ruminants

MDPI

Article

Milking System Changeover and Effects Thereof on the Occurrence of Intramammary Infections in Dairy Cows

Pauline Katthöfer [1], Svenja Woudstra [2], Yanchao Zhang [1], Nicole Wente [1], Franziska Nankemann [1], Julia Nitz [1], Jan Kortstegge [1] and Volker Krömker [2,*]

[1] Department of Microbiology, Faculty of Mechanical and Bioprocess Engineering, University of Applied Sciences and Arts, 30453 Hannover, Germany; pauline.katthoefer@tiho-hannover.de (P.K.); yanchao.zhang@hs-hannover.de (Y.Z.); nicole.wente@hs-hannover.de (N.W.); franziska.nankemann@hs-hannover.de (F.N.); julia.nitz@hs-hannover.de (J.N.); jan.kortstegge@tiho-hannover.de (J.K.)

[2] Department of Veterinary and Animal Sciences, Section for Production, Nutrition and Health, Faculty of Health and Medical Sciences, University of Copenhagen, 1870 Frederiksberg C, Denmark; svenja.woudstra@sund.ku.dk

* Correspondence: volker.kroemker@sund.ku.dk

Simple Summary: The effects resulting from changing the milking system at a dairy farm are manifold. With the installation of an automatic milking system, several management aspects change, such as feeding, cubicle cleaning, and the daily milking routine. Concerning udder health, a deterioration is often assumed after the changeover. The current study investigated the impact of the transition from a conventional milking system to an automatic milking system on udder health at one conventional dairy farm in Northern Germany. A significant increase in the quarter-level somatic cell count was confirmed, as well as an improvement in teat-end condition and udder hygiene during the study period. The transition to the automatic milking system had no impact on the new intramammary infection rate. This trial, therefore, confirmed that changing the milking system does not necessarily have a negative effect on udder health.

Abstract: Adopting a new milking system at a dairy farm causes various changes. This study examined the impact on udder health when changing from a conventional milking system to an automatic milking system. For this purpose, quarter milk samples were taken six times from 138 cows at one conventional dairy farm in Northern Germany over a five-week period around the time of the milking system changeover. To assess udder health, the absolute number of new intramammary infections and the causative pathogen genera and species were analysed for each individual study time point. Pathogen species were detected using matrix-assisted laser desorption ionisation time-of-flight, and the infection dynamics were analysed using two Poisson regression models. In addition, the prevalence and incidence of new intramammary infections and the infection dynamics of the four most frequently isolated pathogen species were calculated. Mixed models were used to determine the development of the new infection rate, the somatic cell count, the teat-end condition, and the udder hygiene between the individual study time points and to compare the new infection rate before and after the changeover of the milking system. After the automatic milking system had been installed, a significant increase in the quarter-level somatic cell count occurred ($p < 0.001$). Two days before the installation of the automatic milking system, the mean quarter-level somatic cell count was 11,940 cells/mL milk; one sampling date later, 8 days after the changeover, a mean quarter-level somatic cell count of 60,117 cells/mL milk was measured. The significant increase in somatic cell count was probably caused by the time between the last milking and the quarter milk sampling. Additionally, significantly more udders were scored as clean

check for updates

Academic Editor: Kiro Petrovski

Received: 24 November 2024
Revised: 21 December 2024
Accepted: 2 January 2025
Published: 4 January 2025

Citation: Katthöfer, P.; Woudstra, S.; Zhang, Y.; Wente, N.; Nankemann, F.; Nitz, J.; Kortstegge, J.; Krömker, V. Milking System Changeover and Effects Thereof on the Occurrence of Intramammary Infections in Dairy Cows. *Ruminants* **2025**, *5*, 1. https://doi.org/10.3390/ruminants5010001

8 days (95%) and 15 days (96%) after the changeover of the milking system compared to at the last sampling date (88%). Also, significantly more teat ends were classified as free of hyperkeratosis 15 days (80%) compared to 22 days (67%) after the changeover of the milking system. The highest number of absolute new intramammary infections was detected 8 days before the transition of the milking system (28.6%). The lowest number of absolute new intramammary infections occurred 8 days after the change to the automatic milking system (11.0%). Minor mastitis pathogens, such as non-*aureus* staphylococci and coryneform bacteria, were mainly responsible for the development of new intramammary infections. The most frequently isolated pathogen species were *Staphylococcus sciuri*, *Staphylococcus chromogenes*, *Staphylococcus haemolyticus*, and *Corynebacterium amycolatum*, with a prevalence of up to 23.9, 10.7, 8.4, and 5.3%, respectively. By comparing the new infection rate before and after the changeover of the milking system, it was possible to establish that the changeover to the automatic milking system had no significant influence on the new intramammary infection rate ($p = 0.988$). Therefore, this trial confirmed that the changeover from a conventional milking system to an automatic milking system had no negative influence on udder health.

Keywords: cattle; udder health; mastitis; mastitis pathogens; somatic cell count; teat-end condition; udder hygiene; quarter level; lactation

1. Introduction

Mastitis remains one of the primary diseases affecting dairy cows [1]. In addition to reduced animal welfare, mastitis leads to economic disadvantages such as treatment costs, discarded milk, and reduced milk yield [2,3]. Cows that have suffered from mastitis are more likely to be culled; moreover, mastitis increases the probability of death [4].

Crucial for the control of mastitis is the reduction of new intramammary infections (NIMIs) [5]. An NIMI is defined as the isolation of a pathogen in a culture of milk that has not been previously isolated from this mammary gland [6]. The development of an NIMI is multifactorial, involving the individual resistance of the cow, the udder quarter, and the presence of microorganisms in the cow's environment [7]. These factors are influenced by farm management practices. Key management factors that significantly affect the development of NIMIs include the milking procedure and the milking system [8].

In addition to conventional milking systems, automatic milking systems (AMSs) are becoming increasingly commonplace [9]. Different milking systems entail different risk factors for the development of NIMIs. Teat cleaning before and teat dipping after milking are not adapted to the individual cow's soiling and have not yet been monitored in most AMSs. However, there is evidence that appropriate udder preparation and post-milking teat disinfection support the prevention of NIMIs [10,11].

Milking in an AMS is quarter-based, which should prevent overmilking of individual udder quarters and thus protect the teat-end condition. Teat-end hyperkeratosis at drying-off increases the risk of NIMI with environmental pathogens, as the teat canal is likely not properly closed, and pathogens could better adhere to the hyperkeratotic teat ends [12]. However, contradictory observations were reported for hyperkeratosis as a risk factor for the development of NIMI during lactation. The probability of an intramammary infection (IMI) increased with deteriorating teat-end condition; in particular, severe teat-end hyperkeratosis was associated with *Staphylococcus aureus* intramammary infections [13,14]. In contrast, no effect of the teat-end condition on the risk of the development of NIMI could be confirmed in a more recent study [15].

In an AMS, the transmission of pathogens between cows cannot be prevented by a previously defined milking order, but a preset intermediate flushing or steaming of the milking liners is possible to prevent the transmission of pathogens. Flushing and steaming are indispensable because many cows are milked with one milking cluster, which increases the likelihood of pathogen transmission between cows.

Unlike in a conventional milking system, the milking interval and milking frequency are individual for each cow in the AMS. A higher milking frequency corresponds to more flushing of the teat canal (i.e., more often milk is ejected from the teat), which prevents the proliferation of pathogens in the udder and thus, reduces the probability of developing subclinical mastitis [16]. However, a high milking frequency leads to higher stress on the teat ends, as the teats have less time to recover between milkings, the teat canal is open more frequently, and pathogens may enter [7]. In contrast, a lower milking frequency and a longer milking interval create more time for pathogens to multiply in the udder tissue.

Another difference concerns mastitis detection. In an AMS, the milk quality is controlled by sensor-based systems. This allows for the detection of both clinical and subclinical mastitis. In a conventional milking system, clinical mastitis can be recognised visually by the milking staff.

The risk factors for the development of NIMI in an AMS have been repeatedly analysed, as described above. However, how udder health is affected as a result of changing the milking system has often only been examined by using the somatic cell count (SCC) of the cows as a reference for udder health. In some studies, no change in the SCC was observed after the milking system changeover [17,18], but in others, an increase in the SCC and mastitis frequency has been reported [19,20].

As AMS technology has evolved in recent years, the present study aimed to observe the infection dynamics of NIMI during the conversion from a conventional milking system to an AMS.

