). Also changes in quarter EC in combination with other data like milk production rate and average milk flow rate have the potential to detect developing mastitis before clinical signs are manifested (Inzaghi et al., 2021). Overall, the sole use of EC, especially that of total milking, is less suitable for CM detection. However, the use of quarter-specific EC, also in the intra-udder comparison, in combination with other parameters promises a high detection performance for mastitis (Hogeveen et al., 2010; Khatun et al., 2018).

3.3.2. Somatic cell count

The determination of SCC is the gold standard for evaluating udder health and is used as a diagnostic tool in different ways, both at cow and herd level (IDF, 2011; Schukken et al., 2003). Somatic cells, i.e., leukocytes, especially lymphocytes, macrophages, neutrophils and epithelial cells, are found in varying amounts in milk at any time (Li et al., 2015; Bobbo et al., 2020). Physiological factors, such as lactation status or lactation number but also environmental influences; such as heat periods and cleanliness of the milking process, influence the amount of SCC (Alhussien and Dang, 2018; Nørstebø et al., 2019; Nyman et al., 2014). However, the main cause of increased SCC is underlying inflammation of the udder tissue (Harmon, 1994). In response to pathogen colonization and growth in the mamma, soluble inflammatory mediators are released which, among other things, lead to an increase in vascular permeability and thus lead to the migration of inflammatory cells from the blood, such as leukocytes, and contribute to inflammation (Oviedo-Boyso et al., 2007; Ibrahim, 2017; Sordillo, 2018). A healthy quarter is considered to have an SCC of $\leq 100,000$ cells/mL (DVG, 2002). In AMS, the determination of SCC can be done by the use of mostly online sensors. For this purpose, a fixed quantity of milk is separated from the milk stream and analyzed separately. Depending on the AMS, the analysis can be automatically performed directly or indirectly (Brandt et al., 2010). Direct determination of SCC is performed by automatic camera-based optical counting of stained fluorescent cell nuclei previously destroyed using an added reagent (Hogeveen et al., 2021). It is used by DeLaval (DeLaval International AB, Tumba, Sweden) system online cell count (OCC), which measures SCC during each milking, depending on the setting (Sørensen et al., 2016). It is largely equivalent (correlation coefficient 0.82) to the laboratory measurements of SCC by the regional DHIA (Nørstebø et al., 2019). Other systems estimate the amount of SCC indirectly similar to the principle of a California mastitis test (CMT) with the viscosity of the milk (Hogeveen et al., 2021). This is done by hydrolyzing the DNA of the cell nuclei and measuring the viscosity of the resulting gel (Sørensen et al., 2016; Deng et al., 2020). It is used in Lely's (Lely Holding B.V., Maassluis, Netherlands) Milk quality controlTM sensor system (MQC-C). One system, DairyMilk M6850TM of GEA Farm Technologies (Bönen, Germany), estimates SCC for each quarter without using a detergent by patented physical measuring method using electrical permittivity thresholds during the whole milking (Klimpel, 2019), but no scientific evaluation of this system has

been done yet. Also SCC determination in AMS could be a good tool, even in combination with other sensor data, to detect udder health problems (Khatun et al., 2019; Dalen et al., 2019; Khatun et al., 2018).

3.3.3. Milk color

Milk color is subject to physiological variations and differs, for example, by breed, milk fat content, milking interval, feed, but also the stage of lactation (Espada, Elena, and Hélène Vijverberg, 2002; Agabriel et al., 2007; Scarso et al., 2017; Calderón et al., 2007; Quist et al., 2008). Immediately after calving, a higher proportion of blood cells, such as immunoglobulins but also red blood cells can be found in the milk due to changes in the capillary membrane of the blood vessels (McGrath et al., 2016). However, this influx of red blood cells can also have pathological causes. Trauma to the teat wall or udder tissue, as well as infection with certain pathogens, can result in blood in the milk (Mulon, 2016; Waage et al., 2001; Bruckmaier and Wellnitz, 2017). Human milkers can detect a blood content of 0.1% (Rasmussen and Bjerring, 2005). This percentage should also be detected independently by AMS and lead to separation of the milk (ISO, 2007). A change in the milk color components during a mastitis period can also be detected in this way. For this purpose, AMS uses online sensors based on the principles of light reflection or transmission to determine the color of the milk (Hogeveen et al., 2021; Ouweltjes and Hogeveen, 2001). Light-emitting diode (LED) sensors, which determine the wavelengths of the reflected light generated by LEDs, can be used to determine the red, green and blue color components of a continuous flow of milk (Espada and Vijverberg, 2002). Milk of mastitis has a higher proportion of blue and green color components (Kamphuis et al., 2008a). Milk with a strong yellowish color has also been found to be an indicator of mastitis, but this is mainly due to the animal's diet (Hovinen et al., 2006). However, the sensitivity of detection mastitis by milk color is 68% and coupled with a high rate of false alerts (Trilk et al., 2006). The transmission method, where light is divided into specific parts, promises higher detection rates (Song et al., 2010). However, due to the influence of fat color, the sole use of milk color for the detection of CM is not suitable (Kamphuis et al., 2010). A combination of milk color with other sensors and data, as well as processing of the data increases the performance for the detection of clinical mastitis (Kamphuis et al., 2010).

3.3.4. Milk temperature

Clinical mastitis with severity level 3 is characterized by disturbances in general condition and fever. Since the milk temperature corresponds to the body temperature (Fordham et al., 1988), measuring the milk temperature can indicate an inflammatory event in the body with a systemic immune reaction. This inline sensor technology is used in some AMS such as robots by Lely or GEA Farm Technologies. However, some physiological variations in body temperature of the cows due to ambient temperature, time of the day and days in milk (Kendall and Webster, 2009; Suthar et al., 2012), as well as some technical issues, like the distance between teat cup and temperature sensor in the milk tube of the robotic arm, milk flow and air leakage, influence the measurement of the milk temperature (Pohl et al., 2014; Fordham et al., 1987). For fever detection the milk temperature should be used with great caution (Pohl et al., 2014). As fever is a general systemic response to inflammation, high and lower grade milk temperature results can only be used in combination with other sensor data to monitor udder health.

3.3.5. Milk yield

The milk yield measurement by AMS can be carried out per quarter by flowmeters or for the overall milking yield volumetrically through measuring containers or gravimetrically through weighing devices (KTBL, 2013b). Milk yield is physiologically associated with a lot of indicators like breed, diet of the cow, age, animal welfare, milking interval etc. (Nocek and Braund, 1985; Løvendahl and Chagunda, 2011; Oltenacu and Broom, 2010; Wathes et al., 2007). During CM, as well as in other diseases such as right abomasal displacement, a sudden decrease in milk yield can be observed (Lukas et al., 2009; Fleischer et al., 2001; Lucey et al., 1986). Therefore, udder milk loss is influenced by both systemic metabolic effects and local inflammation. These local effects are related to the damage of the secretory tissue of the udder due to inflammatory reaction and the pathogen toxins (Zhao and Lacasse, 2008). However, the extent of the decrease also depends on the stage of lactation in which the mastitis occurs (Hagnestam-Nielsen et al., 2009; Lescourret and Coulon, 1994). Milk yield generally decreases one or two weeks prior to diagnosis of CM; while the greatest loss occurs immediately after diagnosis, and reaches physiological niveau after the treatment not at all or only after a longer period depending on the pathogen (Edwards and Tozer, 2004; Gröhn et al., 2004; Wilson et al., 2008). Thus, the respective pathogen, the degree of mastitis, and the

time of mastitis occurrence in the lactation are crucial for the decrease in milk due to mastitis (Heikkilä et al., 2018). In summary, a declined milk yield is a good early warning as a general sign of disease, but is not very specific for udder health (Lukas et al., 2009). Nevertheless, quarters with increased SCC show lower secretion performance (Mungube et al., 2005), so quarter-specific measurement of milk yield in combination with other parameters has potential for a good udder health parameter (Adriaens et al., 2018).

3.3.6. Milk constituents

Components of milk can be analyzed without physical or chemical modification of the milk. Main components which can be determined in laboratories but also in AMS systems are concentrations of milk fat, milk protein and lactose (King et al., 2019). Milk fat and protein concentrations of milk from healthy or mastitic udders hardly differ (Kester et al., 2015). Lactose is a stable milk constituent with low relative diurnal variation and osmotic modulating characteristics (Svennersten-Sjaunja and Pettersson, 2008). It is also strongly associated with SCC and therefore a potential biomarker for mastitis (Antanaitis et al., 2021a). Commercially used in AMS are near-infrared spectroscopy systems (NIR; Zucali et al., 2021). They are relatively cheap and do not require sample preparation while giving immediate results of the flowing milk (Pu et al., 2020). Their disadvantage is that they are somewhat less accurate than medium infrared wavelength diagnostics used in laboratories (Schmilovitz et al., 2007). The commercially used NIR system (IMA, AfiMilk, S.A.E Afikim, Israel) can determine the presence of blood but also the milk components with wavelength ranges of 350nm-1000nm (Giannuzzi et al., 2022). Increased SCC, as it can occur with mastitis, alters the milk spectrum through concomitant electrolyte, protein and lactose changes (Tsenkova et al., 2000; Forsbäck et al., 2009). Therefore, a regular calibration of the sensors before determining the milk components, including the SCC, is crucial to obtain reliable results (Fadul-Pacheco et al., 2018). Overall the use of milk analysis systems in AMS, especially for lactose, may be useful for monitoring udder health (Kester et al., 2015; Antanaitis et al., 2021a; Ebrahimie et al., 2018; Televičius et al., 2021).

3.3.7. Biosensors

The enzyme L-lactate dehydrogenase (LDH) is part of the glycolytic metabolism and is found in the cytoplasm of all cells. Due to the enzymatic reaction during IMI,

it leaks into the milk primarily from epithelial cells destroyed by the body's immune response. In this way it could be used as an indicator for mastitis (Chagunda et al., 2006b). A LDH sensor for AMS is currently commercially available. It is included in the Herd Navigator™ system from DeLaval (Tumba, Sweden) and has been field tested in some studies (Malašauskienė et al., 2019; Antanaitis et al., 2021b; Jørgensen et al., 2016). The methodology of this sensor system is the measurement of LDH activity ($\mu\text{mol}/\text{min}$ per liter) by dry-stick technology. A fixed amount of milk is placed on the indicator rod. An enzymatic reaction of LDH now begins, the intensity of the reaction can be determined by a color change by a digital camera (Jørgensen et al., 2016). Nevertheless, it is not specific to inflammation of the udder, the high concentration especially in the muscle, liver and kidney tissues can also transfer into the milk in case of inflammation of these (Antanaitis et al., 2021a). Additionally, a study found that calving causes LDH in milk to rise and remain at a high level for up to 30 days after calving (Chagunda et al., 2006b). Also influences of breed and season were found. From a sampling perspective, the use of the foremilk, which has higher LDH concentrations, appears to be advantageous for udder health monitoring (Khatun et al., 2019). However, for the detection of IMI, not fully satisfactory detection could be established, even when the above mentioned influences of e.g. season, were applied into the model (Nyman et al., 2016). Otherwise when using LDH of the foremilk there was recently found potential for early detection of IMI (Khatun et al., 2022). Also for CM detection LDH measurement provides better performance (Chagunda et al., 2006a). The use of LDH with additional data, such as SCC, also has potential to differentiate the underlying pathogens into gram positive and gram negative bacteria (Hernández-Castellano et al., 2017; Khatun et al., 2022). Overall, the use of LDH measurements provides a good assistance in identifying udder health problems, especially when extracted from the pre-strip and additional udder health data are available.

3.3.8. Activity

A dairy cow has its own daily rhythm, moving from the feeding area to water intake, cow comfort, lying areas and milking. This general moving activity can be recorded using pedometers or sensor technology in collars and is mostly used to detect oestrus and time of insemination (Mottram, 2016; Talukder et al., 2015; Grinter et al., 2019; Elischer et al., 2013). But also sick, lame or cows under certain environmental conditions, such as heat stress, show changes in the activity pattern

(King et al., 2017; Veissier et al., 2017; Ramón-Moragues et al., 2021). This can be used for mastitis detection, as cows activity may increase due to pain of CM when lying down (Fogsgaard et al., 2015; Siivonen et al., 2011). Also, cows with severe CM may show a decrease in activity to the point of recumbency (Kester et al., 2015; King et al., 2018; Stangaferro et al., 2016). Due to the different causes of changed activity and the different effects of mastitis on the activity behavior of a cow, the sole use of this sensor system for the determination of udder health warning is less suitable (Stangaferro et al., 2016). However, the combination of activity data with EC was able to improve the accuracy of predicting SCM at quarter level (Khatun et al., 2020).

In addition, a dairy cow's activity is not based solely on the distance she walks during the day. Ear tags or rumen boli can record chewing behavior or direct rumination activity of a cow (Hamilton et al., 2019). Furthermore, cows with health problems, even with CM, show a reduced ruminating activity (Fitzpatrick et al., 2013; Paudyal et al., 2018), so that these sensor showed to have potential for the detection of udder health problems (Gusterer et al., 2020; Antanaitis et al., 2022).

3.3.9. Further possible automatic milking system sensorics

In recent years, other sensors and technologies have been developed or adapted from other areas of technology for the detection of mastitis. Sensors that detect physical characteristics of milk altered by CM will most likely provide better detection results for abnormal milk (Hogeveen et al., 2010). Maasen-Franke et al. described in 2004 an algorithm trained by camera-based image analysis to detect and classify homogeneity changes. With this technique, secretory changes of mastitis, such as flakes or clots, could be differentiated well from other particles, like litter or air bubbles. Digital photo-based diagnostic tools and image recognition and processing have already been used successfully in other parts of the food industry for quality assurance (Pounds et al., 2022; Khan et al., 2021). However, despite its potential, this optical sensing technique for detection of abnormal milk is not commercially available. Other visible signs of udder or quarter inflammation, as seen in moderate mastitis, can be detected with the help of sensor technology. The heat of the tissue as an indicator of inflammation can be determined using an infrared thermal camera (Colak et al., 2008). This pictorial detection of increased inflammation-related blood flow has achieved promising results in recent studies (Hovinen et al., 2008; Metzner et al., 2014; Wang et al., 2022; Zaninelli et al.,

2018). But, the detection is critically dependent on animal hygiene, AMS pre-cleaning, and weather conditions and so on limited to clean, clearly visible udders (Sinha, 2018). With increased blood flow, more fluid enters the tissue, consequently the tissue hardens. Even for the inflammation sign of increased hardness, a sensor is developed that can potentially detect inflammation of the udder by measuring pressure at certain points of the udder (Bertulat et al., 2012; Rees et al., 2017). However, this sensor is also highly dependent on the hygiene status of the udder.

3.3.10. Mastitis detection models

A key advantage of AMS is the large amount of data that is recorded daily at herd, cow and quarter level. These data allow a good insight into the production capacity and health of an animal. Alerts on udder health problems are presented to farmers on lists and reports on their end device, but show deficits in terms of low specificity (Hovinen and Pyörälä, 2011). Since mastitis is not only manifested by changes in milk, but also affects the cow's physiology and behavior, combining different sensor data is the most modern approach to improve mastitis detection (Hogeveen et al., 2010; Khatun et al., 2018). Combining data using models and algorithms to generate udder health warning lists are commercially available and used in practice, but these have not been sufficiently publicly validated (King and DeVries, 2018). Several studies have been conducted over the past decade to improve mastitis detection performance by AMS using specific algorithms and models. However, these studies are difficult to compare with each other due to different study designs, methodology, used definitions of mastitis (Rutten et al., 2013). The studies included multi-sensor based approaches such as infection level (Højsgaard and Friggens, 2010), decision tree-based methods (Kamphuis et al., 2010), fuzzy logic algorithm (Kamphuis et al., 2008b; Cavero et al., 2006; Kramer et al., 2009), naive Bayesian networks (Steenefeld et al., 2010; Jensen et al., 2016), moving averages or thresholds (Claycomb et al., 2009; Khatun et al., 2017; Mollenhorst et al., 2010), logistic mixed models (Khatun et al., 2018; Penry et al., 2017), multilayer perceptron models (Anglart et al., 2021) or recurrent neural networks (Cavero et al., 2008; Naqvi et al., 2022). Overall, combining some data in different models improved SN and SP of the mastitis detection, although SP still seems unsatisfactory and in need of improvement. However, the search for a mastitis detection method, which covers all aspects of the disease, legal regulations and preferences of farmers, continues. Also the need for an easy-to-interpret detection

system of mastitis for the farmer remains.

