



## Transmission dynamics of *Staphylococcus aureus* and *Streptococcus agalactiae* in a Dutch dairy herd using an automatic milking system

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### ABSTRACT

*Staphylococcus aureus* and *Streptococcus agalactiae* are important contagious mastitis pathogens and are considered to mainly transmit between cows through the milking machine. Controlling contagious mastitis on dairy farms requires a reduction of the transmission rate or the duration of intramammary infections (IMI), or both. These parameters may differ in dairy herds milked with an automatic milking system (AMS) as compared to those milked with a conventional milking system (CMS). The aims of this prospective longitudinal study were to estimate the transmission rate, the median duration of IMI and the basic reproduction number ( $R_0$ ) of *Staph. aureus* and *Strep. agalactiae* in a Dutch AMS herd. Bacteriological cultures of quarter milk samples were collected every 2 wks. Using 3 different definitions of IMI, we estimated the transmission rate for *Staph. aureus* to be within the range of 0.002 (95 % CI: 0–0.005) quarter-day<sup>-1</sup> to 0.019 (95 % CI: 0.010–0.032) quarter-day<sup>-1</sup>, and for *Strep. agalactiae* of 0.007 (95 % CI: 0.005–0.010) quarter-day<sup>-1</sup> to 0.019 (95 % CI: 0.011–0.032) quarter-day<sup>-1</sup>, the median duration of chronic IMI at 95 (95 % CI: 72–125) days for *Staph. aureus* and at 86 (95 % CI: 67–111) days for *Strep. agalactiae*, and the  $R_0$  between 0.16 (95 % CI: 0.05–0.27) and 0.34 (95 % CI: 0.20–0.48) for *Staph. aureus*, and between 0.64 (95 % CI: 0.41–0.87) and 0.68 (95 % CI: 0.48–0.88) for *Strep. agalactiae*.

Transmission of these two contagious pathogens in this herd was limited and theoretically the IMI would not sustain, given that  $R_0$  of both pathogens was lower than 1. The estimated transmission rate of *Staph. aureus* in this AMS herd was found to be comparable to those described for CMS herds, while for *Strep. agalactiae*, it was slightly higher than in CMS herds. The duration of *Staph. aureus* IMI was in line with results from CMS farms, while the duration of *Strep. agalactiae* was lower than what has been described in CMS herds. The  $R_0$  of these contagious pathogens was found to be lower than the estimates in CMS herds. Our study suggests that the transmission rate of these two contagious pathogens in this AMS herd were comparable to what has been reported about well-performing CMS herds that have a low rate of transmission.

### 1. Introduction

Although contagious mastitis pathogens have long been the subject of research, and the five-point mastitis control plan has been shown to significantly reduce their prevalence (Green and Bradley, 2013), there still is much to gain in this respect in many farms worldwide (Bradley et al., 2015; Gao et al., 2017; Leuenberger et al., 2019). *Staphylococcus*

*aureus* and *Streptococcus agalactiae* are still among the contagious pathogens that are most prevalent on dairy farms in many countries (Gao et al., 2017; Vakkamäki et al., 2017).

Clearly, the milking machine plays an important role in the transmission of mastitis causing pathogens (Mein, 2012). Contagious mastitis causing pathogens, such as *Staph. aureus* and *Strep. agalactiae*, have been reported to primarily spread from cow to cow during milking by a

**Abbreviations:** SCC, Somatic cell count; O-SCC, Online somatic cell count; AMS, automatic milking system; CMS, conventional milking system; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; IMI, intramammary infection;  $R_0$ , basic reproduction number; CFU, colony forming units; 95 % CI, 95 % confidence interval.