2. Materials and Methods

For the study, one conventional farm was selected, which was visited six times. A prerequisite for choosing the farm was the planned milking system changeover from a conventional milking system to milking with an AMS. The farm was located in North Rhine-Westphalia, Germany. In total, the farm kept 198 Holstein Friesian cows. Lactating and dry cows were housed in a free stall barn; periparturient cows were transferred to a deep litter free stall. Before changing the milking system, the cows were milked twice a day in a 2 × 10 steep herringbone milking parlour from GEA Farm Technologies GmbH, Bönen, Germany, with automatic take-off, but without milk yield measurement. The pre-dipping and post-dipping of teats were performed at every milking. The teats were cleaned with reusable cloths. The farm participated in the local monthly dairy herd improvement (DHI) programme. Thus, it was possible to document the milk yield, the cow-level SCC, the lactation number, and the days in milk at the beginning of the trial from the last three DHI records before the AMS was installed (Table 1). The average energy-corrected milk yield (4.0% fat and 3.4% protein) per cow from the DHI five days before the milking system changeover was 38.5 kg. The average SCC from the individual cows was 166,000 cells/mL of milk. The average energy-corrected annual milk production 12 months before the trial started was 11,657 kg. The milking system was changed on 22 May 2023, and four GEA Dairy Robots 9500 (GEA Farm Technologies GmbH) were installed. Stimulation, teat cleaning, forestripping, milking, and dipping of the teats have, since then, been carried out in the milking liner. Each step of the milking process is performed in the respective milking liner. At the same time that the pulsation stimulates the teats, the teats are cleaned with water to remove the dirt from the teats. The wastewater is disposed of via a dump

line. After milking, the individual teats are dipped, while still under vacuum, in a stretched position [21].

Table 1. Characteristics of the cows participating in the trial before the milking system changeover.

	Number of Cows (%)
Number of lactations	
1st lactation	41 (29.7)
2nd lactation	31 (22.5)
≥3rd lactation	66 (47.8)
Stage of lactation	
<100 days in milk	43 (31.2)
100–180 days in milk	38 (27.5)
181–305 days in milk	42 (30.4)
>305 days in milk	8 (5.8)
Missing data	7 (5.1)
Milk yield (kg per day)	
20–30 kg	35 (25.4)
31–40 kg	54 (39.1)
41–50 kg	40 (29.0)
51–60 kg	9 (6.5)
In total	138 (100)

Quarter milk sampling for the study was performed on six sampling dates in May and June 2023. Out of a total of 198 cows on the farm, 138 cows participated in the study. All dairy cows in lactation during the six visits, with four functional udder quarters and no antimicrobial treatment during the previous 30 days and throughout the study, were included. Ten cows were not sampled on any of the six sampling dates, i.e., two cows were treated with antibiotics during the trial, and their samples were therefore no longer taken, and quarter milk samples from the other eight cows were missing during sampling. None of the lactating cows on the farm were treated with medication to prevent NIMI.

After changing the milking system, the following data were obtained from the AMS: milk yield, milking frequency, milk flow, and individual time in hours after the last milking until quarter milk sampling. The installed AMS was not equipped with a cell count sensor. The SCCs shown were therefore determined either from the DHI or from the quarter milk samples. The bulk milk SCC was additionally used for comparison with the quarter milk sample SCC during the period of the milking system changeover. The bulk milk SCC was 98,000 (23 May), 100,000 (25 May), 165,000 (27 May), 111,000 (02 June), 116,000 (04 June), 95,000 (06 June), and 124,000 (08 June) cells/mL of milk. The milking system changeover was accompanied by a modification in the feeding ration and cubicle cleaning. Due to the change in the milking system, the total mixed ration was replaced by a partly mixed ration, and each cow received an additional 3–6 kg of concentrate per day during milking in the AMS. Before the changeover of the milking system, cubicle cleaning was carried out twice a day while cows were being milked. After the transition to the AMS, manure and soiled bedding material were removed from the cubicles irrespective of milking, without moving the cows.

2.1. Quarter Milk Samples

The sampling took place three times before and three times after the AMS had been installed. The first sampling date was 14 days (−14 days), the second sampling date was 8 days (−8 days), and the third sampling date was 2 days (−2 days) before the milking system changeover. The fourth sampling took place 8 days (+8 days), the fifth sampling

15 days (+15 days), and the sixth and last sampling 22 days (+22 days) after the milking system changeover. Thus, there were 5 to 10 days between each sampling date. All milk samples were taken in the milking parlour by three to five veterinarians. During the first three sampling dates, sampling was performed directly before the daily milking in the milking parlour. For the last three sampling dates, the cows were specially driven into the old milking parlour, regardless of the individual time elapsed after the past milking in the AMS.

Quarter milk samples were collected according to the German Veterinary Medical Association guidelines [22]. All veterinarians wore disposable gloves during the trial. First, the teats were pre-dipped and cleaned to remove coarse dirt. Three streams of milk were milked from each teat and discarded. Subsequently, the teat ends were cleaned and disinfected with a disposable wipe previously soaked in 70% ethanol. For quarter milk sampling, the tubes were opened under the cow, held horizontally and closed under the udder after filling to avoid contamination. The test tubes contained the preserving agent boric acid (5%) (Ly20) [23]. The samples were cooled in a cooling box and transferred to the laboratory (Department of Microbiology, Faculty of Mechanical and Bioprocess Engineering, Hannover University of Applied Sciences and Arts, Hannover, Germany) for analysis.

2.2. Laboratory Procedures

The milk samples were analysed according to the guidelines of the German Veterinary Medical Association [22]. The SCC was measured by flow cytometry with the SomaScope™ Smart (Delta Instruments B.V., Drachten, The Netherlands). For microbiological analysis, 10 µL of each milk sample was streaked on one quadrant of an esculin blood agar plate (5% defibrinated sheep blood, Oxoid Deutschland GmbH, Wesel, Germany), incubated at 37 °C, and examined after 24 h and 48 h. The growing colonies were analysed by matrix-assisted laser desorption ionisation time-of-flight (MALDI TOF, Bruker Daltonics, microflex LT/SH smart, MBT Compass Library, V8) for genus and species identification, as described by Randall et al. [24].

Yeasts, moulds, and Prototheca were differentiated by their cell morphology. Therefore, gram staining was performed. If ≥5 colonies of the same environmental-associated mastitis-causing pathogen were growing on the plate, the milk sample was classified as microbiologically positive. One growing colony of *Staphylococcus aureus*, *Streptococcus agalactiae*, *Trueperella pyogenes*, and *Streptococcus dysgalactiae* was sufficient to classify the milk sample as microbiologically positive. If >2 different pathogens were growing on one plate, the milk sample was considered contaminated, but *Staphylococcus aureus*, *Streptococcus agalactiae*, *Trueperella pyogenes*, and *Streptococcus dysgalactiae* were taken into account.

2.3. Data Collection

2.3.1. Udder Hygiene

By using the scoring chart published by Schreiner and Ruegg [25], udder hygiene was scored for every cow at every farm visit. Udder hygiene was scored in the milking parlour during quarter milk sampling. The udders were assigned the following scores: udder free of dirt (Score 1), udder slightly dirty (Score 2), udder covered with 10–30% dirt (Score 3), and udder covered with caked-on dirt on more than 30% of the surface (Score 4).

2.3.2. Teat-End Condition

Teat-end conditions were scored using the classification system of Mein et al. [26]. The change of the teat-end condition is considered a long-term effect. This change takes two to eight weeks to occur and provides information on milking management [26]. Therefore, the teat-end condition was scored at every second farm visit. The four-point scale divides

the teat ends as follows: no ring of hyperkeratosis visible at the teat end (Score 1), a raised ring with either no roughness or slight roughness at the teat orifice (Score 2), a raised rough ring and old keratin shreds visible at the teat end (Score 3), or a raised rough and cracked ring at the teat end (Score 4).

2.4. Definitions

The following definitions were used for the analysis of the NIMI, including the prevalence and NIMI incidence of mastitis pathogens.

An NIMI was considered to be present if a pathogen was cultivated from a quarter milk sample, and this pathogen had not been isolated in the quarter milk sample collected one sample date earlier, irrespective of the result of the penultimate sampling. If two pathogens with different genera were cultured from one quarter milk sample, a mixed NIMI was considered to be present.

For the prevalence and NIMI incidence determination of pathogen species, every cultivated pathogen was counted, regardless of whether it was a mixed or a single NIMI.

The occurrence of certain pathogens was quantified using their prevalence. The prevalence of a pathogen refers to the number of quarters infected with this specific pathogen in relation to the total number of quarters sampled at a given time. For the NIMI incidence (%) of a pathogen species, the number of NIMIs at a study time point was divided by the number of susceptible quarters. The susceptible quarters were all quarters that had not been infected with the respective pathogen in the previous quarter milk sample.

2.5. Statistical Analysis

The SPSS 28.0 program (IBM, Inc., Chicago, IL, USA) and R were used to analyse the data set. Two models were calculated in R to determine the transmission rate of individual pathogens. This rate indicates the rate per unit of time at which an infected quarter will infect an uninfected quarter, whereby reinfection of previously cured quarters was possible. Udder quarters were the statistical units used in this model.

The Poisson regression model was used, as described by Zadoks et al. [13], to estimate the transmission rate β, as follows:

$$\widehat{\log(I_N)} = \log(\beta) + \log\left(\frac{S_{int} * I_{int}}{Nint}\right),$$

with I_N being the number of observed new infections, β the transmission rate, and S_{int}, I_{int}, and N_{int} the number of susceptible, infected, and total quarter days at risk, respectively.