4. Automatic milking system operator

While AMS have been the subject of a large number of studies in recent years, the role of users has hardly been investigated although many aspects of the automated milking are still under the ultimate responsibility and the decision-making of humans. The few studies that have been conducted on the human role in AMS farms focus, for example, on aspects such as purchase motivations (Hogeveen et al., 2004), effects on work habits (Butler et al., 2012; Schewe and Stuart, 2015), perceptions of transition impacts (Tse et al., 2017; Wildridge et al., 2020), additional sensors likely used on farms (Steenefeld and Hogeveen, 2015; Abeni et al., 2019), preferences for animal health warnings (Mollenhorst et al., 2012), and CM treatment decisions (Deng et al., 2020). The human decision making is especially relevant when it comes to assessing and ensuring the health of the udder. On this aspect, data on mastitis diagnostic management of dairy farmers using AMS are still needed. These are of great importance in order to detect possible weaknesses in this area and to work out improvement measures.

5. Objectives

The aim of this thesis is to reveal the roles of milking robots and humans in clinical mastitis detection on Bavarian dairy farms. For this purpose, the following four study objectives were developed:

First, to determine the sensitivity and specificity of clinical mastitis detection by AMSs from the four most common manufacturers on Bavarian dairy farms.

Second, to identify parameters among those routinely collected by the AMS at cow level and originating from the monthly testing of the regional Dairy Herd Improvement Association, that could improve the sensitivity and specificity of clinical mastitis detection.

Third, to evaluate management practices of the farmers for the detection of clinical mastitis in Bavarian AMS farms through an online survey.

Fourth, to present farmers' personal assessment of their work with the milking robot and the performance of mastitis detection of their AMS.

III. PUBLICATIONS

1. Sensitivity and Specificity for the Detection of Clinical Mastitis by Automatic Milking Systems in Bavarian Dairy Herds

Bausewein M, Mansfeld R, Doherr MG, Harms J, Sorge US.

“Sensitivity and Specificity for the Detection of Clinical Mastitis by Automatic Milking Systems in Bavarian Dairy Herds”

Animals. 2022; 12(16):2131. <https://doi.org/10.3390/ani12162131>



Article

Sensitivity and Specificity for the Detection of Clinical Mastitis by Automatic Milking Systems in Bavarian Dairy Herds

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Simple Summary: This cross-sectional study assessed the sensitivity and specificity of clinical mastitis detection by automated milking systems on Bavarian dairy herds in southern Germany. Clinical mastitis affects animal health and food safety, and therefore, its detection is an important task of any automatic milking system. Different manufacturers have different approaches to detecting clinical mastitis, with detection rates (sensitivity) ranging between 31% and 78% and correct rejection rate (specificity) between 79% and 97%. In multivariable models, some cow-level factors were shown to influence these rates.

Abstract: In automatic milking systems (AMSs), the detection of clinical mastitis (CM) and the subsequent separation of abnormal milk should be reliably performed by commercial AMSs. Therefore, the objectives of this cross-sectional study were (1) to determine the sensitivity (SN) and specificity (SP) of CM detection of AMS by the four most common manufacturers in Bavarian dairy farms, and (2) to identify routinely collected cow data (AMS and monthly test day data of the regional Dairy Herd Improvement Association (DHIA)) that could improve the SN and SP of clinical mastitis detection. Bavarian dairy farms with AMS from the manufacturers DeLaval, GEA Farm Technologies, Lely, and Lemmer-Fullwood were recruited with the aim of sampling at least 40 cows with clinical mastitis per AMS manufacturer in addition to clinically healthy ones. During a single farm visit, cow-level milking information was first electronically extracted from each AMS and then all lactating cows examined for their udder health status in the barn. Clinical mastitis was defined as at least the presence of visibly abnormal milk. In addition, available DHIA test results from the previous six months were collected. None of the manufacturers provided a definition for clinical mastitis (i.e., visually abnormal milk), therefore, the SN and SP of AMS warning lists for udder health were assessed for each manufacturer individually, based on the clinical evaluation results. Generalized linear mixed models (GLMMs) with herd as random effect were used to determine the potential influence of routinely recorded parameters on SN and SP. A total of 7411 cows on 114 farms were assessed; of these, 7096 cows could be matched to AMS data and were included in the analysis. The prevalence of clinical mastitis was 3.4% (239 cows). When considering the 95% confidence interval (95% CI), all but one manufacturer achieved the minimum SN limit of >80%: DeLaval (SN: 61.4% (95% CI: 49.0%–72.8%)), GEA (75.9% (62.4%–86.5%)), Lely (78.2% (67.4%–86.8%)), and Lemmer-Fullwood (67.6% (50.2%–82.0%)). However, none of the evaluated AMSs achieved the minimum SP limit of 99%: DeLaval (SP: 89.3% (95% CI: 87.7%–90.7%)), GEA (79.2% (77.1%–81.2%)), Lely (86.2% (84.6%–87.7%)), and Lemmer-Fullwood (92.2% (90.8%–93.5%)). All AMS manufacturers' robots showed an association of SP with cow classification based on somatic cell count (SCC) measurement from the last two DHIA test results: cows that were above the threshold of 100,000 cells/mL for subclinical mastitis on both test days had lower chances of being classified as healthy by the AMS compared to cows that were below the threshold. In conclusion, the detection of clinical mastitis cases was satisfactory across AMS manufacturers. However, the low SP will lead to unnecessarily discarded milk and increased



Citation: Bausewein, M.; Mansfeld, R.; Doherr, M.G.; Harms, J.; Sorge, U.S. Sensitivity and Specificity for the Detection of Clinical Mastitis by Automatic Milking Systems in Bavarian Dairy Herds. *Animals* **2022**, *12*, 2131. <https://doi.org/10.3390/ani12162131>

Academic Editor: George F. W. Haenlein

Received: 13 July 2022

Accepted: 14 August 2022

Published: 19 August 2022

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workload to assess potentially false-positive mastitis cases. Based on the results of our study, farmers must evaluate all available data (test day data, AMS data, and daily assessment of their cows in the barn) to make decisions about individual cows and to ultimately ensure animal welfare, food quality, and the economic viability of their farm.

Keywords: dairy cow; automatic milking system; clinical mastitis detection

1. Introduction

In the last decades, advances in technology and automation have impacted many aspects of modern dairy farming [1]. The promise of reduced workload and more flexible work hours as a quality-of-life benefit is encouraging more and more farmers to switch to an automatic milking system (AMS), despite an initially higher economic burden [2,3]. In Bavaria, a southern region of Germany, the number of AMS farms has more than quadrupled in the last ten years [4].

Regardless of the milking system, mastitis remains a frequent and costly disease on dairy farms [5–7]. In its clinical manifestation, this inflammation of the udder often results in visible milk changes such as flakes, clots, pus, or watery milk [8]. As abnormal milk is unfit for human consumption, milk producers are required, according to EU Regulation 853/2004, to assess the milk organoleptically or with an equivalent method at each milking [9]. Besides the implications for food safety, the accurate detection of clinical mastitis (CM) allows targeted treatments of sick animals and is therefore essential to ensure animal welfare [10]. Unlike conventional milking systems, the inspection, assessment and, if necessary, the decision to separate milk has to be done automatically by the AMS [11]. For this reason, AMSs are equipped with various sensor systems to detect abnormal milk. These raw sensor data are analyzed and summarized as warning lists for the farmer in the respective herd management software [12]. However, no direct alert is given for clinical mastitis; the list will include a more general indication of a potential udder health problem. Different approaches are currently used to detect and process changes in the milk. The commercially available sensor systems provide, for example, information on electrical conductivity (EC), somatic cell count (SCC), milk yield (MY), and milk color as well as inflammation-indicating enzymes.

The most common sensor system in AMSs is the measurement of milk EC [13,14]. During CM, changes in milk ion concentrations can be observed due to increased vascular permeability caused by the inflammatory response [15,16]. However, fluctuations of ion concentrations also occur in the presence of non-disease-related influences, such as lactational period [17,18]. This may lead to inadequate detection of CM based on EC alone [19,20]. Another sensor technique gaining importance in AMSs is the in-line measurement of SCC. These sensor systems use a defined milk sample volume and can determine SCC by either automated counting of stained, fluorescent nuclei [21] or by automated CMT [22]. Despite some non-infectious-related influences on SCC, such as breed [23,24], it is a commonly used gold standard for detecting udder health problems [13]. In addition to a drop in MY due to CM [25,26], the assessment of milk color, measured by light reflectance or transmission, is another means to detect abnormal milk [27,28]. However, this information alone is not suitable for CM detection due its dependence on milk fat content [29]. Therefore, in order to improve the monitoring of udder health, new sensors have been introduced in recent years, such as the measurement of inflammatory enzymes such as L-lactate dehydrogenase [30,31], or a physical sensor such as infrared thermography that determines the temperature of an inflamed udder [32]. The combination of sensor data can lead to improvements in CM detection [33–35]. While the performance of mastitis detection in AMSs has been widely studied in recent years, the detection of clinical mastitis of different AMS types has hardly been considered in the field. Different approaches, study populations, and gold standard definitions further resulted in a variety of perfor-

mance indices for mastitis detection in AMSs [28,36]. In the context of animal welfare and food quality assurance, however, a satisfactory detection of CM irrespective of the system is essential.

Therefore, the first objective of this study was to determine the sensitivity (SN) and specificity (SP) of CM detection by AMSs from the four most common manufacturers in Bavarian dairy farms. The second objective was to identify parameters among those (i) routinely collected by the AMS at cow level and (ii) originating from the monthly testing of the regional Dairy Herd Improvement Association (DHIA) that, when incorporated into a multivariable model, could improve the SN and SP of clinical mastitis detection.

2. Materials and Methods

2.1. Study Design and Herd Selection

For our study, the free web-based sample size calculator EpiTools formulas for estimating a single proportion with a given precision were used to calculate the sample size [37]. To estimate an assumed specificity of 99% with a precision of $\pm 1\%$ and a confidence of 95%, at least 321 healthy cows from a finite population ($N = 10,000$ cows) had to be included in the sample. Our cross-sectional design did not allow selection of cows with regards to clinical udder health status. Therefore, the actual sample size was based on the expected number of clinical mastitis cases, which served as the gold standard for sensitivity estimation. We assumed that, on average, 2% of cows in a herd would have CM at any given point in time. To assess whether an 80% sensitivity was reached with assumed true prevalence of 2% and desired precision of $\pm 1\%$, approximately 2000 cows would need to be screened per AMS manufacturer to obtain 40 cows with CM. Given that one milking robot milks on average about 60 cows [38], at least 30 AMS per manufacturer were needed. The four most common AMS manufacturers in Bavaria, (in alphabetical order) DeLaval (DeLaval International AB, Tumba, Sweden), GEA Farm Technologies (GEA Farm Technologies, Bönen, Germany), Lely (Lely Industries N.V., Maassluis, The Netherlands), and Lemmer-Fullwood (Lemmer-Fullwood GmbH, Lohmar, Germany) were contacted. They provided a list of potential herds from which 30 AMSs per manufacturer were recruited. The inclusion criteria were that the selected herds maintained their AMSs regularly and preferably participated in the monthly testing by the Bavarian regional DHIA (Landeskuratorium der Erzeugerringe für tierische Veredelung in Bayern e.V., München, Germany).

2.2. Data Collection

Each farm was visited once between September 2019 and August 2020 by trained and specifically instructed udder health technicians of the Bavarian Animal Health Services (TGD). On each farm, first the AMS data from the herd management system was downloaded in accordance with manufacturer guidelines. Then the udder health of all lactating cows milked by the AMS was assessed in the barn. For this purpose, cows were fixed in head locks and their udder was examined for pathological changes such as redness, swelling, or hardness visually and by palpation. Foremilk from each quarter was collected on a CMT scoring plate. Pathological changes as well as the quality of abnormal milk (watery, small or large flakes etc.) were recorded and a CMT was performed. In addition, aseptic quarter milk samples were collected, and the teat end condition (score 1–4; highest score recorded per cow [39]) as well as the cow's hygiene (score 1–4 [40]) were assessed at cow level. After the sampling, the herd management strategies, farm structure, and AMS-specific data such as cleaning, disinfection, etc. were recorded (checklist available upon request). The DHIA provided available data for the last six monthly performed test days. These included date of milk recording, test-day milk yields (kg), fat (%), protein (%), and urea concentration (ppm), as well as SCC measurements (cells/mL). The data and sample collection did not require ethical approval under German animal protection law.

2.3. Gold Standard Definition

Based on the findings from clinical examination of the udder, CM was recorded as grade 1 (abnormal milk with change in character, like watery or bloody, and/or the occurrence of flakes, clots, or pus of at least one quarter), grade 2 (abnormal milk in addition to local signs of inflammation of the quarter), or grade 3 (signs of grades 1 and 2 in addition to systemic signs, e.g., off feed, fever) in accord with Bradley and Green [41]. The gold-standard definition of CM in our study included grades 1–3 on a cow level, as the milk of an affected cow would have to be discarded. Cows were classified as having CM (1) or not (0).

2.4. AMS Data

Commonly, the AMS warning lists about udder health include only cows that the system flagged for inconsistencies in their parameters. However, for this study we needed the information on all milking cows of the respective herd. Therefore, a full backup file of the AMS data was extracted on all farms and original lists were generated with the help of the manufacturers in two ways: The software support teams of DeLaval and GEA Farm Technologies helped us to extract the needed lists directly out of backup files with the respective AMS herd management software. Lely and Lemmer-Fullwood created lists for this study prior to the herd visit that expanded the commonly used warning lists to include healthy cows as well. These lists as well as a full backup were electronically saved at the farm visit. The list names and brief descriptions are shown in Table 1.

2.5. Clinical Mastitis Alert

None of the AMS manufacturers provided a definition of CM. Instead they gave reference values for potential udder health problems. Therefore, after consultation with the manufacturers, the following markers were used as alerts for CM for this study:

DeLaval

The mastitis detection index (MDi; DeLaval International AB, Tumba, Sweden) was used as a CM alert for DeLaval. The MDi is a mathematically generated index that considers EC and blood presence, which are both measured at quarter level, and milking interval. It uses values between 1 and 6 [42]. According to DeLaval, cows with an MDi ≥ 1.4 should be checked for udder health problems. An MDi of ≥ 2.0 is considered an acute warning for an udder health problem.

GEA Farm Technologies

The cow's listing in the "AMS_udder_health_monitoring" list was used as the CM alert. This list is divided into three subgroups: "List1", which displays cows that have a deviation in EC value between the quarter with the highest average EC value and the quarter with the lowest average EC value in the last four milkings. The lowest EC quarter value must be greater than 400 (manufacturer's own unitless measurement). The factory setting for the deviation from which cows are displayed in List1 is $\geq 30\%$. "List2", which displays cows with an EC deviation within a quarter of the default setting $\geq 110\%$. This list is identical to the "AMS_Increased_conductivity" list, which is checked daily. The third subgroup "Acute_Udder_Health_warnings" summarized cows which have been flagged on both "List1" and "List2".

Lely

To check udder health, the original lists Report12 and Report23 were modified to show all milking cows and highlight those cows that were normally shown on these alert lists. Report12 ("Action_list") displays cows with a new indication for mastitis within the last 24 h, while cows remain on Report23 ("Monitor_list") until a milking without mastitis marker occurred. Factory settings for these reports were a deviation of daily milk yield (MY) of 4.0 kg or 20% and/or a decrease of daily milk production of more than 7 kg. A 20% deviation of the EC from either the last milking and/or from the 3-day average value, as well as an absolute EC threshold of 100 (manufacturer's own value without unit) were

used as indicators of udder health problems. Milk temperature changes above a deviation factor of 2.0 as well as above the SCC threshold of 500, measured by Lely's MQC-C system, were also considered an indication for udder health problems. Marker thresholds could be customized by farmers, but they were asked to leave them in the default settings for this study.