This model simulates contagious transmission, with the number of new infections being dependent on the number of infected quarter days at risk. Missing data points (missing samples and contaminated samples) were categorised in the analysis as follows: missing data between two positive samples were also considered positive; missing data between two negative samples were considered negative. Missing data between one positive and one negative sample were considered negative. Additionally, we used the following model that assumes a fixed rate of transmission:

$$\widehat{\log(I_N)} = \log(\beta) + \log(S_{int}),$$

as used previously for *Corynebacterium* spp. [27]. The Akaike information criterion (AIC) of both models (at the species level) was compared to assess which model best fitted the observed data.

The individual udder quarter was also used as a statistical unit for further statistical analysis. First, descriptive statistics of the outcome variables were generated by the study time point. The raw NIMI data for the subsequent time points were calculated after

taking into account the IMI at the first time point of the study. Then, various mixed models were calculated to determine whether the target variables (NIMI, teat-end condition, $\log_{10}$SCC, udder hygiene score) differed between the individual study time points. In each case, the time point was considered as a repeated measurement. A diagonal covariance matrix was assumed. The cows and the quarters within cows were considered as random effects. The random effects were not significant in the models but were kept as design variables. Generalised linear mixed models were used for the target variables NIMI, teat-end condition, and udder hygiene score, using logit link and binomial response (logistic regression), and a linear mixed model was used for the target variable $\log_{10}$SCC. The target variables teat-end condition and udder hygiene score were coded binomially (Score 1 or 2 vs. Score 3 or 4). Statistical significance was set at $p \leq 0.05$. Model fit was assessed by checking the normality of the residuals. For the presentation of the development of the quarter-level SCC, the $\log_{10}$SCC was retransformed into the SCC.

3. Results

3.1. Cytomicrobiological Results of the Quarter Milk Samples and the Evaluation of the NIMIs with the Causative Pathogens

The cytomicrobiological results of all quarter milk samples are presented in Table 2. NIMIs were evaluated from the second sampling date, 8 days before the milking system changeover. The results of the first sampling, 14 days before the changeover, counted as the base value. On this sampling date, a total of 552 quarters were sampled, 190 quarter milk samples of which showed no growth, and 120 of which were contaminated. Hence, 43.8% of the sampled quarters were infected with a pathogen 14 days before the milking system changeover. Existing IMIs were mainly associated with non-*aureus* staphylococci (NAS; 28.6%), mixed infections (10.5%), and *Corynebacterium* spp. (2.7%). The cultivated pathogens were mainly minor mastitis pathogens.

Table 2. Microbiological results of all quarter milk samples obtained throughout the study.

Quarter Sample Status	% (Number of Quarters)						
	−14 Days [1]	−8 Days [1]	−2 Days [1]	+8 Days [1]	+15 Days [1]	+22 Days [1]	NIMIs [2] During the Entire Study
In total	100 (552)	100 (548)	100 (524)	100 (536)	100 (536)	100 (540)	100 (558)
No growth	34.4 (190)	50.5 (277)	57.8 (303)	78.9 (423)	64.9 (348)	76.3 (412)	
Contaminated	21.7 (120)	9.9 (54)	9.0 (47)	1.9 (10)	1.9 (10)	2.6 (14)	
NAS [3]	28.6 (158)	25.2 (138)	18.7 (98)	11.4 (61)	17.5 (94)	10.0 (54)	60.8 (339)
Mixed infections	10.5 (58)	10.5 (58)	6.3 (33)	4.1 (22)	11.0 (59)	4.6 (25)	20.4 (114)
Corynebacterium spp.	2.7 (15)	2.9 (16)	6.5 (34)	2.4 (13)	2.4 (15)	4.6 (25)	15.8 (88)
Streptococcus spp.	0.9 (5)	0.2 (1)	0.4 (2)	0.7 (4)	0.6 (3)	0.4 (2)	0.4 (2)
Yeasts	0.4 (2)	0.4 (2)	0.6 (3)	0.4 (2)	0.7 (4)	0.4 (2)	0.7 (4)
Other pathogens [4]	0.4 (2)	0.4 (2)	0.4 (2)	0.2 (1)	0.4 (2)	0.6 (3)	0.9 (5)
Gram-negative pathogens [5]	0.2 (1)					0.6 (3)	0.9 (5)
Enterococcus spp.	0.2 (1)				0.2 (1)		0.2 (1)

[1] −14 days, −8 days, −2 days: 14 days, 8 days, and 2 days before the milking system changeover; +8 days, +15 days, +22 days: 8 days, 15 days, and 22 days after the milking system changeover; [2] new intramammary infection; [3] non-*aureus* staphylococci; [4] *Bacillus* spp., *Lactococcus garviae*, *Glutamicibacter* spp.; [5] *Acinetobacter* spp., *Escherichia coli*, *Proteus vulgaris*.

A total of 558 NIMIs occurred during the entire study. NAS caused 60.8% of the NIMIs; *Corynebacterium* spp. was responsible for 15.8% of the NIMIs. A total of 20.4% of the NIMIs

were mixed NIMIs. Gram-negative bacteria caused 0.9% of all cases of NIMI in the study. Other bacteria, like *Bacillus* spp., *Lactococcus garviae*, and *Glutamicibacter* spp., infected 0.9% of new udder quarters. Gram-negative bacteria included *Acinetobacter* spp., *Escherichia coli*, and *Proteus vulgaris*. Yeasts (0.7%), *Streptococcus uberis* (0.4%), and *Enterococcus* spp. (0.2%) caused the fewest NIMIs.

Absolute NIMIs were analysed five times. Eight days before transition, 28.6% NIMIs were detected. Two days before the changeover, 23.6% of NIMIs had been proven. Eight days after the changeover to the AMS, 11.0% NIMIs were found, presenting the fewest cases of NIMI in the entire study. Fifteen days after the changeover, NIMIs peaked at 27.4%. At the last sampling date, 22 days after the changeover, 13.1% NIMIs were detected (Table 3).

Table 3. Absolute number of quarters with a new intramammary infection detected at each individual study time point.

	% (Number of Quarters)				
	−8 Days [1]	**−2 Days [1]**	**+8 Days [1]**	**+15 Days [1]**	**+22 Days [1]**
NIMI [2]	28.6 (157)	23.6 (124)	11.0 (59)	27.4 (147)	13.1 (71)
No NIMI [2]	71.4 (391)	76.3 (400)	89.0 (477)	72.6 (389)	86.9 (469)
In total	100 (548)	100 (524)	100 (536)	100 (536)	100 (540)

[1] −8 days, −2 days: 8 days and 2 days before the milking system changeover; +8 days, +15 days, +22 days: 8 days, 15 days, and 22 days after the milking system changeover; [2] new intramammary infections.

3.2. Most Frequently Isolated Pathogen Species

The most frequently isolated pathogen species that caused an NIMI were Staphylococcus sciuri (*S. sciuri*), Staphylococcus chromogenes (*S. chromogenes*), Staphylococcus haemolyticus (*S. haemolyticus*), and Corynebacterium amycolatum (*C. amycolatum*). The highest incidence of NIMI with *S. sciuri* (14.5%) occurred 8 days before the milking system changeover, and the lowest NIMI incidence occurred at the first study time point after the changeover (4.1%). *S. chromogenes* showed the lowest NIMI incidence at the last study time point (2.3%), whereby most NIMIs with *S. chromogenes* in susceptible quarters were detected 15 days after the milking system changeover (8.0%). Like *S. sciuri*, the incidence of NIMI with *S. haemolyticus* was the highest at the beginning of the trial (5.8%) and the lowest 8 days after the changeover (1.7%). The lowest incidence of NIMI with *C. amycolatum* was also detected 8 days after the changeover (1.8%), and the highest NIMI incidence was detected 2 days before the changeover (4.3%) (Table 4).

Table 4. New intramammary infection incidence of the most frequently detected pathogen species during the trial.

Pathogen Species	**Number of Quarters**				
	−8 Days [1]	**−2 Days [1]**	**+8 Days [1]**	**+15 Days [1]**	**+22 Days [1]**
Staphylococcus sciuri					
Susceptible quarters	420	417	478	498	464
NIMI [2]	61	22	19	62	19
NIMI [2] incidence	14.5%	5.3%	4.0%	12.4%	4.1%
Staphylococcus chromogenes					
Susceptible quarters	507	490	498	503	482
NIMI [2]	28	20	15	40	11
NIMI [2] incidence	5.5%	4.1%	3.0%	8.0%	2.3%

Table 4. *Cont.*

Pathogen Species	Number of Quarters				
	−8 Days [1]	−2 Days [1]	+8 Days [1]	+15 Days [1]	+22 Days [1]
Staphylococcus haemolyticus					
Susceptible quarters	517	502	515	523	508
NIMI [2]	30	16	9	28	14
NIMI [2] incidence	5.8%	3.2%	1.7%	5.4%	2.8%
Corynebacterium amycolatum					
Susceptible quarters	541	533	514	522	522
NIMI [2]	7	23	9	11	20
NIMI [2] incidence	1.3%	4.3%	1.8%	2.1%	3.8%

[1] −8 days, −2 days: 8 days and 2 days before the milking system changeover; +8 days, +15 days, +22 days: 8 days, 15 days, and 22 days after the milking system changeover; [2] new intramammary infections.