Lemmer-Fullwood

The 4QCM-System gives an indication of cows suspected of mastitis based on a quarter-level EC deviation of at least 35% from the 10-day average and/or a threshold value of >7.5 mmho (manufacturer's own unit) for the current milking. This indication was set as the CM alert. The farmers were able to adjust these thresholds, but they were asked to leave them in the default settings for this study.

Table 1. Udder health lists of each manufacturer's herd-management software used for this study.

AMS/Software	Lists	Content and Explanation
DeLaval/ DelPro Farm Manager 5.5	cow_monitoring ¹	Sensor (e.g., EC ^{2,*} , MY ^{3,*} , blood occurrence *, etc.) and cow data (e.g., MDi ^{4,**} , MI ⁵ , DIM ⁶ etc.).
	Milking_data_last_30_days ¹	Sensor and cow data of the last 30 days.
GEA Farm Technologies/Dairy Plan C21	Daily_checked_lists ¹	Summary of lists to be checked daily in the program. Indicates whether cows appear on these lists or not (1/0).
	AMS_udder_health_monitoring-List1	Displays cows with a deviation in EC ² value between the quarter with the highest average EC ² value and the quarter with the lowest average EC ² value in the last 4 milkings.
	AMS_udder_health_monitoring-List2	Displays cows with an EC ² deviation within a quarter.
	AMS_udder_health_monitoring-Acute_warnings	Summarized cows which have been flagged on both List1 and List2.
	AMS_increased_conductivity	Displays cows with an EC deviation within a quarter.
	Mrobot_milk_decline	Displays cows with a milk decline.
	Mrobot_to_be_milked	Displays cows overdue for milking.
	Herd_status_current_last_milking ¹	Sensor (e.g., EC ^{2,*} , MY ^{3,*} , blood occurrence *, MT ^{7,*} etc.), and cow data (e.g., MI ⁵ , DIM ⁶ etc.).
	Milking_data_for_the_last_10_days ¹	Sensor and cow data of the last 10 days.
QuarterCellCount_alert	Alert list using SCC ^{8,*} .	
Lely/ T4C-Time for cows	Dailymilkproduction ¹⁰	Sensor (e.g., MYD ^{9,**} , milk fat **, milk protein **, etc.) and cow data (e.g., feed intake, DIM ⁶ , etc.) of current milking.
	Milkings_last_7_days ¹⁰	Sensor (e.g., EC ^{2,*} , milk color *) and cow data (e.g., DIM ⁶ etc.) of the last seven days.
	Action_list ^{10,11}	Displays cows and their sensor data with a new indication such as MYD ^{9,**} , EC ^{2,*} , MT ^{7,**} , SCC ^{8,**} for 24 h on this list.
	Monitor_list ^{10,11}	Displays cows until a milking without mastitis indicator (MYD ⁹ , EC ^{2,*} , MT ^{7,**} , SCC ^{8,**}) occurred.
Lemmer-Fullwood/ Chrystal Fusion	Control_report_10_days ¹⁰	Sensor (e.g., milk protein, milk fat, lactose, etc.) and cow (e.g., DIM, MI) data of the last 10 days including an alert for suspected mastitis, based on EC ^{2,*} .
	Kick_off ¹⁰	Kick-off event (yes/no) of the teat cups per quarter of the last 10 days.
	4qcm_10_days ¹⁰	Displays data of EC ^{2,*} at quarter level for each milking of the last 10 days.
	Control_report_milking ¹⁰	Displays cows for udder health monitoring.

¹ Study lists created in cooperation with employees of the respective manufacturers on the basis of herd management program lists; ² EC = Electrical conductivity of milk (manufacturer's internal unit); ³ Milk yield (kg) ⁴ MDi = Mastitis detections index. The MDi is a mathematically generated index that considers EC and blood presence (both measured at quarter level) as well as milking interval; ⁵ MI = Milk interval; ⁶ DIM = days in milk; ⁷ MT = milk temperature, (°C); ⁸ SCC = somatic cell count, (manufacturer's internal unit); ⁹ MYD = Milk yield per day, (kg); ¹⁰ Created by the milking equipment service team for this study based on originally used lists; ¹¹ Original lists, modified by software service staff of the companies to show all lactating cows, but marked cows which were originally indicated on each list; * = at quarter level; ** = at cow level.

2.6. DHIA Data

Missing monthly test day data, e.g., during the dry period, were excluded. Then, eight new variables were generated to represent the changes in the cow's SCC (Table 2). The DHIA data were then aggregated at the most current test day.

Table 2. Overview of raw and generated cow test day data from the regional Dairy Herd Improvement Association (DHIA) for this study on AMS system accuracy in Bavarian dairy herds.

Source	Variable
Test day data	Cow identification
	Date of birth
	Breed
	Lactation number
	Days in milk at the test day
	Date of monthly test day
	Milk yield (kg)
	Fat (%)
	Protein (%)
	Urea concentration (ppm)
	SCC (cells/mL)
Generated ¹	Test day with SCC \geq 700,000 cells/mL (1/0)
	Number of test days with SCC \geq 700,000 cells/mL (<i>n</i>)
	Test day with SCC \geq 400,000 cells/mL (1/0)
	Number of test days with SCC \geq 400,000 cells/mL (<i>n</i>)
	Number of missing test day data (<i>n</i>)
	Udder health status (categorization, based on two subsequent test days in that lactation):
	chronic: two subsequent tests with >100,000 cells/mL
	new IMI ² : previous SCC < 100,000 and current SCC > 100,000 cells/mL
	cured: previous SCC > 100,000 and current SCC < 100,000 cells/mL
	healthy: both tests < 100,000 cells/mL
no current test data: only data of 1 test day available	
no DHIA data available	

¹ New variables were generated after the data for monthly test days from cows with DIM \leq 5 (no measurement) and a dry period or both were excluded; ² IMI: Intramammary infection.

2.7. Statistical Analysis

Data arrangement and analysis was completed in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The respective manufacturers' lists were merged at the last recorded milking. The AMS data lists were merged with the clinical observations for each cow and corresponding DHIA data. This resulted in one data set for each AMS manufacturer. Cows that were not clearly identifiable, <3 DIM, not milked for >24 h prior to backup, not milked by the AMS on the quarter that showed clinical signs of mastitis on examination, and cows that had missing CM alert values were excluded from the data set (Table 3). Data sets for each AMS manufacturer containing all available variables are available upon request. Using the abovementioned alerts for CM, sensitivity (SN) and specificity (SP) were assessed for each manufacturer individually. Binomial proportions were derived using the statement PROC FREQ with the method BINOMIAL and option EXACT. Target proportions were set for SN ($p = 0.8$) and SP ($p = 0.99$), and the alpha level of statistical significance was set to $\alpha = 0.05$.

For the second objective, two generalized logistic mixed models were used for each manufacturer to identify factors associated with SN and SP, respectively. To represent the populations for each model, observations were divided into two groups according to the occurrence of CM (as diagnosed by the technicians during the on-site visit). Thus, the SN models were run on a dataset that included only CM-positive cows, and the SP models used only data from healthy CM-negative, cows. The outcome, i.e., the binary dependent variable (coded 0/1) for the SE model was defined as true positive CM detection (1), and for the SP model as true negative CM detection (1). In a first step, quarterly individual

measurements were scaled to cow level and analyzed for associations with CM status using PROC NPAR1WAY. To identify potential predictor variables for SN and SP, respectively, continuous variables and categorical variables were screened by PROC NPAR1WAY and by PROC FREQ, respectively. They were potential predictors of the multivariable model if $p < 0.25$. To avoid collinearity of the possible predictors, variables were screened using PROC CORR SPEARMAN and PROC FREQ AGREE for continuous and categorical predictors, respectively. Variables with a Spearman correlation coefficient or kappa > 0.6 were excluded. Prior to the multivariable model approach, all potential predictors were tested individually for their association with the dependent variable in a mixed logistic regression (PROC GLIMMIX) that included herd as a random effect. In order to achieve a better fit, some variables were subjected to a transformation or categorization process, e.g., the grouping of the days in milk (DIM) into <60 d, 60–120 d, >120 d. The final generalized logistic mixed model was performed with PROC GLIMMIX with option IC = Q for computation of model fit information criteria and herd as random effect. Using a manual stepwise elimination procedure, the variable with the highest p -value was excluded from the model after each run until all remaining variables had $p \leq 0.05$. Then, the excluded variables were individually reentered into the model in the same order in which they were excluded to test for confounding. If a change in regression parameter estimate of $\geq 20\%$ occurred in other variables, then that variable remained in the model as a potential confounder. Interactions between predictors could not be considered due to the large number of independent variables with missing biological connections. For all models, goodness of fit was assessed using the -2 Res Log Pseudo-Likelihood.

Table 3. Overview of data-cleaning process in a study on AMS system accuracy in Bavarian dairy herds.

	DeLaval	GEA	Lely	Lemmer-Fullwood	Overall
Study herds, n	27	29	31	27	114
AMS data					
Backup at farm visit, n (restored ¹)	20 (7)	29	31	26 ²	113
Last milking data, n	2047	1721	2247	1974	7989
Evaluated cows at farm visit, n	1904	1664	2152	1691	7411
Cows excluded due to, n					
Incorrect identification	13	8	21	31	73
Not matching with AMS data	12	6	16	68	102
DIM < 3 d	22	21	18	11	72
>24 h since last milking	11	9	11	6	37
3-teater cows, i.e., quarter with CM ³ not milked by AMS	15	4	10	8	37
No alert information available	-	-	-	22	22
Cows in final statistical analysis, n	1831	1616	2076	1545	7090
Additional DHIA ⁴ Data	1665	1462	1879	1534	6540
Last three test day data available	1517	1309	1636	1425	5887
Only last test day data available	99	107	156	45	407
No test day data available	166	154	197	33	550
Cows with CM ³ , n (affected quarters, n)	70	54	78	37	239
Grade 1—mild: abnormal milk	60 (62)	52 (59)	69 (76)	31 (42)	212 (239)
Grade 2—medium: abnormal milk and/or swollen quarter	9 (10)	2 (2)	8 (8)	5 (5)	24 (25)
Grade 3—Severe: grade 1 or 2 with systemic signs	1	-	1	1	3

¹ AMS Data restored of the automated daily backups from 1 or 2 am; ² AMS data of one Lemmer-Fullwood herd could not be restored; ³ CM = clinical mastitis; ⁴ DHIA = regional Dairy Herd Improvement Association.

3. Results

Between September 2019 and August 2020, 114 dairy farms with a total of 126 AMSs were visited once by a team of two technicians from the Bavarian Animal Health Services. In total, 23 trained technicians were involved in the data-collection process. The characteristics of participating herds has been summarized in Table 4.

Table 4. Overview of the characteristics of participating herds summarized per manufacturer. Farm visits between September 2019 and August 2020 for a study on AMS system accuracy in Bavarian dairy herds. Unless otherwise stated, the median (25th–75th percentile) is reported.

Characteristic		DeLaval	GEA	Lely	Lemmer-Fullwood	Overall
Participating herds, <i>n</i>		27	29	31	27	114
Number of AMSs, <i>n</i>		31	30	35	30	126
Year of AMS installation,	median	2014	2018	2015	2017	2017
	(min–max)	(2007–2020)	(2016–2020)	(2009–2019)	(2011–2019)	(2007–2020)
Herd size ¹	mean, ±SEM	71 ± 4.8	57 ± 3.1	69 ± 4.3	63 ± 2.9	65 ± 1.9
	(min–max)	(40–139)	(28–106)	(35–127)	(31–100)	(28–139)
Herd average milk yield ² , kg		8424	7700	8949	8400	8525
		(7905–8875)	(7126–8573)	(8515–9325)	(7981–9106)	(7700–9135)
Bulk tank ³ (×10 ³ /mL)	Somatic cells/mL	176	126	202	130	155
		(140–240)	(103–154)	(155–241)	(101–178)	(124–210)
	Bacterial count, cfu/mL	13	17	12	17	15
		(10–19)	(11–25)	(9–17)	(13–26)	(10–21)
Clinical mastitis prevalence ⁴ , %		4.1	2.7	3.8	2.9	3.4
Herds without clinical mastitis, <i>n</i>		3	4	3	6	16
Operating structure, % herds	Conventional	85	86	97	93	90
	Organic	15	14	3	7	10
	DHIA ⁵ member	96	97	97	100	
Breed, % herds	Simmental	19	86	58	82	61
	Mixed	37	7	23	11	19
	Brown Swiss	26	3	10	7	11
	Other (incl. Holstein Friesian)	19	4	10	-	8
Period of the farm visits (2019–2020)		Oct–Aug	Apr–Aug	Sep–Mar	Feb–Aug	Sep–Aug

¹ Number of lactating cows, ² Result of herd performance (365 d)/number of tested cows, ³ Data of last available bulk tank analysis, ⁴ Median intra-herd prevalence of herds with cows with clinical mastitis, ⁵ DHIA = Dairy herd improvement association is the Landeskuratorium der Erzeugerringe für tierische Veredelung in Bayern e.V.

3.1. Sensitivity and Specificity of CM Alerts

DeLaval

For 7 of the 27 DeLaval herds, only data from the daily routine backup at 1 or 2 am could be obtained. Exclusion of these seven herds did not change the results, and therefore their data remained in the statistical analysis. Data for 1831 cows, including 70 CM cases, were available for the analysis (Table 3). The results of SN and SP for different MDi thresholds are shown in Table 5. The highest value for SN and SP was reached with an MDi threshold of 1.4 (SN: 61.4%, 95% CI: 49–72.8%; SP: 89.3%, 95% CI: 87.7–90.7).

GEA Farm Technologies

All backup data for the herds milking with a GEA AMS (*n* = 29) could be used for final analysis. This included data for 1616 cows, of which 54 cows were diagnosed with CM (Table 3). SN and SP of the CM alert of the AMS from the list “AMS_udder_health_monitoring” is shown in Table 5. The subgroup “List1” resulted in the highest SN of 75.9% (95% CI: 62.4–86.5) and SP of 79.2% (95% CI: 77.1–81.2).

Lely

The data from the AMS herd programs of all Lely study herds (*n* = 31), including 2076 cows (78 CM cases), were used in the analysis (Table 3). The SN and SP of the udder health monitoring lists provided by Lely are shown in Table 5. Use of the Lely “Monitor_list” resulted in the highest SN of 78.2% (95% CI: 67.4–86.7) with an SP of 86.2% (95% CI: 93.8–95.8).

Table 5. Sensitivity and specificity of clinical mastitis (CM) alerts for AMSs from DeLaval, GEA, Lely and Lemmer-Fullwood.