3.3. Prevalence of the Four Most Frequently Isolated Pathogen Species

The prevalence of the most frequently isolated pathogens is shown in Figure 1. The highest prevalence of *S. sciuri* was detected 14 days and 8 days before the milking system changeover (23.9%), and the lowest prevalence was detected 22 days after the changeover (3.9%). *S. chromogenes* was the only pathogen to show the highest prevalence of 10.7% after the milking system changeover but also the lowest prevalence 22 days after the changeover (5.7%). *S. haemolyticus* was most frequently detected 8 days before the changeover (8.4%), and the fewest udder quarters were infected 8 days after the changeover (3.0%). *C. amycolatum* was most frequently detected 2 days before the milking system changeover (5.3%) and was the only pathogen with the lowest prevalence 14 days before the changeover (2.0%). For visualisation, the NIMI incidence and prevalence of the four most frequently isolated pathogen species are shown in Figure 1.

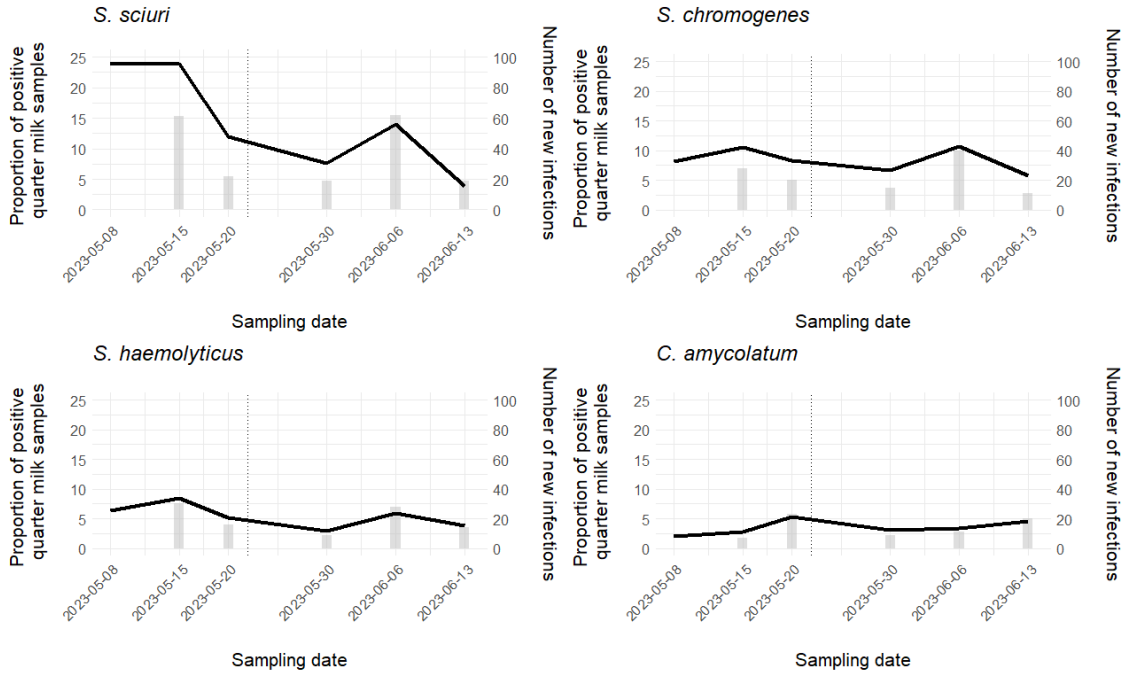

Figure 1. Pathogen species prevalence (proportion of positive quarter milk samples = black line) and the number of new intramammary infections (number of new infections = bars) of the four most frequently isolated pathogen species at the respective sampling dates. The dotted lines visualise the time of the milking system changeover.

3.4. New Intramammary Infection Rate

The NIMIs were analysed for every sampling date. These data were used to calculate a generalised linear mixed model that compared the NIMI rate before and after the changeover of the milking system (Table 5). No significant difference was determined for the NIMI rate before and after the changeover ($p = 0.988$).

Table 5. Generalised linear mixed model for the comparison of the new intramammary infection rate before and after the milking system changeover.

Dependent Variable	Independent Variable (Reference Category)	ß [1]	SE [2]	OR [3]	*p*-Value	95% CI [4] (OR)
New intramammary infection rate	After the changeover [5] (before the changeover [6])	0.548	13.261	0.578	0.988	0.000–1.002
	After the changeover [5] (before the changeover [6])	0				

[1] regression coefficient; [2] standard error; [3] odds ratio; [4] confidence interval; [5] sampling dates +22 days, +15 days, +8 days after the milking system changeover; [6] sampling dates −2 days, −8 days before the milking system changeover.

In order to investigate the possible effect of the different time periods between sampling on the NIMI rates, the effect was estimated in a further model using estimated means, and then the NIMI rates were corrected accordingly. The corrected estimated NIMI rates were compared using post hoc tests. The *p* values were corrected according to the Bonferroni–Holm correction (Figure 2).

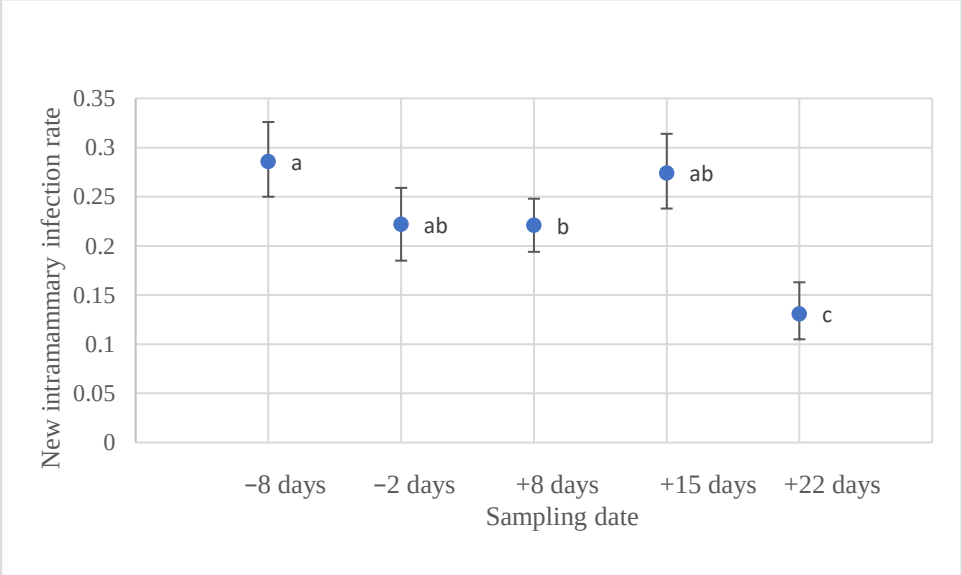

Figure 2. Estimated mean values of the new intramammary infection rate for each study time point. The different time periods between the study time points were taken into account, and the *p*-values were corrected according to the Bonferroni–Holm correction. Significances are labelled, whereby significantly different results were assigned different letters. The new intramammary infection rate was significantly different between sampling date −8 days and +8 days ($p = 0.036$). In addition, the new intramammary infection rate was significantly different at the last sampling date (+22 days) compared to that at all other sampling dates.

3.5. The Somatic Cell Count

The SCC was determined six times for each udder quarter throughout the trial by measuring the SCC of each quarter milk sample. The $\log_{10}$SCC was subsequently calculated

from these values. The quarter-level SCC was significantly different between the individual study time points ($p < 0.001$). An increase in the quarter-level SCC was observed after installing the AMS (Figure 3).