AMS	Cows, <i>n</i>	CM, <i>n</i> Cases	AMS Alert Used	Sensitivity, %	95% CI, % ¹	Specificity, %	95% CI, % ¹
DeLaval	1831	70	MDi ²	≥1.4	61.4	49.0–72.8	89.3
				≥2.0	31.4	20.9–43.6	97.2
GEA	1616	54	AMS_udder_health_monitoring ³				
			List1 ⁴	75.9	62.4–86.5	79.2	77.1–81.2
			List2 ⁵	48.2	34.3–62.2	93.5	92.1–94.6
			Acute_Udder_Health_warnings ⁶	38.9	25.9–53.1	94.9	93.7–95.9
Lely	2076	78	Monitor_list ^{7,9}	78.2	67.4–86.7	86.2	84.6–87.7
			Action_list ^{7,9}	28.2	18.6–39.5	94.9	93.8–95.8
Lemmer-Fullwood	1545	37	4QCM alert ^{8,9}	67.6	50.2–82.0	92.2	90.8–93.5

¹ Exact confidence interval, ² MDi = Mastitis detections index. The MDi is a mathematically generated index that considers EC and blood presence, which are both measured at quarter level and milking interval. It uses values between 1 and 6; ³ CM alert list based on deviations in EC. This list is divided into three subgroups: List1, List2 and Acute_Udder_Health_warnings; ⁴ List1: displays cows that have a deviation of ≥30% in EC value between the quarter with the highest average EC value and the quarter with the lowest average EC value in the last 4 milkings. The lowest value must be greater than 400 (manufacturer's own unitless measurement); ⁵ List2: displays cows with an EC deviation within a quarter of the default setting of ≥110%; ⁶ Acute_Udder_Health_warnings: displays cows which are indicated on List1 and List2; ⁷ Two udder health lists, modified from the pre-installed lists Report12 and Report23 in the system to show all cows, but those cows originally shown on these lists were marked. Action_list, e.g., Report12, displays cows with a new indication for 24 h on this list, while on Monitor_list, e.g., Report23, cows remain on this list until a milking without indication occurs. Factory settings of the indication limits for these reports are: milk yield deviation of daily milk production of 4.0 kg or 20%; decrease of daily milk production of more than 7 kg; EC deviation from last milking of 20%, EC deviation from the 3-day average of 20%; absolute EC threshold of 100; milk temperature above a deviation factor of 2.0%; exceeding the SCC threshold of 500, measured by the MQC-C system; ⁸ 4QCM system (quarter conductivity measurement system): measures the EC at the quarter level and with standard limits for the conductivity per quarter; deviation by 35% from the 10-day average and threshold value of the measured value of >7.5 mmo. ⁹ CM alert thresholds could be customized by farmers.

Lemmer-Fullwood

Of the 27 herds provided by Lemmer-Fullwood, one backup file could not be used due to unrecoverable data. The associated herd was removed from the data set, resulting in available data for 1545 cows from 26 herds, with 37 CM cases, for statistical analysis (Table 3). In addition, some variables, such as milk lactose, milk fat, and milk protein measured by the AMS from the specifically created list “control_report_milking_10 days” could not be assigned without doubt to their respective given meaning, so that these variables were excluded from analysis. The SN and SP of the list provided by Lemmer-Fullwood for udder health monitoring “4QCM_10_days” are shown in Table 5. The 4QCM system achieved an SN of 67.6% (95% CI: 50.2–82) and an SP of 92.2% (95% CI: 90.8–93.5) for the detection of CM.

3.2. Sensitivity and Specificity Predictors

SN Predictors

Table 6 shows these models and their predictors by manufacturer. For DeLaval and GEA Farm Technologies, only EC could be identified as a factor that improved SN for CM detection. For Lely, with every 1 log increase in log-transformed SCC (logSCC) from Lely's MQC-C system the odds of correctly identifying a sick animal increased (OR: 4.1; *p* = 0.002). No additional SN predictor was identified for Lemmer-Fullwood.

Table 6. Factors improving the sensitivity of clinical mastitis detection (outcome: true positive) in a cow-level multivariable logistic regression analysis including automatically recorded data from AMS and DHIA test days. Only cows with clinical mastitis were used and herd was included as random effect.

AMS	CM, n	AMS Alert	Predictor	β ¹	SEM	Odds Ratio	95% CI	p-Value
DeLaval	70	MDi ² \geq 1.4	Intercept	−3.56	1.76			0.05
			EC of current milking	0.22	0.10	1.24	1.03–1.51	0.03
GEA	54	List 1 ³	Intercept	−4.43	1.77			0.02
			Δ QEC ⁴	0.03	0.01	1.03	1.01–1.05	0.01
Lely	78	Monitor list ⁵	Intercept	−7.56	2.62			0.01
			LogSCC ⁶	1.41	0.42	4.10	1.75–9.52	<0.01
Lemmer-Fullwood	37	4QCM ⁷ Alert	-	-	-	-	-	-

¹ β = Regression coefficient; ² MDi = Mastitis detections index. The MDi is a mathematically generated index that considers EC and blood presence, which are both measured at quarter level and milking interval. It uses values between 1 and 6; ³ List1: a warning list for udder health problems and displays cows that have a deviation of \geq 30% in EC value between the quarter with the highest average EC value and the quarter with the lowest average EC value in the last four milkings. The lowest value must be greater than 400 (manufacturer's own unitless measurement); ⁴ Δ QEC = Difference of the highest to lowest quarter EC measurement; ⁵ Monitor list, e.g., Report23, is a warning list for udder health problems and displays cows until a milking without indication occurs. Factory settings of the indication limits for these reports are: milk yield deviation of daily milk production of 4.0 kg or 20%; decrease of daily milk production of more than 7 kg; EC deviation from last milking of 20%, EC deviation from the 3-day average of 20%; absolute EC threshold of 100; milk temperature above a deviation factor of 2.0%; exceeding the SCC threshold of 500, measured by the MQC-C system; ⁶ SCC determined by the Lely MQC-C System, log transformed; ⁷ 4QCM system (quarter conductivity measurement system): measures the EC at the quarter level and with standard limits for the conductivity per quarter; deviation by 35% from the 10-day average and threshold value of the measured value of >7.5 mmho (manufacturer-specific unit).

SP Predictors

Table 7 shows the four models and their predictors associated with the correct negative detection of the specified alerts from the four manufacturers. Among other predictors, the odds of being correctly classified as a healthy cow decreased with increasing milking interval (MIH, in hours) for DeLaval (OR: 0.8; $p < 0.01$) and GEA Farm Technologies (OR: 0.9; $p < 0.01$). For both Lely and Lemmer-Fullwood the EC at the quarter level (dichotomized) were also identified as helpful predictors for healthy cows. Lely cows with a quarter-level EC of less than 72 (manufacturer internal unitless score; OR: 3.73; $p < 0.01$) and Lemmer-Fullwood cows with a quarter-level-based EC below 5.6 mmho (manufacturer internal unit; OR:13; $p < 0.01$) were more likely to be correctly classified as healthy cows. For all AMS manufacturers, the udder health status based on DHIA tests was useful as a predictor for SP. Cows classified as “healthy” here had up to five times the odds of being correctly considered not to be affected by CM than cows classified as “chronic”: DeLaval (OR: 5; $p < 0.01$), GEA Farm Technologies (OR: 5; $p < 0.01$), Lely (OR: 2.2; $p < 0.01$), and Lemmer-Fullwood (OR: 5; $p < 0.01$).

Table 7. Factors improving the specificity (outcome: healthy cows, i.e., no clinical mastitis, without alert) on a cow level, including only healthy cows and DHIA as well as AMS data, in a cow-level multivariable logistic regression analysis with herd as random effect.

AMS, n Cows	Predictor	β ¹	SEM	Odds Ratio	95% CI	p-Value	
DeLaval 1761	Intercept	−1.93	2.11			0.37	
	Milking interval in hours	−0.20	0.03	0.82	0.78–0.88	<0.01	
	Δ highest and lowest quarter EC ²	−0.59	0.10	0.56	0.46–0.67	<0.01	
	DHIA ³ lactose concentration	1.60	0.42	4.94	2.16–11.29	<0.01	
	Udder health status ⁴	chronic	−1.70	0.27	0.18	0.10–0.30	<0.01
		new IMI ⁵	−1.11	0.34	0.33	0.17–0.64	<0.01
		cured	−0.20	0.44	0.82	0.35–1.93	0.65
		no DHIA data	−0.83	0.61	0.44	0.13–1.46	0.18
		no current test data	−0.64	0.67	0.53	0.14–1.96	0.34
		healthy	Referent				
GEA 1562	Intercept	1.72	0.28			<0.01	
	Milk yield of last milking, kg	0.01	0.02	1.12	1.08–1.17	<0.01	
	Milking interval in hours	−0.10	0.02	0.90	0.87–0.93	<0.01	
	Lactation number	1	0.60	0.17	1.82	1.30–2.55	<0.01
		2	0.11	0.16	1.11	0.81–1.54	0.51
		≥ 3	Referent				
	Udder health status ⁴	chronic	−1.64	0.20	0.19	0.13–0.29	<0.01
		new IMI ⁵	−1.46	0.23	0.23	0.15–0.36	<0.01
		cured	−0.74	0.25	0.48	0.29–0.79	<0.01
		no DHIA data	−1.03	0.24	0.37	0.23–0.58	<0.01
	no current test data	−0.98	0.34	0.38	0.19–0.73	<0.01	
	healthy	Referent					
Lely, 1998	Intercept	8.89	0.72			<0.01	
	Quarter based EC threshold of 72	0	1.32	0.20	3.73	2.54–5.47	<0.01
		1	Referent				
	Fat content (measured by AMS)	−0.31	0.10	0.74	0.60–0.90	0.03	
	LogSCC ⁶	−1.19	0.12	0.30	0.24–0.38	<0.01	
	Udder health status ⁴	chronic	−0.80	0.25	0.45	0.27–0.73	<0.01
		new IMI ⁵	−0.33	0.33	0.72	0.38–1.38	0.32
		cured	−0.57	0.38	0.95	0.45–2.01	0.88
		no DHIA data	−0.68	0.38	0.51	0.24–1.06	0.07
		no current test data	−0.09	0.45	0.91	0.38–2.21	0.84
	healthy	Referent					
Lemmer- Fullwood, 1508	Intercept	1.19	0.33			<0.01	
	Quarter based EC threshold of 5.6	0	2.58	0.32	13.13	7.03–24.51	<0.01
		1	Referent				
	Udder health status ⁴	chronic	−1.60	0.31	0.20	0.11–0.37	<0.01
		new IMI ⁵	−0.35	0.45	0.71	0.29–1.72	0.44
		cured	−0.40	0.55	0.67	0.23–1.97	0.47
		no DHIA data	−0.20	0.60	0.82	0.26–2.63	0.74
		no current test data	−0.80	0.58	0.45	0.14–1.41	0.17
		healthy	Referent				

¹ β = Regression coefficient; ² EC: Electrical conductivity measurement; ³ DHIA: Dairy herd improvement association is the Landeskuratorium der Erzeugerringe für tierische Veredelung in Bayern e.V.; ⁴ Udder health status: Categorization, based on DHIA somatic cell count measurement (SCC). SCC from two subsequent test days in that lactation with a cutoff value of 100,000 cl/mL are compared; ⁵ IMI: Intramammary infection; ⁶ SCC determined by the Lely MQC-C System, log transformed.

4. Discussion

This study investigated the performance of CM detection by currently used AMSs from the four most common AMS manufacturers in Bavaria, southern Germany. The strengths of the study were a sufficiently large overall sample size, inclusion of multiple commercial farms for each manufacturer, and the use of the true gold standard for clinical mastitis, i.e., clinical observation. In addition, we were able to identify automatically

recorded parameters that could improve the sensitivity and specificity of the AMS mastitis classification (“alerts”) when considered by the farmer in post-AMS analysis of the data collected at each milking.

The detection of clinical mastitis is critical for the farmer’s decision-making. For one, sick animals need to be identified to be treated or their clinical development closely observed. Furthermore, abnormal milk must not enter the food supply chain and needs to be discarded. The gold standard is the organoleptic detection of abnormal milk by the human milker. Thus, AMS must be able to guarantee the legal regulations for ensuring safe food to at least the same extent as human milkers. It has been estimated that milking technicians will find approximately 80% of CM cases going through the parlor [43]. It was therefore positive to note that the AMS of all manufacturers achieved the minimum SN of >70% as required by Annex C of ISO 20966:2007 [44]. However, the slightly higher minimum value of 80% for SN called for by Hogeveen et al. [45] was achieved by only three of four manufacturers; the SN of DeLaval fell slightly short. Since the point estimates for each manufacturer provide only an average, the confidence intervals are a better estimate of the SN range and precision. These included the required 70% SN of all AMSs and the required SN of 80% by three AMS manufacturers. However, while one might argue that the range of the intervals are fairly wide, we had enough statistical power to find potential differences, i.e., to test our hypothesis. Therefore, the identification of clinical mastitis cases has to be considered sufficient for food quality and animal welfare, especially in view of the low prevalence of the disease, i.e., 70–80% of the few cows with clinical mastitis per herd were identified. A large cost factor of CM is discarded milk [46].

Focusing on the SP, none of the evaluated systems reached the >99% SP required by ISO 20966:2007 and Hogeveen et al. [44,45]. Since the vast majority of cows in a herd will not have clinical mastitis, a farmer would suffer substantial economic losses due to falsely discarded “abnormal” milk (false positive cases) [47,48] if they use the system alerts to automatically separate milk. Whether the alert lists have a high SN or SP for subclinical mastitis was not answered in this study, since the focus of this study was clinical cases. However—purely from a legal perspective—visually normal milk in cases of subclinical mastitis does not warrant the automatic discarding of milk unless the bulk tank SCC would exceed legal limits.

Our SN and SP estimates are in agreement with previous studies [10,49]. Slight differences between studies are likely due to different gold standards, evaluated alerts, or sensors, as well as different sampling timeframes [19,28,34,50]. Brandt [51] found an SN mostly below 60% and SP above 90% for three different AMS manufacturers in 12 northern German herds, with comparable gold standard (alteration in homogeneity of the foremilk) and time window (milking right after sampling). Castro et al. [49] evaluated three different AMS types in ten Galician herds and estimated an average SN of 58% and SP of 94% for the detection of a mastitis case, based on positive CMT. Only Dalen et al. [10] found higher SN values of 80% and SP values of 90% by evaluating the online cell count (OCC) device of the DeLaval AMS operating on a Norwegian research farm, where they used veterinary mastitis treatments as gold standard.

While the results for all evaluated manufacturers were comparable, slight differences were observed and likely due to the different underlying sensor technology and proprietary algorithms. The reviewed alerts of GEA Farm Technologies and Lemmer-Fullwood are purely based on measuring EC [19]. Using EC alone is not considered sufficient for CM detection [16,20,34], due to the impact of milk temperature, fat content, or milk fractions [52,53]. Nevertheless, these alerts reached the required minimum SN thresholds—probably because not the absolute EC but the variation at quarter level was considered by system alerts. This has been shown to improve the usefulness of EC for mastitis detection [18,54]. DeLaval and Lely processed multiple sensor data into an indication of udder health problems. Although combining EC with other sensor data should improve the detection of CM [35,55], the AMS by DeLaval did not achieve the minimum SN required by Hogeveen et al. [45]. This might be because MDi is advised more as a

probability of an udder health problem [35] and less for detection of abnormal milk as it occurs mainly in CM. With a higher MDi threshold, no better SN was detected in our study. This contradicts in part the approach of Lusi et al. [56], which suggested an MDi threshold of ≥ 2 for abnormal milk detection to keep the SCC of bulk tank milk at a low level. While the higher threshold markedly increased the SP, the SN decreased drastically. Subsequently, milk from many undetected cows with CM would still be collected. The use of detection of blood in milk as an additional sensor in MDi may be insufficient. However, in a field study by Hovinen et al. [57] every case of bloody milk could be detected, but many were detected due to the yellow color of the milk, which in turn depends on the milk fat color and thus on feed and breed [29]. Lely's SN values are likely to be obtained by combining several sensor data and especially the overall use of SCC data in these study herds [13,21,58,59]. Data from other manufacturers' SCC sensors could not be utilized in our study due to low numbers (e.g., only seven DeLaval herds had OCC data). Besides the measurement of SCC, there are several additional sensor technologies for each manufacturer on the market that could have potentially improved the accuracy of mastitis detection, such as lactate dehydrogenase detection [60,61] or rumination activity [62,63]. However, these technologies were not sold as a standard part of the AMS mastitis detection sensor package [64]. Therefore, they were not evaluated in this study. Furthermore, we assessed the standard udder health alert lists of each system based on discussions with manufacturer personnel. Unfortunately, one Lemmer-Fullwood list ("control_report_milking_10 days") could not be used due to missing headers. However, the data might have been indicative of udder health problems, as they included cow activity [65] and milk lactose [66]. Due to these limitations, one has to assume that when considering the stated and theoretically available additional sensor data the SN and SP could have actually been higher than we were able to determine with the given data. Likewise, it must be considered that farmers using a Lely or Lemmer-Fullwood AMS could adjust the limits of the pre-installed warning lists. A check of the settings at the time of the data backup was carried out, but due to this technically conditioned snapshot at the herd visit, an adjustment of the threshold values shortly before cannot be ruled out. For this reason, possible herd-specific limit value adjustments were not taken into account and a possible improvement or deterioration of the SN and SP with farm-specific values could not be evaluated.

The low specificity estimates of this study highlight a known problem [67]. A high number of false positive alerts as a result of low prevalence of CM and a lower SP of the detection system can lead to economic losses due to wrongly discarded milk [67] and increased labor cost to assess each of these animals' udder health in the barn. This will decrease the farmer's confidence in the AMS' udder health alerts [68]. The low SP may be due to several factors: first, CM is a dynamic event that passes through different stages [69], second, the AMS alerts do not distinguish the grade of mastitis (e.g., subclinical or clinical), and third, the manufacturers did not provide system definitions for clinical mastitis. Therefore, this purely binary classification may lead to higher false positive rates since marked cows may not yet have an apparent change in milk but may already show changes in milk components detectable by the sensors [33,46]. The gold standard definition of ISO 20966:2007 also proposed by Kamphuis et al. [44,70], i.e., the presence of clots or flakes in two out of three consecutive milkings, thus tries to counteract this and capture the evolution process of CM. The single assessment of the udder in our study was selected for several reasons: (1) the farmer has to assess cows on the lists at least twice a day and new mastitis cases should be present; (2) the visual assessment allowed for the assessment of the quality of visually abnormal milk, watery character of the milk, or single abnormal milk [71]. Therefore, we are closer to the basis of legal assurance of milk fit for human consumption (EU Directive EC/853/2004) based on the organoleptic examination of milk and udder pre-milking by milkers [43,72]. The main concern was that none of the manufacturers provided a definition of "clinical mastitis" that would allow for a simple "yes/no" answer. Solutions to this dilemma could be probability indications of potential clinical mastitis as suggested by Friggens et al. [69] and different alerting

approaches for different forms of mastitis or udder health situations [45]. We were able to identify several automatically collected parameters that were associated with SN and SP in multivariable regression models. These factors may be used by the farmer to improve CM detection. However, although EC was included in many algorithms already, the additional consideration of high EC improved the identification of CM by DeLaval and GEA systems. This suggests that cows with CM have higher milk EC values [73] and the effects of, for example, temperature, fat, and milk fractions on EC [18,52,53] play a lesser role in EC than the underlying inflammation [20]. Due to the relatively small number of CM cases, only a few additional predictors could be identified in the SN models, and none for Lemmer-Fullwood. In contrast, several parameters associated with SP were identified, which can partly be attributed to the substantially larger sample size. For example, it has been found that cows with longer milking intervals in DeLaval and GEA systems have a lower chance of being correctly classified as healthy cows. This could be because the likelihood of a cow having abnormal milk increases as the milking interval increases [74], and the flakes that may be present may affect the measurement of EC [18], making it difficult to alert correctly; additionally, a milking visit could be protracted due to pain caused by the onset of udder inflammation.

The one factor that was consistently helpful across all systems was the trend of SCC between the last two monthly milk tests. Cows with “chronically” high SCC (i.e., two subsequent tests with SCC > 100,000 cells/mL) had lower chances of being correctly classified as healthy by the AMS. One explanation could be that “chronic” cows had persistent or repeated subclinical mastitis and this triggered the alert by the AMS. As there is no specific alert for CM — only udder health “abnormalities”, this may drive the misclassification for clinical mastitis [45]. Regular checking of these cows with repeated high SCC values should be performed to detect CM early on the one hand, and to prevent unnecessary automatic separation of milk by the AMS on the other hand. Although the models included a large number of automatically recorded parameters, they did not include all possible parameters, especially in the Lemmer-Fullwood data set. Similarly, farm-specific factors such as setting alert limits were not part of the available data. The herd-level random effect accounts for some differences among these unknown factors. Inclusion of additional interactions in the model could have potentially improved the model fit. However, due to the lack of biologically plausible interactions to be considered and the low sample size in all SN models they were not further explored.

Although AMSs sufficiently identify cows with clinical mastitis, farmers must continue to know and monitor their animals several times per day in the barn. Otherwise, cows that were unable to visit the AMS due to acute severe disease (e.g., mastitis), might be identified too late if flagged simply based on milking interval, or the milk of cows with CM not yet identified by the machine could enter the food supply as well. On the other hand, the milk of healthy cows would be discarded longer than necessary, if the farmer does not assess the udder status of the cow. While the benefits of an AMS are more flexible working hours and a daily overview over each animal’s health and production data [38,64], the farmer needs to understand the provided data and machine functions and evaluate their animals in the barn to ensure food safety and animal health. In addition, AMS producers need to revise their approaches to detect udder health problems, especially CM. For example, there are promising studies on intelligent data processing and machine learning to use the amount of data collected to identify sick cows [75–77]. In addition, new sensor technologies closer to the origin of the gold standard definition, the organoleptic (especially visual) inspection of the milk, as well as information from other sensors that measure other more general health parameters should be explored. The former could include camera-based milk quality assessment of the fore stripping. These technologies are already being used in other livestock production chains, for example, to detect and assess footpad health in poultry [78]. Until then, a more comprehensive implementation of DHIA data in herd management software along with AMS data may be helpful for monitoring udder health.

5. Conclusions

The present study shows the SN and SP of CM detection from different AMSs used in Bavaria. Overall, the detection of clinical mastitis by different AMSs was found to be sufficient, but the low specificity could cause unnecessarily discarded milk and additional workload for farmers to check on their animals. Some automatically collected parameters, such as EC and monthly test day results, are related to the current detection performance of CM by AMSs and can be helpful to farmers in their assessment of AMS udder-health alerts. Because there is currently no official definition of visibly abnormal milk (i.e., clinical mastitis) by the manufacturers, farmers need to consider test day data and results of udder health evaluations of each cow in the barn to interpret and act on AMS lists appropriately to warrant food safety, milk quality, animal welfare, and the economic viability of their farm.

Author Contributions: M.B.: data collection, data analysis, manuscript preparation; R.M., M.G.D. and J.H.: manuscript review; U.S.S.: study design, data collection, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding: All funding received during this specific study came from the Free State of Bavaria and the Bavarian Joint Founding Scheme for the Control and Eradication of Contagious Livestock Diseases (Bayerische Tierseuchenkasse). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Institutional Review Board Statement: The sampling procedures of this study do not require ethics approval under the German animal protection law.

Informed Consent Statement: Informed consent was obtained from all of the owners of the animals (or an authorized agent for the owner) involved in the study.

Data Availability Statement: The data of this study are property of the Bavarian Animal Health Services. None of the data were deposited in an official repository.

Acknowledgments: The authors thank all participating dairy farms and the service teams of DeLaval, GEA Farm Technologies, Lely, and Lemmer-Fullwood for providing herds, AMS data, and general support.

Conflicts of Interest: The authors declare no conflict of interest.

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2. Survey on dairy farmers' management practices for and satisfaction with the detection of clinical mastitis by automatic milking systems in Bavaria, Germany.

Bausewein M, Mansfeld R, Doherr MG, Harms J, Sorge US.

“Survey on dairy farmers' management practices for and satisfaction with the detection of clinical mastitis by automatic milking systems in Bavaria, Germany.”

Milk Science International (76) 2023 P. 28-34. ISSN 2567-9538;
<https://doi.org/10.48435/MSI.2023.5>

Survey on dairy farmers' management practices for and satisfaction with the detection of clinical mastitis by automatic milking systems in Bavaria, Germany

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Date submitted: 23/12/2022

Date accepted: 18/05/2023

Page(s): 28-34

Abstract

The objectives of this study were to identify (i) management practices for the detection of clinical mastitis (CM) in dairy farms with automatic milking systems (AMS), and (ii) the farmers' personal assessment of their work with the AMS as well as the mastitis detection performance of the AMS through an online survey. Complete responses of 47 of the 108 contacted Bavarian dairy producers were available for analysis. Warning lists of AMS, highlighting cows with potential udder health problems, were checked twice a day by 68% and once per day or less frequently by 27% of the farmers. Checking warning lists reportedly took five minutes per day (median). Besides the presence of flakes on the milk filter (75%), data from the AMS (78%) was another important factor that farmers considered for their decision to assess an indicated cow in the barn. Electrical conductivity (EC; 50%), milk color/ blood presence (49%), and, if available, somatic cell count (66%) were selected most frequently as "extremely important" from provided options in the survey. Flagged cows were commonly checked within 12 hours of the alert (23%) in the barn. Most commonly, these cows were assessed by organoleptic examination of the udder and/or the first milk strains (50%). Most farmers (68%) agreed with the statement of being very satisfied with the detection performance of CM by the AMS. However, almost half of the farmers (44%) perceived the number of false-positively flagged cows by the AMS as too high. While farmers were overall positive towards the detection of CM in AMS, some management factors such as the frequency of monitoring the warning list and cows in the barn could be intensified.

Keywords: dairy cows; milking robots; mastitis monitoring; questionnaire

Introduction

Dairy farmers are responsible for the health of their animals [1], the

production of a high-quality food, and maintaining the profitability of their farm [2, 3]. Clinical mastitis (CM) of dairy cows affects all of these production areas as it impacts animal welfare and food quality and causes high economic losses [4–6]. Rapid diagnosis and appropriate treatment of CM are therefore crucial [7]. In conventionally milking dairy farms, the milker monitors udder health of each animal during the milking preparation process, e.g., by prestripping. This way, food safety is – from a legal point of view – ensured by organoleptic examination of the udder and milk for pathological changes [8]. Due to the increasing popularity of automatic milking systems (AMS), fewer humans are physically present at the milking of cows. This is related to both the Europe-wide trend of decreasing number of farms whilst simultaneously increasing farm sizes [9]. Another reason is that farmers might seek a better work-life balance through the installation of an AMS that allows for flexible working hours [10, 11]. Hence, also in Bavaria, the proportion of farms with AMS has risen from 3% to 16% in the last decade [12]. In farms using an AMS, the inspection of milk and udder health relies on the performance of the AMS sensors due to the absence of a milker [13–15]. The AMS indicates animals via warning lists to farmers if the animals are likely to have udder health problems. The sensor technology is able to detect and indicate inflammatory processes that may be minor and without visible changes to the milk [16]. However, the detection of CM by AMS has its limitations [17, 18]. For this reason, the German Federal Ministry of Food, Agriculture and Consumer Protection established a list of action items for dairy farms with AMS to ensure adequate udder health and an ongoing monitoring in 2012 [19].

Since the final (physical) assessment and maintenance of udder health (e.g., treatment decisions, consultation of farm veterinarian) remain the responsibility of the farmer, the "interaction" between AMS and humans is crucial. Few studies have addressed the role of the farmer on udder health in AMS herds: In 2012, Mollenhorst et al. investigated

the requirements for CM detection systems desired by farmers and concluded that CM alerts should have a low false positive rate, occur in a short time, and be graded by severity [20]. A Dutch study found that most Dutch farmers milking with an AMS made inspection decisions based on intuition and only the minority of farmers reported using non-AMS information about cows or detailed alerts to decide which cows to visually inspect [21]. However, which data of different AMS are important to farmers for the detection of CM and how farmers deal with these warnings has not been investigated yet.

Therefore, the objectives of this study were (i) to evaluate management practices for the detection of CM in Bavarian AMS farms, and (ii) to present farmers' personal assessment of their work with the AMS and the performance of mastitis detection of their AMS.

Materials and Methods

Herd selection and contact: Dairy farms equipped with AMS, that participated (n=114) in a previous study of the Bavarian Animal Health Services [17], were invited by personal e-mail to participate in this anonymous online survey. The personal invitation e-mail included a description of the study objectives, a note that subjects should also be the primary users of the AMS on their farms, and a link to the online survey. In addition, it provided information about the chance to participate in a prize draw for ten milk sample test kits in case of successful participation on the survey. To maintain anonymity of the main survey, an URL to a second independent input mask of the survey tool was provided at the end of the questionnaire for participating the prize draw. There, the respondents could enter their e-mail address, which was used to randomly select and contact the winners at the end of the survey period.

Questionnaire development: A survey with 22 questions was developed for the study. Question content, structure and organization of the questionnaire were revised and validated based on feedback from specialists and AMS manufacturer support personnel (n=8) as well as existing literature. The survey covered six main topics: general herd structure (3 questions), work with the dairy herd in the barn (4), work with the AMS software (4), mastitis diagnostic (6), personal opinion (2), and demographic data (3). Open ended and closed questions as well as Likert scale answer options [22] were included.

Subsequently, the questionnaire was pretested in personal interviews with three not-study-related AMS-using farmers, and the adapted version was transferred into the open-source online tool LimeSurvey (LimeSurvey Project Team/Carsten Schmitz, 2012). This online version was pretested with two farmers and four other specialists (veterinarians and AMS manufacturer staff). The final survey in the target population ran from June 18th to July 17th, 2021. To increase participation in the survey, a reminder e-mail was sent to all participants one week before the deadline [23]. The final version of the survey (in German) is available as a PDF file as supplements under <https://openjournals.hs-hannover.de/milkscience/issue/view/198>.

The questionnaire content and implementation procedure were approved by the ethical committee of the Freie Universität Berlin (ZEA-Nr.2021-009).

Statistical Analysis: The raw survey data were exported to MS Excel (microsoft.com) and analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC; USA). Only fully completed surveys were included in the final statistical analysis. The data were then checked for plausibility and excluded if illogical errors were found. Continuously measured items were evaluated for normal distribution by Q-Q-plots and the Kolmogorov-Smirnov test by PROC UNIVARIATE. Descriptive summary

statistics were used by PROC FREQ and PROC MEANS. Associations between AMS, gender, and age and continuous variables were analyzed with a Mann-Whitney U test by PROC NPAR1WAY WILCOXON test and for three or more groups by using the Kruskal Wallis test. Correlations between ordinal variables were assessed with non-parametric statistic Spearman's rho using PROC CORR SPEARMAN. The significance level was set at P<0.05. Figures were designed using Tableau version 2022.1 (Tableau Software, Seattle, Washington, USA).

Results and Discussion

Survey response rate: In the previous study [17], 114 herds had participated. Of these, e-mail addresses from 111 farmers were available. However, due to invalid or incorrect e-mail addresses, three farms could not be contacted. Therefore, a total of 108 farmers were invited and 62 participated (57%). Since 15 of the 62 collected questionnaires were incomplete, 47 questionnaires remained for the statistical analysis.

Of the 15 excluded surveys, five were completely empty, five responders had answered only 14% of the questions, and the remaining five dropped out after answering up to 50% of the questions. This net response of 43.1% was above the average response rate of other web-based questionnaires with dairy producers [24, 25]. This could be due to the underlying design which included a clearly defined and understandable study topic, professional layout, provision of the estimated processing time, invitation via a personalized e-mail, follow-up contact with resending survey link, length of the questionnaire minimized, and a prize draw as an incentive to participate [26–28]. The fact that the farmers were more interested in the topic as they had already participated in the earlier study certainly provided another incentive to complete the survey. Considering the selection process and response rate, any generalization of the results of this study should be done with caution.

Demographic data and herd structure of the sample: The participants were predominantly male (79%) and reported to be in the age category 31 to 50 years (62%). They had been working with AMS for a median of four (interquartile range [IQR]: 3–8) years. The majority of producers reported that they worked almost exclusively alone in monitoring the AMS udder health lists (80%) as well as subsequently inspecting the indicated cows in the barn (76%). This implies that the respondents to the questionnaire were remarkably familiar with the topic. At the time of the survey the herds milked 65 cows (median, IQR: 59–74). This herd size is consistent with the normal herd size for the most common use of one AMS, i.e., 60 cows/AMS unit, and the Bavarian average number of cows on AMS farms [12, 14]. The median annual bulk tank somatic cell count was reported to be 165 (IQR: 105–190) x1000 cells/mL for the herds in 2020 and was below the Bavarian average [12]. This may be due to the fact that the AMS of these herds were all maintained regularly by their AMS companies and the farmers were concerned about the udder health of their herd, which was expressed in the participation in the previous study.