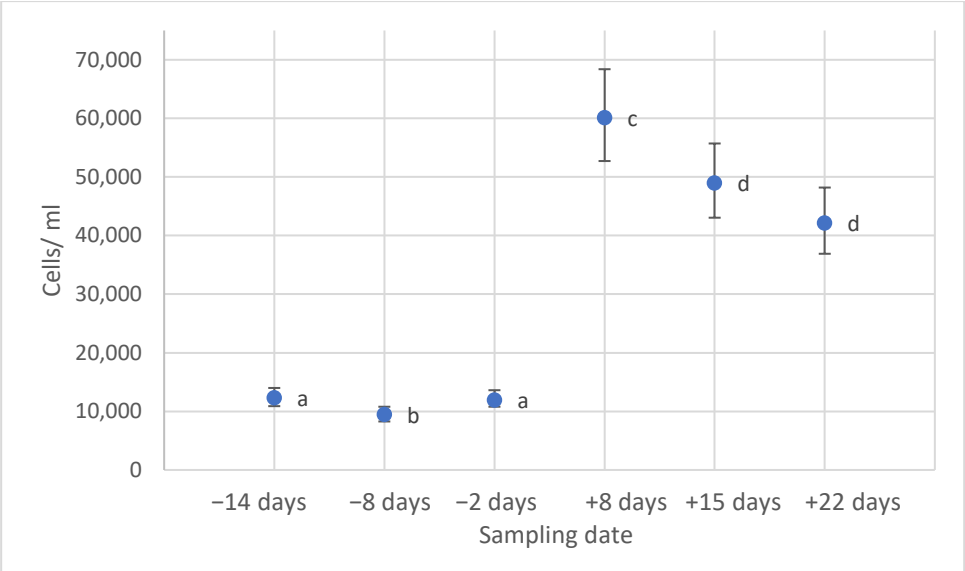

Figure 3. Change in the somatic cell count over the course of the study. Estimated mean values and 95% confidence interval (upper and lower bound) of the quarter-level SCC are shown at the respective sampling date. The values were estimated by calculating a generalised linear mixed model for the $\log_{10}$SCC (dots = estimated mean; error bars = 95% confidence interval). Significances are labelled, whereby significantly different results are assigned different letters.

3.6. Teat-End Condition

Scoring of the teat-end condition was performed at every second quarter milk sampling and additionally at the last sampling date. Teat-end scores were clustered, and comparisons were made between teat ends with no ring of hyperkeratosis (Score 1) or a raised ring with no roughness or slight roughness (Score 2) and teat ends with a raised rough ring (Score 3) or cracked teat ends (Score 4). Teat-end conditions were significantly different at the sampling date +15 days after the milking system changeover compared to teat-end conditions +22 days after the transition ($p < 0.001$) (Table 6). At the sampling date 15 days after the transition, more teats were scored as teat ends with no ring of hyperkeratosis (Score 1) or with a raised ring with no roughness or slight roughness (Score 2).

Table 6. Final generalised linear mixed model for the teat-end condition.

Dependent Variable	Independent Variable (Reference Category)	ß [1]	SE [2]	OR [3]	*p*-Value	95% CI [4] (OR)
Teat-end condition	intercept	0.693	0.914	2.0	<0.001	1.672–2.392
	−14 days [5] (teat-end condition +22 days)	0.116	0.1299	1.124	0.370	0.871–1.449
	−2 days [5] (teat-end condition +22 days)	−0.134	0.1282	0.874	0.295	0.680–1.124
	+15 days [5] (teat-end condition +22 days)	0.707	0.1419	2.028	<0.001	1.536–2.679
	+22 days [5] (teat-end condition +22 days)	0				

[1] regression coefficient; [2] standard error; [3] odds ratio; [4] confidence interval; [5] −14 days, −2 days: 14 days and 2 days before the milking system changeover; +15 days, +22 days: 15 days and 22 days after the milking system changeover.

3.7. Udder Hygiene

Scoring of udder hygiene was carried out at every farm visit, resulting in a total of six scores per cow. Udders with scores of 1 or 2 (udder free of dirt, udder slightly dirty)

were clustered and compared to udders with scores of 3 or 4 (udder covered with dirt, udder covered with caked-on dirt). Udder hygiene at the sampling date +8 and +15 days after the changeover was significantly different from udder hygiene +22 days after the changeover (Table 7). More udders were given the score of 1 or 2, i.e., were clean at these sampling dates.

Table 7. Final generalised linear mixed model for udder hygiene.

Dependent Variable	Independent Variable (Reference Category)	ß [1]	SE [2]	OR [3]	*p*-Value	95% CI [4] (OR)
Udder hygiene	intercept	2.007	0.133	7.438	<0.001	5.727–9.658
	−14 days [5] (udder hygiene +22 days)	0.109	0.1837	0.896	0.552	0.625–1.285
	−8 days [5] (udder hygiene +22 days)	0.297	0.1784	0.743	0.096	0.523–1.054
	−2 days [5] (udder hygiene +22 days)	0.143	0.1839	0.866	0.436	0.604–1.243
	+ 8 days [5] (udder hygiene +22 days)	0.900	0.2356	2.459	<0.001	1.549–3.902
	+15 days [5] (udder hygiene +22 days)	1.098	0.2515	2.999	<0.001	1.831–4.911
	+22 days [5] (udder hygiene +22 days)	0				

[1] regression coefficient; [2] standard error; [3] odds ratio; [4] confidence interval; [5] −14 days, −8 days, −2 days: 14 days, 8 days, and 2 days before the milking system changeover; +8 days, +15 days, +22 days: 8 days, 15 days, and 22 days after the milking system changeover.

3.8. Infection Rates

S. sciuri was the most frequently detected pathogen species. The mean infection rate calculated with a Poisson regression model was 0.08 cases per quarter per day (Table 8). As the second most frequently detected pathogen, *S. chromogenes* had an average infection rate of 0.07 cases per quarter per day. Quarters infected with *S. haemolyticus* amounted to 0.10 cases per quarter per day. *C. amycolatum* showed the highest infection rate of 0.10 cases per quarter per day.

Table 8. Poisson regression model for the four most frequently isolated pathogen species.

Pathogen	Poisson Estimate	Poisson Confidence Interval 2.5%	Poisson Confidence Interval 97.5%	AIC [1] Poisson with Offset	AIC [1] Poisson Without Offset
Staphylococcus sciuri	0.07923525	0.06830256	0.0912758	74	106
Staphylocccus chromogenes	0.07354711	0.06085905	0.08788587	52	59
Staphylococcus haemolyticus	0.09644336	0.07850171	0.11692896	43	54
Corynebacterium amycolatum	0.1013182	0.079399	0.1269408	48	51

[1] Akaike information criterion.

The AIC of the model, which also took the number of infected quarters into account (with offset), was lower for all four pathogens compared to the results for the model that only included the suspectable quarters (without offset). Therefore, the model that included the already infected quarters best fitted the observed data.

4. Discussion

A milking system changeover leads to numerous changes on a dairy farm. Whether or not the change from a conventional milking system to an AMS affects udder health was investigated in the present study. Due to the current increase in the number of

AMSs on dairy farms worldwide [9], several studies have investigated risk factors for the development and transmission of NIMIs by milking dairy cows in an AMS. However, to date, how udder health changes as a result of the transition and how the occurrence of mastitis-causing pathogens is affected have rarely been investigated.

4.1. Impact of the Milking System Changeover on the Somatic Cell Count, the New Intramammary Infections, and the Cultivated Pathogen Species

Various studies have tried to assess the effects on udder health due to such a milking system changeover. However, most previous studies analysed the individual cow SCC to determine udder health. Weiss et al. [18] reported no change in SCC immediately after switching from conventional milking to an AMS. Additionally, Klungel et al. [17] obtained comparable results, although the farms in this experiment already exhibited elevated SCC levels before the milking system changeover. Recent studies detected a higher number of cows with high SCCs after the changeover and a sudden increase in the individual cow-level SCC, as well as an increase in the frequency of mastitis [19,20]. As the AMS technology has improved in recent years since the two aforementioned studies were carried out, contemporary studies are of great importance. An observational study analysing cow-level SCC data from 2007 to 2019 was able to demonstrate a deterioration in udder health surrounding the transition to an AMS. However, the deterioration in udder health in 2019 was not as severe as that noted in 2007 [28]. Significant increases in the quarter-level SCC were also observed in the present study following the installation of the AMS. The SCC provides information on whether an inflammation is present. It should be considered that the milking interval also affects the SCC and is therefore of particular importance when changing to an AMS [7].

The observed increase in quarter-level SCC after the changeover may be due to the interval between the last milking and the quarter milk sampling in the trial. During milking with the conventional milking system, the samples were taken directly before milking; after the milking system changeover, sampling was independent of the previous milking. This would suggest that the increased quarter-level SCC was not due to inflammation, but merely to too short an interval between the previous milking and the quarter milk sampling. To verify this assumption, the SCC of the bulk milk was reviewed for the applicable period. No increase in the bulk milk SCC was observed after the installation of the AMS. This confirms that the increase in the SCC from the quarter milk samples after the changeover was a result of the time interval between the previous milking and the sampling. Due to an observed sudden significant increase in SCC, Rasmussen et al. [19] assumed the presence of more NIMIs during the first year after the introduction of an AMS. The assumption of Rasmussen et al. [19] could not be confirmed in our study, as the detected NIMIs were the lowest after the changeover. Furthermore, no significant difference in the NIMI rate was observed in the comparison of NIMI rates from before the changeover to after the changeover of the milking system. As the SCC does not provide information on which bacteria are involved in the NIMI, quarter milk samples were cytomicrobiologically analysed in the current study. It should also be considered that different pathogens influence the somatic cell count individually [29].