Daily management of monitoring the udder health of the AMS herd: The majority of the surveyed farmers reported conducting daily measures in adherence to the list of measures aimed at ensuring udder health. It includes to check the AMS udder health warning lists and the herd for udder health in the barn at least twice per day. The check of the AMS warning list took a median of 5 minutes daily (IQR 5–10 min./day) and was performed by 68% of the farmers twice a day, as recommended. Also, the additional assessment of the herd for udder health in the barn took a median of 10 minutes per day (IQR 5–20 min./day) and was performed by 53% of respondents twice per day. There

Milk production

was a positive correlation between the amount of time spent checking warning lists and the amount of time spent checking the udders of the herd each day: the more time spent on warning lists, the longer it took to check the udder health of the herd in the barn (Spearman's rho=0.4; p=0.01). This could be an indication that more intensive use of the udder health warning lists lead to longer and possibly more careful udder health control of the herd. Alternatively, this could also be due to the high number of warnings needing to be checked. However, the time taken and the frequency with which the recommended measures are carried out allow only a cautious assessment of the quality of daily udder health monitoring. About 70 to 80% of CM cases are flagged by the system, but the number of false-positive cases is fairly high [17]. The farmer should therefore follow the guidelines of the catalogue of measures to look at data and cows at least twice a day, since each milking adds information about that animal's health and will help to increase overall accuracy of warnings. The better the farmer knows cows on the list, the better potential udder health problems could be detected and false positive alerts distinguished by the farmer. In addition, detection of udder health problems by AMS is limited by the sensor technology used [18, 29]. For instance, AMS currently do not detect udder-related diseases such as udder cleft dermatitis or acute trauma to the skin. Also, irregular control of the herd may result in slower and delayed detection and treatment of, for example, immobilized cows due to acute CM caused by *Escherichia coli*-infection, which can no longer visit the AMS. This has both economic and animal welfare consequences as the severity of the disease increases rapidly [30–32]. For these reasons, in addition to frequent monitoring of AMS warning lists, the farmer must continue to physically monitor his herd in his daily routine to identify problem cows or to prevent the spread of (udder) disease. Therefore, it is concerning, that about one third of the respondents checked only once a day or less the udder health warning lists (27%) or their herd in the barn (34%) for udder health problems.

Detection management of new CM cases: Given the absence of a specific AMS alert for cows with CM, which results in legally unmar-

ketable milk, the decision to take action on an indicated cow ultimately rests with the farmer. Consequently, farmers were asked about their handling of new AMS udder health alerts. Newly indicated cows in this study were defined as having not generated an udder health warning in the previous seven days. Approximately 53% of the participants reported often performing a check within 12 hours of the warning, while 42% of farmers only went to look at flagged cows after four or more consecutive warnings. This observation is consistent with findings of other studies, where farmers were selective about assessing indicated cows in the barn due to the high workload coupled with the low specificity of the detection of mastitis by AMS [21, 33]. However, this behavior does not meet the suggested measures, which advise an immediate examination of all indicated cow [19]. One has to assume that if cows on warning lists are not examined immediately in the barn, many specifically milder mastitis cases will likely be overlooked. This assumption is supported by the finding that AMS farms detected mostly severe cases of CM [34].

The decision to assess flagged cows in the barn was based on various factors. Of particular importance was examination of the milk filter for abnormal milk components such as flakes or clots ("very important" (74%)) and additional AMS data (78%). In contrast monthly test day data or the milk yield of the cow relative to herd mates were reportedly of minor importance to farmers in this study. While Steeneveld et al. did not find non-AMS data helpful in distinguishing between true-positive and true-negative alerts [33], another study found that inclusion of somatic cell count (SCC) of monthly test day data was related to CM detection performance of AMS [17]. There, the inclusion of monthly test day data was identified as a previously overlooked tool that has potential to improve udder health monitoring. To assess the importance of AMS data for the decision to check a newly indicated cow in the barn, the respondents were asked to rate the importance of AMS data on a seven-point Likert scale (1=extremely unimportant to 7=extremely important) as well as the answer options "no answer" and "sensor data not available". The list of the AMS data provided to farmers to be ranked

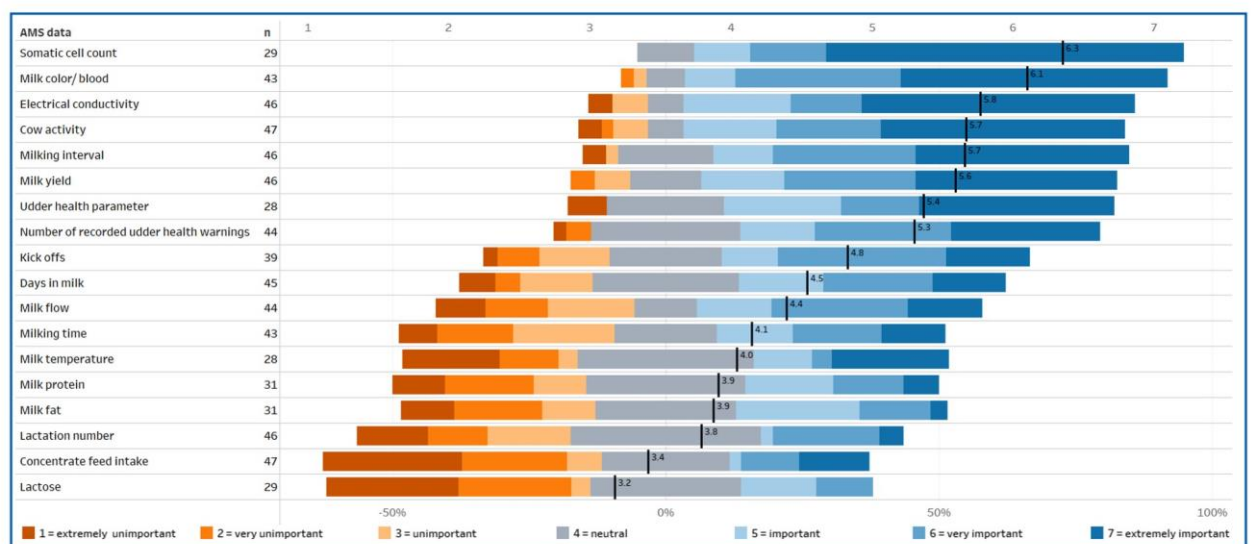


Figure 1: Comparison of the subjective relevance of information displayed to farmers (n=47) on udder health lists of the automatic milking system (AMS). Percentage rating of subjective importance of the participants, which AMS data is helpful for assessing whether an indicated cow will be also controlled in the barn (Gantt percent, lower axis). Importance on a seven-point Likert scale shown in stacked bar charts, sorted in descending order by average Likert score values (upper axis) shown as a black line. The number of answers is not equal in total, since the answer options "sensor data not available" and "no answer" were excluded from the respective bars.

included the most common commercially available sensors and AMS data. Other sensors are offered by some AMS manufacturers that also have reported to be helpful in detecting udder health problems such as sensors for lactate dehydrogenase [16] or rumination activity [35]. They were not considered in our study because they are not widely available as upgradeable sensor technology. Based on the average answer scoring (AAS), the most important information was the SCC (AAS: 6.3; n=29) for farms of this study equipped with such a device (38%), followed by blood or color sensor alerts (AAS: 6.1; n=43), and the electrical conductivity (EC; AAS: 5.8; n=46). Other information was ranked lower in relevance (Figure 1). This is in line with the study of Steeneveld and Hogeveen [36], who investigated the frequency of sensors in daily use and found SCC and EC data were frequently used while fat, protein and milk temperature were less commonly used sensor information. Other studies have shown that the use of the SCC [37, 38] as well as the EC [39] can help to detect udder health problems. The value of the milk color sensor on its own is considered controversial in other studies, as the detection of CM by this sensor alone does not seem suitable due to the influence of fat color [40–42]. However, combining the information from different sensor data is considered a good tool to detect udder health problems [43, 44]. Based on the finding that no sensor data had a high rejection rate, it can be assumed that some farmers combine different information provided by AMS in their decision-making process. The inclusion of additional AMS data showed improved detection performance for CM in some studies [7, 17] and thus can be considered a good state of practice to identify CM. In conclusion, the majority of farmers were applying suitable management procedures to detect CM in AMS herds. Although not all udder health alerts were addressed promptly, they were evaluated in conjunction with sufficient AMS data and information obtained from the barn. In addition, a more extensive utilization of DHIA data for this purpose should be considered.

Examination of new udder health warnings in the barn: The examination of the indicated cow for udder health in the barn was done at least “often” by inspection and palpation of the udder (87% of the study

participants), by evaluation of the foremilk for abnormal milk such as flakes or blood (78%), or a California Mastitis Test (CMT, 64%), while a quarter milk sample for pathogen determination in the laboratory was almost never taken by about 42% of farmers. Since detection of even mild CM cases by sensory clinical examination has a sensitivity of 80% [45], the farmer’s assessment of udder health status is considered sufficient and in general agreement with the methodology proposed by Hogeveen et al. [46]. However, AMS and their udder health alerts are supposedly designed as an early warning system. Therefore, the AMS often detects invisible changes in the milk composition, which can indicate, for example, subclinical mastitis. A purely organoleptic examination of the milk for abnormalities of the indicated cows will therefore lead to a high number of false positive alerts. In this case, regular monitoring of all cows or specific CMT-based checking of those cows that have an AMS warning but no (or not yet visibly detectable) clinical symptoms will be useful to confirm early signs of new infections and subclinical udder inflammation [47, 48].

Agreement with statements about mastitis detections management:

Farmers were able to rank statements related to mastitis detections management according to their personal experiences and subjective feelings using a Likert scale from 1 to 5 (1=strongly disagree to 5=strongly agree). Most farmers saw themselves as competent in the understanding (AAS: 4.1) and use (AAS: 4.4) of the displayed data and in spending sufficient time (AAS: 4.0) at interpreting the udder health lists (Figure 2). Interestingly, farmers that reported to be less confident with the AMS lists take longer in working time with the AMS program (Spearman rho=0.4; p=0.01) and udder health assessments in the barn (Spearman rho=0.4; p=0.02). One explanation could be that those farmers with limited operating ability of the AMS program tried to compensate for this with more time spent on the computer and for assessing the herd in the barn. On the other hand, the unidentified different levels of education and character of the participants as well as influences of the operational structure may be a cause of slower handling of tasks than others. The lowest AAS was achieved by the

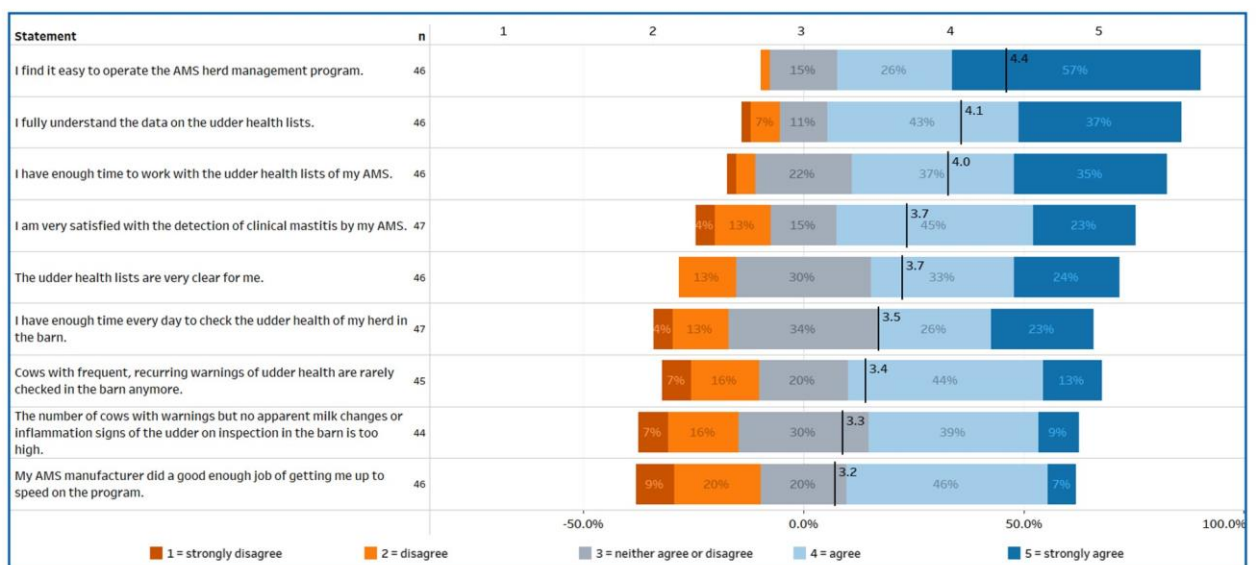


Figure 2: Agreement with various statements regarding udder health management on dairy farms operating with automatic milking systems of 47 dairy farmers. Percentage assessment of subjective agreement with these statements (Gantt percent, lower axis). Agreement on a five-point Likert scale shown in stacked bar charts, sorted in descending order by average Likert score values (upper axis) shown as a black line. The number of answers is not equal in total, since the answer option “no answer” were excluded from the respective bars.

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statement on good instruction by the AMS companies with 3.2. Here 29% of the subjects disagreed with having had a good instruction. This result is seen as critical, because a good instruction how to best use a highly complex system, that constitutes a central part of the daily work in AMS farms, has to be considered essential for farmers to work economically and efficiently.

The majority of respondents (68%) were satisfied with the detection of CM by the AMS; only 17% of the respondents did not agree with this statement. A comparison of the agreement scores between the AMS manufacturers showed no difference. Overall, this is consistent with Mollenhorst et al., who found that Dutch farmers are overall satisfied with the detection of udder health problems [20]. Nevertheless, the data found here must be interpreted with caution, as they are based on the personal assessment of the farmers through their experience in daily work with AMS. Furthermore, the satisfaction with the CM detection of an AMS leaves room for different interpretations and does not allow direct conclusions on the quality of the CM detection. An AMS gives warnings after analyzing a milking process, which cannot be directly checked for correctness due to the absence of a human during milking. The farmer could only estimate the sensitivity of an AMS for the detection of CM, i.e., at least the pathological occurrence of organoleptically abnormal milk, with considerable additional effort. On the other hand, it is much easier to compare the alerts with the udder health status of the cow in the barn. In this regard, our study showed that a large proportion of farmers (48%) agreed with the statement that the number of false positive alerts for CM was too high, and only 23% of farmers disagreed. This is in line with the results of other studies that have reported low specificity for detection of CM by AMS [17, 49], as well as farmers' desire for improved specificity for detection of udder health problems by AMS [20]. However, in herds where farmers agreed with poor CM detection rates or excessive numbers of false-positive cows flagged, no associations were found with other counteracting management practices, such as more frequent or longer inspection of cows in the barn. Critical in the evaluation of statements is that 57% of farmers agreed that "chronic" cows (i.e., cows that repeatedly produced an alert but do not show visibly signs of CM) were no longer checked in the barn in case of further alerts. However, cows with subclinical or chronic mastitis, which can trigger the alert of the AMS, may also develop acute CM [50] and would be likely overlooked. This would affect animal welfare and food safety.