Zecconi et al. [30] examined quarter milk samples bacteriologically. That study did not focus on the effects of the milking system changeover but examined heifers 12 months after calving that were milked in an AMS. In agreement with the current study, Zecconi et al. [30] found that milking with an AMS did not lead to an increase in the frequency of IMI in heifers when no contagious pathogens were previously present in the herd. They concluded that the AMS had no negative effects on the animals if the health status of the cows and the herd management had been previously good. In the present study, the most frequently detected pathogens before the milking system changeover were NAS (18.7% up to 28.6%),

followed by mixed infections and *Corynebacterium* spp. (2.7% up to 6.5%). Even after the changeover, NAS, *Corynebacterium* spp., and mixed infections were the most frequently isolated types. A similar result can also be seen for NIMI; NAS caused most NIMIs before the changeover (63.3%) and after the changeover (58.1%). *Corynebacterium* spp. and mixed infections were equally isolated from NIMIs before the changeover. After the changeover to the AMS, mixed infections caused 23.1% of NIMIs, and *Corynebacterium* spp. caused 14.4%. Thus, no effect of the AMS on the pathogens present on the farm could be proven. Moreover, the NIMIs were also caused by the same pathogen genera before and after the changeover. The most frequently detected pathogens in the present study, both before and after the milking system changeover, were minor mastitis pathogens. Additionally, the changeover did not influence the NIMI rate; therefore, the conclusion drawn by Zecconi et al. [30] could be confirmed. Another study, which did not investigate the conversion of a milking system, but rather the udder health in two herds milked with an AMS, showed an increase in the incidence of subclinical mastitis [31]. Environmental streptococci and NAS were identified in that study as the main causes of subclinical and clinical mastitis. The authors suspected a connection between the increase in NIMIs with environmental pathogens and the cubicle cleaning and pre-milking teat cleaning in the AMS. The majority of NIMIs in the conducted study were also caused by NAS. Hamann and Reinecke [32] carried out a trial with 80 cows which were all milked with a conventional milking system at the beginning of the trial. After two months, half of the cows were switched to milking with an AMS. The study used quarter milk sampling and compared the cytobacteriological results. The cows that were transferred to an AMS showed a slight improvement in udder health when compared to the other group of cows milked with the conventional milking system. Although this improvement was not statistically significant, those authors concluded that milking with an AMS does not deteriorate udder health. Similarly, in that study, no effect of the installation of the AMS could be recognised with regard to the absolute number of NIMIs, the NIMI rate, and the detected pathogen genera. In order to prove a possible effect of the milking system changeover, the prevalence and NIMI incidence of the pathogen species was also determined in that conducted study.

NAS were responsible for most NIMIs in this study. The most frequently isolated pathogen species were *S. sciuri*, *S. chromogenes*, *S. haemolyticus*, and *C. amycolatum*. NAS and Coryneform bacteria are repeatedly detected as the cause of NIMI [12,33]. NAS and *Corynebacterium* spp. are classified as minor mastitis pathogens. However, minor mastitis pathogens have also been detected in subclinical and clinical cases of mastitis [34,35]. Depending on the species, the presence of minor mastitis-causing pathogens in the udder can lead to an increase in SCC, an increased risk for infection with major mastitis-causing pathogens, and a reduced milk yield [35,36]. Nonetheless, contradictory observations have also been reported. In two studies by Piepers et al. [37,38], minor mastitis pathogens did not lead to a decline in milk yield and even acted as a protection against colonisation with major mastitis pathogens. Due to their presence in the environment, in the teat canal, and on the teat skin, minor pathogens may contaminate quarter milk samples and lead to an incorrect interpretation of udder health if the pathogens detected are classified as an NIMI instead of a contamination [39,40]. In the present study, *S. sciuri* was the most commonly detected pathogen species that led to NIMI. With a prevalence of up to 23.91%, *S. sciuri* was also the most often detected pathogen species in the examined quarter milk samples. De Visscher et al. [41] isolated *S. sciuri* in 13% of quarter milk samples from postpartum dairy cows and heifers. They observed a connection between dirty teats and the presence of *S. sciuri*, thus confirming the transmission of this species from the environment to the udder. The study by Piessens et al. [42] resulted in similar conclusions; they were able to isolate *S. sciuri* from slatted flooring and bedding material. However, the evaluation of the AIC in

the present study fitted better with the model, which also included the already infected quarters. Thus, AIC does seem to have an effect on NIMI with *S. sciuri* if quarters of the herd are already infected with this pathogen.

S. chromogenes was detected as the second most common cause of NIMI, with an NIMI incidence of 2.3% to 8.0% and a prevalence of up to 10.7%. Compared to *S. sciuri*, *S. chromogenes* is mentioned in numerous studies as the most frequently detected NAS [40–42]. *S. chromogenes* can cause long-lasting infections, probably due to its good adaptability to the udder [42]. Hamel et al. [40] were able to detect *S. chromogenes* with a prevalence of 12.4%, with 90% of these detected infections with *S. chromogenes* being confirmed as NIMI. In addition, unlike *S. sciuri*, *S. chromogenes* has rarely been isolated from the environment [42]. An infection rate of 0.07 cases per quarter per day for *S. chromogenes* was calculated. Furthermore, for this pathogen species, the model with the already infected quarters also fitted better with the available data.

Like *S. sciuri*, *S. haemolyticus* can survive in the environment, leading to numerous NIMIs [42]. The transmission of *S. haemolyticus* is often considered non-contagious [29]. Hamel et al. [40] detected this pathogen species with a prevalence of 36%; however, 30% of these detected *S. haemolyticus* were categorised as contaminations or very short infections. Taking those results into consideration, the detection of *S. sciuri* and *S. haemolyticus* might not represent colonisation of the udder but only contaminants from the environment. Therefore, the proven prevalence and NIMI incidence in the present study would be overestimated. By comparing the AIC values of the two models, a contagious transmission of *S. haemolyticus* cannot be ruled out either. However, udder quarters infected with *S. haemolyticus* may not always be infected with the same strain of *S. haemolyticus*, as a large strain diversity of *S. haemolyticus* has been detected in previous studies [29].

Among the other minor pathogens, the coryneforms, *C. amycolatum* was detected most predominantly in this study. *C. amycolatum* is known to persist in the udder parenchyma for a long time, leading to subclinical or latent infections [36]. *Corynebacterium* spp. were detected with varying prevalence; Kirkeby et al. [43] detected a prevalence of 24% and 8% in two different herds, with a transmission rate of 0.016 and 0.018 cases, respectively, per quarter day. In comparison, the present study detected a prevalence of 2.0% up to 5.3% and an infection rate of 0.10 cases per quarter per day. The very different prevalence can be explained by the fact that the current study did not investigate the pathogen group *Corynebacterium* spp. but only the particular species *C. amycolatum*. However, the farm-specific occurrence of pathogens and the much shorter sampling interval could also contribute to the different prevalence [42]. A short sampling interval is particularly beneficial for a species with a short infection duration, as it allows for a more precise estimation of the number of NIMIs. Although the infection rate in the current study was higher, the trial by Kirkeby et al. [43] also assumed that contagious transmission of *Corynebacterium* spp. is possible. In addition to the isolation from the environment, *C. amycolatum* was also cultivated on milking clusters with a low strain diversity, which would confirm contagious transmission [44].

The species differentiation made it possible to draw more precise conclusions about the significance of the infection with the respective pathogens. *S. sciuri* and *S. haemolyticus* might originate from contaminations from the environment, and thus the number of NIMIs, as well as the prevalence of these pathogens, would be overestimated. Apart from that, infections with *S. chromogenes* are not likely to be due to contaminants. However, even with the possible overestimation of the NIMI of individual pathogens, no influence of the milking system changeover on the new infection rate could be observed. Conversely, the NIMI incidence and prevalence of *S. sciuri*, *S. haemolyticus*, and *C. amycolatum* were the

lowest after the milking system changeover. Only *S. chromogenes* exhibited the highest NIMI incidence and prevalence after the changeover.

4.2. Teat-End Conditions and Udder Hygiene

Teat-end conditions 15 days after the milking system changeover were significantly better than the teat-end conditions at the last sampling date. As the change in teat-end conditions is described as a long-term effect and can change between two to eight weeks, it is unlikely that teat-end condition deteriorates within one week [26]. This observation can probably be explained by an observation bias, which was present because three to five veterinarians assessed teat-end conditions. Additionally, varying levels of significance have been attributed to the impact of teat-end condition during lactation on the development of NIMI [13–15].

Udder hygiene scores were significantly better at the two sampling dates directly after the milking system changeover compared to the udder hygiene at the last sampling date. Schreiner and Ruegg [25] showed a correlation between the udder hygiene score and the occurrence of IMI caused by contagious and environmental pathogens. Cows with udder hygiene Scores 3 and 4 had a 1.5 times higher chance of major mastitis pathogens being detected in the mammary gland [45]. The significant difference in the scored udder hygiene illustrates the change in the hygiene management of the cubicles associated with the change in the milking system.