Limitations: This questionnaire provides valuable insights but may not definitively determine the presence of recorded management practices and farmers' perceptions on their farms. Since the recording of management practices related to udder health monitoring, the assessment of own skills, and the CM detection performance of the AMS are based exclusively on farmers' subjective experiences, these results should be evaluated carefully. As a result, we cannot completely avoid the possibility of bias and misinterpretation. Therefore, due to its content structure, the questionnaire could also be answered from the point of view of the detection of subclinical mastitis. However, this was addressed by the clear formulation of the study objective in the invitation e-mail as well as the topic in the respective group headings. Furthermore, to prevent a purely intuitive processing of the questionnaire, instructions were given at the beginning of question groups that encouraged to refer to personal experiences from daily work with AMS. To prevent agreement bias, i.e., the likelihood that respondents would agree with the statement regardless of its content, we also used extensive pretesting of the questionnaire, the use of a five-point Likert scale, and two reversed statements in the question group on personal

evaluation of statements [51, 52]. Nevertheless, a generalization of our results to all dairy farms with AMS should be made with caution due to the small sample size and pre-selection by participation in the first study. Thus, only farms with one of the four most common, regularly maintained AMS systems in Bavaria were included in the study. These farmers participated voluntarily in both studies and may therefore be more interested in udder health than other farmers. However, humans and commercially available AMS operate under similar conditions regardless of region, and thus the results of this study provide important insights for the dairy industry and leads for further studies addressing the factors that are critical for farmers to diagnose mastitis through AMS.

Conclusion

The majority of participating farms performed the daily management practices recommended to ensure udder health with AMS. However, some of the farmers reported not immediately checking cows newly indicated by the AMS as having udder health problem in the barn. Instead they used a combination of AMS data and knowledge about the cow for a decision. Also, one-fifth of the farmers reported monitoring their herd in the barn and on the warning lists once or less per day. These practices are considered insufficient for maintaining udder health on AMS farms in relation to officially recommended measures. Farmers perceived the detection of clinical mastitis by the AMS to be satisfactory. This was independent of the AMS type. They rated themselves as having a good understanding of their AMS software program around udder health monitoring. Nevertheless, some felt insufficiently instructed in the use of the AMS software by their manufacturer. Overall, this survey showed that good udder health monitoring practices were being implemented on the majority of the participating AMS farms.

Disclosure of Conflicts of Interest

The study was made possible with financial support of the Free State of Bavaria and the Bavarian Joint Founding Scheme for the Control and Eradication of Contagious Livestock Diseases (Bayerische Tierseuchenkasse). The authors declare no conflict of interest.

Compliance with Ethical Standards

The survey was anonymous and was conducted in compliance with ethical standards and legal privacy protections and was approved by the Ethics Committee of Freie Universität Berlin (ZEA-No.2021-009)

Annex - Questionnaire (Language German)

The questionnaire is available as separate pdf-file under following link: <https://openjournals.hs-hannover.de/milkscience/issue/view/198>

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IV. DISCUSSION

The aim of this thesis was to assess and evaluate the detection of CM on Bavarian dairy farms using AMS. For this purpose, the roles of milking machine and the human operator, the farmer, were investigated. The detection performance of AMS for CM of the four most commonly present AMS manufacturers on Bavarian dairy farms was assessed and additional data associated with SN and SP were identified and evaluated (Publication I). To assess the farmers interactions with the AMS and the data, management practices for CM detection on these farms as well as farmers' personal opinions on this subject were collected through an online survey and analyzed (Publication II). In this way, problem areas in the detection of CM were identified and practical guidance could be provided to ensure food safety and animal welfare.

After an a priori sample size calculation to meet the objectives, the number of sampled cows from several commercial dairy farms allowed an adequate amount of data from the field. In addition, the distribution of the farms throughout Bavaria and a study period of eleven months reduced geographical, seasonal and climatic influences. Although a large number of farms per manufacturer participated, the results should not be used for an udder health comparisons between farms or AMS types, because the farms were selected from list of potential participants provided by manufacturers based on having a regularly maintained machine and the farmers participated voluntarily. Furthermore, it can be assumed that due to the voluntary participation and the selection criterion of regularly maintained AMS, particularly farms with a strong interest in good udder health were included. This, and the fact that not all AMS manufacturers used in Bavarian AMS farms were investigated, means that generalization of the results to Bavarian dairy farms using AMS should be made with caution.

The evaluation of udder health by AMS showed that, compared to the proposed SN and SP limits from literature and DIN ISO 20996: 2008, the detection performance for CM of the AMS used on the study farms reached sufficient SN but insufficient SP. However, it needs to be acknowledged that the proposed methodologies for determining detection performance from the literature (Kamphuis et al., 2016) or DIN ISO 20996: 2008, could not be fully adopted in this study due to the nature of

a cross-sectional study. A follow-up of both, an udder health alert or a sign of CM over three milkings, was not be carried out. Nevertheless, the comparison used in this study between the one-time clinical assessment of udder health in the barn with a contemporary udder health alert offered great advantages. It reflected the legal methodology of organoleptic monitoring of udder health to ensure food safety and was therefore closer to farmers' everyday practice of ensuring the supply of legally normal milk while providing a rapid detection of abnormal milk and diseased cows with subsequent treatment options. Furthermore, the results of the detection performance observed were similar to the results of other studies in other regions (Brandt, 2012; Castro et al., 2015; Rasmussen, 2004). A direct comparison of the detection performance of these studies may be complicated by the different study designs, the different definitions of CM, or the different warning systems evaluated (Hogeveen et al., 2021). However, the clear trend of adequate detection of CM (SN) with inadequate SP observed in these studies was confirmed by the results of our study (publication I). Thus, the recorded results showed for the first time in the field with this large sample size that after almost 30 years of commercially used AMS, there is still insufficient detection performance of CM by AMS in Bavaria, not at all in terms of SN, but especially in terms of SP.

The practical importance of this issue can be illustrated by the low prevalence of CM and the number of possible alerts (at each milking time, i.e., on average 2-3 times per day/animal). In our study, CM was detected in an average of 3.5% of a herd of approximately 65 cows during the farm visit. This corresponds to about six milkings per day with abnormal milk, respectively CM, of which at least 4.5 (SN >70%) were also recognized as such by the AMS. If this low number is additionally compared with the detection performance of abnormal milk of the conventional milker (80%, Rasmussen, 2005; Hillerton, 2000), the maintenance of food safety and animal welfare can be considered satisfactory. On the other hand, at least 155 of the 156 milkings (SP >99%) with normal milk should not trigger an udder health warning. If a farmer, as required in the study of Steeneveld et al. (2010), would inspect every alert with the in this thesis recorded insufficient SP in the barn, a large number of these indicated animals would also show no sign of visually abnormal milk. This could result in the following: on the one hand, it could lead to a considerable cost factor in case of activated automatic milk separation based on the evaluated alerts due to discarding of visually normal milk, and therefore legally fit

for human consumption. On the other hand, the huge amount of work involved, coupled with the frustration of a false alert, could lead to farmers losing confidence in their AMS's detection system. The results of the survey conducted for this thesis support this assumption. While a majority of the respondents were satisfied with the detection of CM by their AMS, it was also stated that the number of alerts for cows with visually normal milk is too high. These statements were, of course, based on the subjective experiences and leave room for bias, as the farmer in AMS farms has no comparative cow observations of each milking and its data results. However, these results are in line with the opinion of Dutch farmers who were interviewed in another study (Neijenhuis et al., 2009). The finding that some surveyed farmers do not immediately check every udder health warning of their AMS in the barn, confirms the statements of Mollenhorst et al. of 2012 and seems understandable from practical point of view in regard to the low SP. Rather, farmers conducted to weigh AMS warnings based on other AMS data and using background knowledge about those cows from the barn. While this practice may save labor time and supposedly unnecessary inspections, it carries the risk that some cases of CM will go undetected, which in turn will affect bulk milk quality and the health of the individual animal. For this reason, but especially to address the insufficient SP, it is recommended to immediately check animals with udder health warnings (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz, 2012). This should be done both to quickly separate the milk and animal for treatment in an emergency and to prevent the waste of visually normal milk suitable for human consumption due to incorrect automatic milk separation.

In order to address the issue of improving the detection performance for CM, efforts have been made to identify additional parameters that have positive effects on SN and SP. Several parameters were worked out in a statistical model, which are associated with CM detection performance. It is shown that the combination of multiple sensor data provides better detection performance (Hogeveen et al., 2021), and so some studies have attempted to improve the detection performance of AMS by incorporating additional data in various models with promising results (Steeneveld et al., 2012; Khatun et al., 2020; Steeneveld et al., 2010; Jensen et al., 2016; Khatun et al., 2018). Nevertheless, due to the relatively small number of CM cases used for model calculation, only few parameters were identified in the study to have potential in improving SN. One of these parameters was EC at the quarter

level. The use of quarter conductivity measurements to improve mastitis detection performance is supported by other studies (Khatun et al., 2017; Norberg et al., 2004; Claycomb et al., 2009). In addition, farmers in our survey indicated to check further AMS data, especially EC, to evaluate alerts. Thus, this can be seen as a beneficial practice as cows with CM respective milk changes have increased EC levels (Norberg et al., 2004; Bansal et al., 2005). However, due to the much larger number of healthy cows, several factors could be extracted for the associations with SP. Most importantly, the trend in SCC from the monthly test days of DHIA was associated with SP for all producers and thus could help in the alert assessment by the farmer. This could be explained by the use of AMS detection systems as an early warning system. An animal with elevated cell count over a prolonged period of time may suffer from SCM with associated milk changes. Sensor systems of AMS detect mainly the non-visible changes in milk and generate alerts from this, so these animals are more likely to be misclassified in terms of CM detection. Use of DHIA data would counteract this and help identify such cows and better classify their AMS alerts. This result, in contrast to the results of Steeneveld et al. (2010), confirmed a benefit of non-AMS data in improving the detection of udder health problems by AMS. However, our survey found that only few farmers use DHIA data to decide whether that indicated cow also needs to be checked in the barn. Thus, there is an underutilized potential in combining DHIA data with AMS data for improved daily monitoring udder health in an AMS herd (Fredebeul-Krein et al., 2022). Cows with prolonged elevated cell counts should therefore be examined on a regularly basis, as on the one hand, legally normal milk may be falsely automatically separated, but on the other hand, these cows are at risk for development CM and eventually spreading mastitis pathogens such as *S. aureus* (Rainard et al., 2018). Consequently, it is critical to see that in many of the surveyed farms such cows are hardly ever subjected to a clinical examination in the event of an udder health warning.

With this in mind, the management practices in the catalog of measures for udder health in AMS need to be further encouraged to farmers (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz, 2012). The suggested control of the warning lists as well as the control of the udder health of the herd during the inspection in the barn, were carried out in their recommended frequency (twice a day) by a large part of the surveyed farms. However, a small number of farms do

this with insufficient frequency, which should be regarded as a risk to udder health and food safety, especially when considering the SN and SP of CM detection by AMS.

In conclusion, for CM detection on AMS farms, human monitoring of both the machine and the animals under care remains essential. The role of farmers and their management practices in ensuring udder health could be assessed for the first time and considered largely appropriate. Nevertheless, farmers should additionally consider, on the one hand, the test day data in combination with the AMS data and, on the other hand, especially the results of their udder health assessment of each cow in the barn in order to correctly interpret the AMS udder health warning list and thus ensuring food safety, milk quality, animal welfare and farm profitability.

This study is intended to help raise awareness of the above-mentioned issues and generate further discussion. Future studies should continue to examine commercial AMS farms working in the field. In particular, the effect of non-maintained AMS for udder health and detection of mastitis should be studied more closely. Furthermore, the possibilities of a follow-up of the mastitis occurrence have to be considered. From the author's point of view, there is a need for action on different levels: First, on the side of the manufacturers, who have to push the development of new cost-effective sensor technology for the exact determination of legally abnormal milk, as well as the design of warning lists for different mastitis stages. Second, on the side of experts and politicians, who should define the term abnormal milk more clearly for the discussion and distinction of the term clinical mastitis and abnormal milk, especially in connection with their diagnosis by AMS. And finally, on the side of the farmers, who should focus even more on the professional training for the work with an AMS and, at the same time, should not neglect the essential contact between human being and animal.

V. SUMMARY

On dairy farms with automatic milking systems (AMS), the detection of clinical mastitis (CM) is performed autonomously using sensors. This must be done reliably, as animal welfare as well as economy and food safety depend on this through autonomous separation of legally abnormal milk. Therefore, the objectives of this cross-sectional study were to determine the sensitivity (SN) and specificity (SP) of CM detection of AMS from the four most common manufacturers in Bavarian dairy farms and to identify routinely collected cow data (AMS and monthly test day data from the regional Dairy Herd Improvement Association (DHIA)) that could improve the SN and SP of CM detection. In addition, an online survey was designed to determine management practices for CM detection on AMS farms as well as farmers' personal opinions of their work with the AMS and the mastitis detection performance of the AMS. Bavarian dairy farms with AMS from the manufacturers DeLaval, GEA Farm Technologies, Lely, and Lemmer-Fullwood were recruited with the aim of sampling at least 40 cows with CM per AMS manufacturer in addition to clinically healthy cows. During a single farm visit, cow milking data were first extracted electronically from each AMS and then all lactating cows in the barn were examined for udder health status. Clinical mastitis was defined at least as the presence of visibly abnormal milk. In addition, available DHIA test results from the previous six months were collected. None of the producers provided a clear definition or alert for CM (i.e., visibly abnormal milk), so the SN and SP of the AMS udder health alert lists were evaluated individually for each producer based on clinical assessment results. Generalized linear mixed models (GLMMs) with herd as a random effect were used to determine the potential influence of routinely recorded parameters on SN and SP. A total of 7411 cows on 114 farms were surveyed; of these, 7096 cows could be matched to AMS data and were included in the analysis. The prevalence of CM was 3.4% (239 cows). When considering the 95% confidence interval (95% CI), all but one producer met the SN minimum threshold of >80%: DeLaval (SN: 61.4% (95% CI: 49.0%-72.8%)), GEA (75.9% (62.4%-86.5%)), Lely (78.2% (67.4%-86.8%)), and Lemmer-Fullwood (67.6% (50.2%-82.0%)). However, none of the AMSs evaluated met the SP minimum of 99%: DeLaval (SP: 89.3% (95% CI: 87.7%-90.7%)), GEA (79.2% (77.1%-81.2%)), Lely (86.2% (84.6%-87.7%)), and Lemmer-Fullwood (92.2%

(90.8%-93.5%)). With potential for an influence on SN and SP, different parameters could be found in a model. For all AMS producers, a correlation in between SP and cow classification based on somatic cell count (SCC) measurement from the last two DHIA test results could be detected: Cows that were above the threshold of 100,000 cells/ml for subclinical mastitis on both test days had lower odds of being classified as healthy by the AMS than cows that were below the threshold. Some findings were also reflected in the recorded experience of the farmers. From the survey, 47 complete responses from the 108 study participants contacted could be analyzed. A proportion of 68% of farmers agreed with the statement that they were very satisfied with the detection performance of CM by the AMS. However, almost half of the farmers (44%) felt that the number of cows reported as false positives by AMS was too high. Some management practices to counteract that were reported as follows: AMS warning lists indicating cows with potential udder health problems were checked twice a day by 68% and once a day or less by 27% of farmers. In addition to the presence of flakes on the milk filter (75%), recorded AMS data (78%), such as electrical conductivity, somatic cell count and milk color, were the most important factor in farmers' decisions about whether an indicated cow should also be examined in the barn.

In conclusion, the detection of CM was satisfactory in all AMS producers. However, the low SP results in unnecessary discarded milk and increased workload in assessing potentially false-positive mastitis cases. In this context, it is essential that farmers take into account all available data but more importantly the monitoring of their animals in the barn to make decisions about individual cows and ultimately ensure animal welfare, food quality and the economic viability of their farm.

VI. ZUSAMMENFASSUNG

In Milchviehbetrieben mit automatischen Melksystemen (AMS) wird die Erkennung von klinischer Mastitis (CM) sensorgestützt automatisch durchgeführt. Dies muss zuverlässig erfolgen, da hiervon sowohl Tierwohl als auch die Wirtschaftlichkeit der Milchviehbetriebe sowie die Lebensmittelsicherheit durch automatische Abtrennung rechtlich abnormer Milch abhängig ist. Die Ziele der Arbeit waren daher in einer Querschnittsstudie die Bestimmung der Sensitivität (SN) und Spezifität (SP) der CM-Erkennung von AMS der vier gängigsten Hersteller in bayerischen Milchviehbetrieben sowie die Identifizierung von routinemäßig erhobenen Kuhdaten (AMS und monatliche Testtagsdaten des regionalen Landeskontrollverbandes, Landeskuratorium der Erzeugerringe für tierische Veredelung in Bayern e.V. (LKV)), die die SN und SP der CM Erkennung verbessern könnten. Zusätzlich sollten durch eine Onlineumfrage Managementpraktiken zur Erkennung von CM in AMS-Betrieben und die persönliche Einschätzung der Landwirte zu ihrer Arbeit mit dem AMS sowie die Mastitiserkennungsleistung des AMS ermittelt werden. Bayerische Milchviehbetriebe mit AMS der Hersteller DeLaval, GEA Farm Technologies, Lely und Lemmer-Fullwood wurden mit dem Ziel rekrutiert, zusätzlich zu den klinisch gesunden Kühen mindestens 40 Kühe mit CM pro AMS-Hersteller zu beproben. Während eines einmaligen Betriebsbesuchs wurden zunächst die Melkdaten der Kühe elektronisch von jedem AMS extrahiert und dann alle laktierenden Kühe im Stall auf ihren Eutergesundheitsstatus untersucht. Klinische Mastitis wurde zumindest als das Vorhandensein von sichtbar abnormaler Milch definiert. Zusätzlich wurden die verfügbaren LKV-Testergebnisse der letzten sechs Monate gesammelt. Keiner der Hersteller gab eine klare Definition oder Alarm für CM (d.h. sichtbar abnorme Milch) an, daher wurden die SN und SP der AMS-Warnlisten für die Eutergesundheit für jeden Hersteller individuell auf der Grundlage der klinischen Untersuchungsergebnisse bewertet. Verallgemeinerte lineare gemischte Modelle (GLMM) mit der Herde als Zufallseffekt wurden verwendet, um den potenziellen Einfluss routinemäßig erfasster Parameter auf SN und SP zu bestimmen. Insgesamt wurden 7411 Kühe in 114 Betrieben untersucht; von diesen konnten 7096 Kühe den AMS-Daten zugeordnet werden und wurden in die Analyse einbezogen. Die Prävalenz der CM betrug 3,4% (239 Kühe). Bei

Betrachtung des 95 %-Konfidenzintervalls (95 % KI) erreichten alle Hersteller bis auf einen die SN-Mindestgrenze von >80 %: DeLaval (SN: 61,4 % (95 % KI: 49,0 %-72,8 %)), GEA (75,9 % (62,4 %-86,5 %)), Lely (78,2 % (67,4 %-86,8 %)) und Lemmer-Fullwood (67,6 % (50,2 %-82,0 %)). Keines der bewerteten AMS erreichte jedoch die SP-Mindestgrenze von 99 %: DeLaval (SP: 89,3% (95% KI: 87,7%-90,7%)), GEA (79,2% (77,1%-81,2%)), Lely (86,2% (84,6%-87,7%)), und Lemmer-Fullwood (92,2% (90,8%-93,5%)). Mit Potential für einen Einfluss auf SN und SP konnten verschiedene Parameter in einem Model gefunden werden. Bei allen AMS-Herstellern konnte ein Zusammenhang zwischen SP und der Klassifizierung der Kuh auf der Grundlage der Messung der somatischen Zellzahl aus den letzten beiden LKV-Testergebnissen erfasst werden: Kühe, die an beiden Testtagen über dem Schwellenwert von 100.000 Zellen/ml für subklinische Mastitis lagen, hatten geringere Chancen, vom AMS als gesund eingestuft zu werden, als Kühe, die unter dem Schwellenwert lagen. Einiger dieser Erkenntnisse spiegeln auch die erfassten Erfahrungen der Landwirte wider. Aus der Umfrage konnten 47 vollständigen Antworten der 108 kontaktierten Studienteilnehmer analysiert werden. Ein Anteil von 68% der Landwirte stimmte der Aussage zu, dass sie mit der Erkennungsleistung von CM durch das AMS sehr zufrieden sind. Allerdings empfand fast die Hälfte der Landwirte (44%) die Zahl der Kühe, die vom AMS als falsch-positiv gemeldet wurden, als zu hoch. Managementpraktiken um jenem entgegenzuwirken wurden folgendermaßen berichtet: Warnlisten des AMS, die auf Kühe mit potenziellen Eutergesundheitsproblemen hinweisen, wurden von 68% der Landwirte zweimal täglich und von 27% einmal pro Tag oder seltener überprüft. Neben dem Vorhandensein von Flocken auf dem Milchfilter (75%) waren die aufgezeichneten Daten des AMS (78%), insbesondere elektrische Leitfähigkeit, somatische Zellzahl und Milchfarbe, der wichtigste Entscheidungsfaktor für die Landwirte, ob eine angezeigte Kuh auch im Stall untersucht werden sollte.

Zusammenfassend lässt sich sagen, dass die Erkennung von CM bei allen AMS-Herstellern zufriedenstellend war. Die niedrige SP kann jedoch zu unnötig verworfener Milch und erhöhtem Arbeitsaufwand bei der Beurteilung potenziell falsch-positiver Mastitisfälle führen. In diesem Zusammenhang ist es unabdingbar, dass Landwirte weiterhin alle verfügbaren Daten aber auch die Überwachung ihrer Tiere im Stall in Entscheidungen über einzelne Kühe berücksichtigen, um letztlich Tierwohl, Lebensmittelqualität sowie Wirtschaftlichkeit ihres Betriebs sicherzustellen.

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VIII. APPENDIX

Survey on dairy farmers' management practices for and satisfaction with the detection of clinical mastitis by automatic milking systems in Bavaria, Germany.

Language: German

(EN) The aim of this questionnaire is to assess the practices with warnings of the milking robot on Bavarian dairy farms regarding udder health problems. Furthermore, the personal experiences and assessments of the farmer in this regard are queried, there are no wrong answers. The questionnaire is voluntary and anonymous. All data will be treated absolutely discretely.

[The survey was conducted via the open-source online tool LimeSurvey (LimeSurvey Project Team/Carsten Schmitz, 2012). In the following, question groups and text of the questions are presented true to the original]

(DE) Dieser Fragebogen dient dem Ziel, die Vorgehensweisen bei Warnhinweisen des Melkroboters zu Eutergesundheitsstörungen auf bayerischen Milchviehbetrieben zu erfassen. Des Weiteren werden die persönlichen Erfahrungen und Einschätzungen des Landwirtes diesbezüglich abgefragt, es gibt keine falschen Antworten. Der Fragebogen ist freiwillig und anonym. Alle Daten werden absolut vertraulich behandelt.

[Die Umfrage wurde über das open-source online tool LimeSurvey (LimeSurvey Project Team/Carsten Schmitz, 2012) durchgeführt. Im Folgenden werden Fragengruppierungen und Text der Fragen originalgetreu dargestellt.]

I. ALLGEMEINE BETRIEBSDATEN

1. Welchen Melkroboter (Automatisches Melksystem, kurz: AMS) besitzen Sie?

- | | | |
|--|---|--|
| <input type="checkbox"/> GEA - Sonstiges | <input type="checkbox"/> Lely Astronaut A3 | <input type="checkbox"/> Lemmer-Fullwood - Sonstiges |
| <input type="checkbox"/> GEA Mlone | <input type="checkbox"/> Next | <input type="checkbox"/> DeLaval - Sonstiges |
| <input type="checkbox"/> GEA Monobox | <input type="checkbox"/> Lely Astronaut A4 | <input type="checkbox"/> DeLaval VMS Classic |
| <input type="checkbox"/> GEA DairyRobot R9500 | <input type="checkbox"/> Lely Astronaut A5 | <input type="checkbox"/> DeLaval VMS V300 |
| <input type="checkbox"/> Lely - Sonstiges | <input type="checkbox"/> Lemmer-Fullwood Merlin | <input type="checkbox"/> DeLaval VMS V310 |
| <input type="checkbox"/> Lely Astronaut A2 | <input type="checkbox"/> Lemmer-Fullwood Merlin 225 | |
| <input type="checkbox"/> Lely Astronaut A2 Evolution | <input type="checkbox"/> Lemmer-Fullwood M ² erlin | |
| <input type="checkbox"/> Lely Astronaut A3 | | |

2. Wie groß ist zurzeit Ihre AMS-Herde (ohne Trockensteher)?

_____ Kühe

3. Wo lag die Tankzellzahl Ihrer Herde 2020 im Durchschnitt (laut MLP-Jahresbericht o. Ä.) ?

_____ Zellen in Tsd. /ml

II. ARBEIT MIT DER MILCHVIEHHERDE

Bitte denken Sie bei der Beantwortung der Fragen an einen normalen Arbeitstag mit Ihrer im AMS gemolkenen Herde.

- 4. Wie lange benötigt die gesamte tägliche Arbeit für die Milchviehherde im Stall (dies umfasst z.B. Füttern, Liegeboxen reinigen, etc.) ?**

Durchschnittlich _____ Stunde(n) pro Tag

- 5. Wie oft pro Tag wird Ihre Milchviehherde mittels Stallrundgang auf Eutergesundheit inspiziert?**

Bitte wählen Sie nur eine der folgenden Antworten aus:

- | | |
|--|--|
| <input type="checkbox"/> seltener als einmal | <input type="checkbox"/> drei- bis viermal |
| <input type="checkbox"/> einmal | <input type="checkbox"/> öfter als viermal |
| <input type="checkbox"/> zweimal | <input type="checkbox"/> keine Antwort |

- 6. Wie lange benötigt diese Euterinspektion der Herde pro Tag?**

Durchschnittlich _____ Minute(n) pro Tag

- 7. Wie viele Personen (Sie eingeschlossen) sind für diese Kontrolle der Eutergesundheit Ihrer Herde im Stall verantwortlich?**

_____ Person(en)

III. ARBEIT MIT DER MELKROBOTERSOFTWARE

- 8. Wie lange benötigt die gesamte tägliche Arbeit mit dem Melkroboterprogramm (bspw. zur Brunsterkennung, Futtereinstellung, Melkreihenfolge etc.) ?**

Durchschnittlich _____ Minute(n) pro Tag

- 9. Wie lange benötigt die Kontrolle der Warnlisten des AMS zur Eutergesundheit?**

Durchschnittlich _____ Minute(n) pro Tag

- 10. Wie oft werden die Warnlisten zur Eutergesundheit pro Tag kontrolliert?**

Bitte wählen Sie nur eine der folgenden Antworten aus:

- | | |
|--|--|
| <input type="checkbox"/> seltener als einmal | <input type="checkbox"/> drei- bis viermal |
| <input type="checkbox"/> einmal | <input type="checkbox"/> öfter als viermal |
| <input type="checkbox"/> zweimal | <input type="checkbox"/> keine Antwort |

- 11. Wie viele Personen (Sie eingeschlossen) arbeiten mindestens einmal pro Woche mit dem Melkroboterprogramm zur Überwachung der Eutergesundheit?**

_____ Person(en)

15. Gibt es sonstige Informationen, die Sie für die Überprüfung von neu angezeigten Kühen berücksichtigen? Falls ja, welche?

Bitte geben Sie Ihre Antwort hier ein: _____

16. Wie wird die neu angezeigte Kuh im Stall überprüft?

Bitte wählen Sie die zutreffende Antwort für jeden Punkt aus:

	sehr selten	selten	gelegentlich	oft	sehr oft	keine Antwort
das Euter angeschaut und durchgetastet	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
die ersten Milchstrahlen auf Veränderungen begutachtet	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
ein Schalmtest durchgeführt	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
eine aseptische Viertelgemelksprobe genommen und in ein Labor zur Erregerbestimmung eingeschickt	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

17. Wie viele Personen (Sie eingeschlossen) sind verantwortlich für die Kontrolle der auf den Warnlisten neu angezeigten Kühe im Stall?

_____ Person(en)

V. PERSÖNLICHE EINSCHÄTZUNG

18. Bitte bewerten Sie Ihr tägliches Arbeitsvolumen zu folgenden Tätigkeiten in Ihrem Betrieb:

	ich arbeite gar nicht in diesem Bereich	ich arbeite eher weniger in diesem Bereich	ich übernehme die Hälfte der gesamten Arbeit	ich arbeite vermehrt in diesem Bereich	nur ich arbeite in diesem Bereich
Eutergesundheitsüberwachung im Melkroboterprogramm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Kontrolle der Milchviehherde in Bezug auf Eutergesundheit	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Kontrolle der auf den Warnlisten des Melkroboters angezeigten Kühe im Stall	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

VI. DEMOGRAPHIE**20. Wie alt sind Sie?**

- | | | |
|------------------------------------|------------------------------------|------------------------------------|
| <input type="checkbox"/> unter 20 | <input type="checkbox"/> 31 bis 40 | <input type="checkbox"/> 51 bis 60 |
| <input type="checkbox"/> 21 bis 30 | <input type="checkbox"/> 41 bis 50 | <input type="checkbox"/> über 60 |

21. Geschlecht?

- | | | |
|-----------------------------------|-----------------------------------|---------------------------------|
| <input type="checkbox"/> weiblich | <input type="checkbox"/> männlich | <input type="checkbox"/> divers |
|-----------------------------------|-----------------------------------|---------------------------------|

22. Wie lange arbeiten Sie selbst bereits mit einem Melkroboter?

_____ Jahr(e)

IX. ACKNOWLEDGEMENTS

Mein besonderer Dank gilt meinem Doktorvater Herrn Prof. Dr. Rolf Mansfeld für die Überlassung des Dissertationsthemas sowie die Übernahme der Betreuung. Vielen Dank für Ihre stets herzliche Unterstützung, die Durchsicht der Manuskripte und aufmunternden Worte.

Besonders möchte ich mich bei meiner Mentorin, Frau Dr. Ulrike Sorge, bedanken. Ohne Sie wäre diese Doktorarbeit so nicht zustande gekommen. Herzlichen Dank Ulrike, für die geduldige und aufopferungsvolle Betreuung. Die vielen Stunden der Erklärungen, Durchsichten und Verbesserungsvorschläge und deine Mühen um uns Doktoranden sind unbezahlbar. Danke, dass du stets an uns geglaubt und uns während schwierigen Zeiten motiviert und aufgerichtet hast!

Ein weiterer herzlicher Dank gilt Herrn Prof. Dr. Marcus Doherr und Herrn Dr. Jan Harms für Ihre fachliche und menschliche Unterstützung. Ihre wertvollen Anmerkungen, Hilfestellungen und Ihr Fachwissen waren immer wieder Wegbereiter für neue Ideen.

Ohne die MitarbeiterInnen des Tiergesundheitsdienstes Bayern e.V. und der AMS-Firmen hätte es diese Studie nicht gegeben. Mein Dank gilt den TGD-MelktechnikerInnen für die Hilfe bei der Probennahme, den MitarbeiterInnen des Milchlabors und des Sekretariats für die Unterstützung in der Probensammlung, sowie den SpezialistInnen der AMS-Firmen und der Firmen DeLaval, GEA Farm Technologies, Lely und Lemmer Fullwood selbst, für die Bereitstellung der AMS-Daten und die stetige Ansprechbarkeit und Hilfe bei Fragen.

Mein Dank gilt auch allen LandwirtInnen und BetriebshelferInnen der an der Studie teilgenommenen Betriebe.

Ein nicht unwesentlicher Anteil am Gelingen dieser Arbeit haben auch meine Freunde, denen ich an dieser Stelle meinen Dank aussprechen möchte. Vielen Dank für eure aufbauende Worte, vor allem jedoch für die schöne gemeinsame Zeit!

Von ganzem Herzen möchte ich mich bei Lucie bedanken. Ohne dich wäre ich nie so weit gekommen. Deine Bedeutung und Unterstützung kann man nicht in Worte fassen - Danke Lucie!

Zu guter Letzt gilt ein tiefer Dank meiner Familie, insbesondere meinen Eltern, Geschwistern und Großeltern für die stetige und unermüdliche Unterstützung. Ihr habt mir immer den Rücken freigehalten, mich ermutigt und seid mir ein liebevolle Stütze. Danke, einfach für alles!
Danke auch Dir, Paschi, für die seelisch wichtigen Streicheleinheiten.