4.3. Limitations of the Study

The present study evaluated the influence of the milking system changeover to an AMS on the development of NIMI. Therefore, quarter milk samples were collected three weeks before and three weeks after the transition. Most studies that accompany a changeover of the milking system analyse data one year before and one year after the changeover [19,20]. Compared to this, the evaluation time of six weeks was very short. However, the research question in this trial did not attempt to determine whether or how an AMS can influence udder health, but whether the time of the changeover has an effect on udder health. An examination period of six weeks was sufficient to assess udder health at the time of the changeover. Nevertheless, the study period was too short to prove a reliable effect of the AMS on teat-end condition.

Moreover, it should be noted that the intervals between samplings were not uniform in our study. Unfortunately, it was not possible to sample the cows exactly every 7 days due to a staff shortage. The sampling interval varied between 5 to 10 days. A short interval between two sampling dates is nevertheless advantageous to detect infections of short duration. In the calculated Poisson regression models, however, the exact sampling interval was taken into account.

The presented study was carried out exclusively on one farm. To confirm the results of the study, a similar investigation should be carried out on other farms.

Furthermore, it must be noted that many of the quarter milk samples were contaminated (Table 3), especially at the beginning of the trial.

5. Conclusions

The NIMI rate changed from study time point to study time point. Since no significant difference between the NIMI rate before and after the changeover could be demonstrated, the variation in the NIMI rate cannot be attributed to the changeover of the milking system. The main pathogens isolated were minor mastitis pathogens such as *S. sciuri*, *S. chromogenes*, *S. haemolyticus*, and *C. amycolatum*, the dynamics of which are not yet fully understood. By calculating Poisson models, a contagious transmission could not be ruled out in the

study. Additionally, no effect of the milking system changeover on the occurrence of the pathogen species could be proven. In contrast, a significant increase in the quarter-level SCC was observed after the installation of the AMS. However, this cellular reaction does not necessarily represent a deterioration in udder health and was probably caused by the time interval between the last milking and the quarter milk sampling. This trial, therefore, confirmed that the milking system changeover does not necessarily have a negative effect on udder health.

Author Contributions: Conceptualisation, V.K. and P.K.; methodology, V.K., S.W. and P.K.; software, S.W. and V.K.; validation, V.K.; formal analysis, S.W. and V.K.; investigation, F.N., J.N., J.K., V.K. and P.K.; resources, N.W., Y.Z. and P.K.; data curation, V.K., S.W. and P.K.; writing—original draft preparation, P.K.; writing—review and editing, V.K.; visualisation, S.W. and P.K.; supervision, V.K.; project administration, V.K.; funding acquisition, V.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Steinbeis Research Center Milk Science, Kirchlengern, Germany, Fund 23/02.

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the animal welfare committee of the University of Veterinary Medicine Hannover, Foundation, Germany; file reference: TVO-2022-V-61; date of approval: 12 September 2022.

Informed Consent Statement: Not applicable.

Data Availability Statement: The dataset is available upon request from the authors.

Acknowledgments: The authors would like to thank the dairy farm that made it possible to carry out this study. We would also like to thank the laboratory staff for analysing the milk samples. Furthermore, we would like to thank Frances Sherwood-Brock for proofreading the English manuscript.

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

References

1. Østerås, O.; Solbu, H.; Refsdal, A.O.; Roalkvam, T.; Filseth, O.; Minsaas, A. Results and evaluation of thirty years of health recordings in the Norwegian dairy cattle population. *J. Dairy Sci.* **2007**, *90*, 4483–4497. [CrossRef] [PubMed]
2. Hertl, J.A.; Schukken, Y.H.; Welcome, F.L.; Tauer, L.W.; Gröhn, Y.T. Pathogen-specific effects on milk yield in repeated clinical mastitis episodes in Holstein dairy cows. *J. Dairy Sci.* **2014**, *97*, 1465–1480. [CrossRef] [PubMed]
3. Donnelly, M.R.; Hazel, A.R.; Hansen, L.B.; Heins, B.J. Health Treatment Cost of Holsteins in Eight High-Performance Herds. *Animals* **2023**, *13*, 2061. [CrossRef] [PubMed]
4. Hertl, J.A.; Schukken, Y.H.; Bar, D.; Bennett, G.J.; González, R.N.; Rauch, B.J.; Welcome, F.L.; Tauer, L.W.; Gröhn, Y.T. The effect of recurrent episodes of clinical mastitis caused by gram-positive and gram-negative bacteria and other organisms on mortality and culling in Holstein dairy cows. *J. Dairy Sci.* **2011**, *94*, 4863–4877. [CrossRef]
5. Reyher, K.K.; Dohoo, I.R.; Muckle, C.A. Evaluation of clustering of new intramammary infections in the bovine udder, including the impact of previous infections, herd prevalence, and somatic cell count on their development. *J. Dairy Sci.* **2013**, *96*, 219–233. [CrossRef]
6. Bulletin of the International Dairy Federation, 448/2011, Suggested Interpretation of Mastitis Terminology (Revision of Bulletin of IDF No 338/1999). Available online: https://fil-idf.org/wp-content/uploads/woocommerce_uploads/2011/03/Bulletin-of-the-IDF-No.-448_2011-Suggested-Interpretation-of-Mastitis-Terminology-revision-of-Bulletin-of-IDF-N%C2%B0-338_1999-1-fdvlh1.pdf (accessed on 1 July 2024).
7. German Veterinary Medical Association (GVA). *Leitlinien Bekämpfung der Mastitis des Rindes als Bestandsproblem, [Guidelines for Combating Bovine Mastitis as a Stock Problem]*, 5th ed.; German Veterinary Medical Association: Gießen, Germany, 2012.
8. Volling, O.; Krömker, V. Managementmassnahmen zur Senkung der bovinen Mastitisinzidenzrate im milcherzeugenden Betrieb [Udder health management practices in dairy enterprises to reduce the incidence of bovine mastitis]. *Dtsch Tierarztl Wochenschr.* **2008**, *11*, 410–420.
9. Cogato, A.; Brščić, M.; Guo, H.; Marinello, F.; Pezzuolo, A. Challenges and Tendencies of Automatic Milking Systems (AMS): A 20-Years Systematic Review of Literature and Patents. *Animals* **2021**, *11*, 356. [CrossRef]

10. Dufour, S.; Dohoo, I.R.; Barkema, H.W.; Descôteaux, L.; Devries, T.J.; Reyher, K.K.; Roy, J.P.; Scholl, D.T. Manageable risk factors associated with the lactational incidence, elimination, and prevalence of *Staphylococcus aureus* intramammary infections in dairy cows. *J. Dairy Sci.* **2012**, *95*, 1283–1300. [CrossRef]
11. Deng, Z.; Koop, G.; Lam, T.J.G.M.; van der Lans, I.A.; Vernooij, J.C.M.; Hogeveen, H. Farm-level risk factors for bovine mastitis in Dutch automatic milking dairy herds. *J. Dairy Sci.* **2019**, *102*, 4522–4535. [CrossRef]
12. Nitz, J.; Wente, N.; Zhang, Y.; Klocke, D.; Tho Seeth, M.; Krömker, V. Dry Period or Early Lactation-Time of Onset and Associated Risk Factors for Intramammary Infections in Dairy Cows. *Pathogens* **2021**, *10*, 224. [CrossRef]
13. Zadoks, R.N.; Allore, H.G.; Barkema, H.W.; Sampimon, O.C.; Gröhn, Y.T.; Schukken, Y.H. Analysis of an outbreak of *Streptococcus uberis* mastitis. *J. Dairy Sci.* **2001**, *84*, 590–599. [CrossRef] [PubMed]
14. de Pinho Manzi, M.; Nóbrega, D.B.; Faccioli, P.Y.; Troncarelli, M.Z.; Menozzi, B.D.; Langoni, H. Relationship between teat-end condition, udder cleanliness and bovine subclinical mastitis. *Res. Vet. Sci.* **2012**, *93*, 430–434. [CrossRef] [PubMed]
15. Zoche-Golob, V.; Haverkamp, H.; Paduch, J.H.; Klocke, D.; Zinke, C.; Hoedemaker, M.; Heuwieser, W.; Krömker, V. Longitudinal study of the effects of teat condition on the risk of new intramammary infections in dairy cows. *J. Dairy Sci.* **2015**, *98*, 910–917. [CrossRef] [PubMed]
16. Schunig, R.; Busanello, M.; Nogara, K.F.; Zopollatto, M. Cow-level risk factors associated with the increase in somatic cell count and the occurrence of subclinical mastitis in Brazilian Holstein and Jersey dairy cows. *Prev. Vet. Med.* **2024**, *227*, 106208. [CrossRef] [PubMed]
17. Klungel, G.H.; Slaghuis, B.A.; Hogeveen, H. The effect of the introduction of automatic milking systems on milk quality. *J. Dairy Sci.* **2000**, *83*, 1998–2003. [CrossRef]
18. Weiss, D.; Helmreich, S.; Möstl, E.; Dzidic, A.; Bruckmaier, R.M. Coping capacity of dairy cows during the change from conventional to automatic milking. *J. Anim. Sci.* **2004**, *82*, 563–570. [CrossRef]
19. Rasmussen, M.D.; Blom, J.Y.; Nielsen, L.A.H.; Justesen, P. Udder health of cows milked automatically. *Livest. Prod. Sci.* **2001**, *72*, 147–156. [CrossRef]
20. Hovinen, M.; Rasmussen, M.D.; Pyörälä, S. Udder health of cows changing from tie stalls or free stalls with conventional milking to free stalls with either conventional or automatic milking. *J. Dairy Sci.* **2009**, *92*, 3696–3703. [CrossRef]
21. In-Liner Everything-Robotic Milking. Available online: https://www.gea.com/en/assets/254971/ (accessed on 30 October 2024).
22. German Veterinary Medical Association (GVA). Leitlinien Entnahme von Milchproben unter antiseptischen Bedingungen und Isolierung und Identifizierung von Mastitiserregern. In *Guidelines for Antiseptic Milk Sampling and Guidelines to Isolate and Identify Mastitis Pathogens*, 2nd ed.; Veterinärmed, G.V.D.D., Ed.; German Veterinary Medical Association (GVA): Gießen, Germany, 2009; pp. 1–92.
23. Heeschen, W.; Reichmuth, J.; Tolle, A.; Zeider, H. Die Konservierung von Milchproben zur bakteriologischen, zytologischen und hemmstoffbiologischen Untersuchung. *Milchwissenschaft* **1969**, *24*, 729–734.
24. Randall, L.P.; Lemma, F.; Koylass, M.; Rogers, J.; Ayling, R.D.; Worth, D.; Klita, M.; Steventon, A.; Line, K.; Wragg, P.; et al. Evaluation of MALDI-ToF as a method for the identification of bacteria in the veterinary diagnostic laboratory. *Res. Vet. Sci.* **2015**, *101*, 42–49. [CrossRef]
25. Schreiner, D.A.; Ruegg, P.L. Effects of tail docking on milk quality and cow cleanliness. *J. Dairy Sci.* **2002**, *85*, 2503–2511. [CrossRef] [PubMed]
26. Mein, G.A.; Neijenhuis, F.; Morgan, W.F.; Reinemann, D.J.; Hillerton, J.E.; Baines, J.R.; Ohnstad, I.; Rasmussen, M.D.; Timms, L.; Britt, J.S.; et al. Evaluation of bovine teat condition in commercial dairy herds: 1. Non-infectious factors. In Proceedings of the AABP-NMC International Symposium on Mastitis and Milk Quality, Vancouver, BC, Canada, 13–15 September 2001.
27. Dalen, G.; Rachah, A.; Nørstebø, H.; Schukken, Y.H.; Gröhn, Y.T.; Barlow, J.W.; Reksen, O. Transmission dynamics of intramammary infections caused by *Corynebacterium* species. *J. Dairy Sci.* **2018**, *101*, 472–479. [CrossRef] [PubMed]
28. van den Borne, B.H.P.; van Grinsven, N.J.M.; Hogeveen, H. Trends in somatic cell count deteriorations in Dutch dairy herds transitioning to an automatic milking system. *J. Dairy Sci.* **2021**, *104*, 6039–6050. [CrossRef]
29. Woudstra, S.; Wente, N.; Zhang, Y.; Leimbach, S.; Gussmann, M.K.; Kirkeby, C.; Krömker, V. Strain diversity and infection durations of *Staphylococcus* spp. and *Streptococcus* spp. causing intramammary infections in dairy cows. *J. Dairy Sci.* **2023**, *106*, 4214–4231. [CrossRef]
30. Zecconi, A.; Piccinini, R.; Casirani, G.; Binda, E.; Migliorati, L. Effects of automatic milking system on teat tissues, intramammary infections and somatic cell counts. *Ital. J. Anim. Sci.* **2003**, *2*, 275–282. [CrossRef]
31. Petermann, M.; Wolter, W.; Rittershaus, C.; Kloppert, B.; Seufert, H.; Zschock, M. Automatic milking systems: Udder health and milk flow profiles. In *Physiological and Technical Aspects of Machine Milking, Proceedings of the International Conference, Nitra, Slovak Republic, 26–27 June 2001*; Rosati, A., Mihina, S., Mosconi, C., Eds.; ICAR: Rome, Italy, 2001.
32. Hamann, J.; Reinecke, F. Machine milking effects on udder health—Comparison of a conventional with a robotic milking system. In Proceedings of the 1st North American Conference on Robotic Milking, Toronto, ON, Canada, 20–22 March 2002; Section IV. pp. 17–27.

33. Katthöfer, P.; Zhang, Y.; Wente, N.; Preine, F.; Nitz, J.; Krömker, V. The Influence of Milk Leakage, Udder Pressure and Further Risk Factors on the Development of New Intramammary Infections during the Dry Period of Dairy Cows. *Pathogens* **2024**, *13*, 430. [CrossRef]
34. Gonçalves, J.L.; Tomazi, T.; Barreiro, J.R.; Beuron, D.C.; Arcari, M.A.; Lee, S.H.; Martins, C.M.; Araújo Junior, J.P.; dos Santos, M.V. Effects of bovine subclinical mastitis caused by *Corynebacterium* spp. on somatic cell count, milk yield and composition by comparing contralateral quarters. *Vet. J.* **2016**, *209*, 87–92. [CrossRef]
35. Heikkilä, A.M.; Liski, E.; Pyörälä, S.; Taponen, S. Pathogen-specific production losses in bovine mastitis. *J. Dairy Sci.* **2018**, *101*, 9493–9504. [CrossRef]
36. Lücken, A.; Woudstra, S.; Wente, N.; Zhang, Y.; Krömker, V. Intramammary infections with *Corynebacterium* spp. in bovine lactating udder quarters. *PLoS ONE* **2022**, *17*, e0270867. [CrossRef]
37. Piepers, S.; Opsomer, G.; Barkema, H.W.; de Kruif, A.; De Vliegher, S. Heifers infected with coagulase-negative staphylococci in early lactation have fewer cases of clinical mastitis and higher milk production in their first lactation than noninfected heifers. *J. Dairy Sci.* **2010**, *93*, 2014–2024. [CrossRef]
38. Piepers, S.; Peeters, K.; Opsomer, G.; Barkema, H.W.; Frankena, K.; De Vliegher, S. Pathogen group specific risk factors at herd, heifer and quarter levels for intramammary infections in early lactating dairy heifers. *Prev. Vet. Med.* **2011**, *99*, 91–101. [CrossRef] [PubMed]
39. Hiitiö, H.; Simojoki, H.; Kalmus, P.; Holopainen, J.; Pyörälä, S.; Taponen, S. The effect of sampling technique on PCR-based bacteriological results of bovine milk samples. *J. Dairy Sci.* **2016**, *99*, 6532–6541. [CrossRef] [PubMed]
40. Hamel, J.; Zhang, Y.; Wente, N.; Krömker, V. Non-*S. aureus* staphylococci (NAS) in milk samples: Infection or contamination? *Vet. Microbiol.* **2020**, *242*, 108594. [CrossRef] [PubMed]
41. De Visscher, A.; Piepers, S.; Haesebrouck, F.; De Vliegher, S. Intramammary infection with coagulase-negative staphylococci at parturition: Species-specific prevalence, risk factors, and effect on udder health. *J. Dairy Sci.* **2016**, *99*, 6457–6469. [CrossRef]
42. Piessens, V.; Van Coillie, E.; Verbist, B.; Supré, K.; Braem, G.; Van Nuffel, A.; De Vuyst, L.; Heyndrickx, M.; De Vliegher, S. Distribution of coagulase-negative *Staphylococcus* species from milk and environment of dairy cows differs between herds. *J. Dairy Sci.* **2011**, *94*, 2933–2944. [CrossRef]
43. Kirkeby, C.; Halasa, T.; Farre, M.; Chehabi, G.N.; Græsbøll, K. Transmission Dynamics of *Corynebacterium* spp. Within Two Danish Dairy Cattle Herds. *Front. Vet. Sci.* **2021**, *8*, 735345. [CrossRef]
44. Woudstra, S.; Lücken, A.; Wente, N.; Zhang, Y.; Leimbach, S.; Gussmann, M.K.; Kirkeby, C.; Krömker, V. Reservoirs of *Corynebacterium* spp. in the Environment of Dairy Cows. *Pathogens* **2023**, *12*, 139. [CrossRef]
45. Schreiner, D.A.; Ruegg, P.L. Relationship between udder and leg hygiene scores and subclinical mastitis. *J. Dairy Sci.* **2003**, *86*, 3460–3465. [CrossRef]