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contaminated milking machine (Keefe, 2012). Although some evidence suggests that environmental infections also occur (Fox and Gay, 1993; Barlow et al., 2013), *Staph. aureus* is generally considered to be contagious and transmitted between cows, with the presence of a predominant genotype in a herd reflecting the epidemiological features of contagious mastitis pathogens (Sommerhäuser et al., 2003; Capurro et al., 2010; Klaas and Zadoks, 2018). The prevalence of *Strep. agalactiae* has been significantly reduced since the adoption of the five point mastitis control plan (Hillerton and Booth, 2018). However, recent research found its prevalence to be relatively high in herds using an automatic milking system (AMS) (Svennesen et al., 2019) and proved the existence of an environmental transmission route of *Strep. agalactiae* (Jørgensen et al., 2016). Transmission rates of these pathogens have been estimated in herds using conventional milking systems (CMS) (Zadoks et al., 2002; Leelahapongsathon et al., 2016; Kirkeby et al., 2019), but to the best of our knowledge, only once in an AMS herd, that evaluated data at the cow level rather than quarter level (Dalen et al., 2019).

In AMS herds, contacts between individual cows and interactions with the milking machine differ from those in CMS herds, which may have an effect on the transmission dynamics of contagious mastitis pathogens. For instance, automated cleaning of teats and automated post-milking teat disinfection may affect the transmission rate of these pathogens. Additionally, the use of sensors to identify infected animals may have an effect on the time until diagnosis of subclinical mastitis and therefore possibly on the time until interventions are implemented by herdsmen. The distinctive difference between AMS and CMS herds regarding the milking process is that cows are milked with a single milking unit per AMS. Hence, they may have a higher exposure to contaminated milking equipment, especially when cleaning and disinfection procedures are suboptimal. Intramammary infections, as indicated by a high SCC, seem to occur more frequently in AMS compared to CMS herds (Deng et al., 2019). Although reducing the transmission of IMI is one of the most important parts of mastitis control programs (Østerås and Sølverød, 2009; Down et al., 2013), the transmission rate of these pathogens in AMS herds is not fully clear. Therefore, the aim of this study was to estimate the transmission rate, the duration of IMI and the basic reproduction number ( $R_0$ ) of *Staph. aureus* and *Strep. agalactiae* in a Dutch dairy herd using an AMS and compare this to previous findings in CMS herds. Additionally, the effect of different definitions of IMI on transmission parameter estimates was evaluated.

## 2. Materials and methods

In a prospective longitudinal study, during 6 mo, bi-weekly quarter milk samples were collected for bacteriological culturing in a group of approximately 60 cows milked with an AMS. With the bacteriological culture data, we estimated transmission rate, median duration and  $R_0$  of IMI for both *Staph. aureus* and *Strep. agalactiae*.

### 2.1. Farm and cow selection

A farm was selected based on the use of AMS, the availability of online SCC sensors (MQC-C, Lely Industries N.V., Maassluis, The Netherlands), the location of the farm relatively close to Utrecht University and the farmer's willingness to participate in this study. The selected dairy farm had four separate groups of cows, each milked with a single Lely Astronaut A4 AMS (Lely Industries N.V.). The AMS automatically performed cleaning of teats and post-milking teat disinfection by spraying the teats of each cow as well as rinsing and steaming of the milking unit between milkings. Data about the disease history of the cows in all the 4 groups were recorded by the farmer and were available. One of the four groups of cows was selected for quarter milk sampling based on the farmer's indication that this group of cows had the highest incidence of contagious mastitis among the 4 groups. The selected group consisted of approximately 60 lactating Holstein cows (24 cows in parity

2 and 21 cows in parity 3, the remaining cows were in parity 4–8, the median of days in milk for cows enrolled in sampling was 138 (2.5 %–97.5 % quantile: 20–300). Cows were milked on average 2.8 times/day with an average milk production of 12.3 kg/milking. During the study period, there was an influx of fresh cows and an efflux of cows being dried off using selective dry cow therapy. Cows were fed automatically using a total mixed ration and had free access to feeding. Sawdust was used for bedding, and beddings were cleaned and refilled with sawdust every morning.

### 2.2. Data collection

The milking robot in this group was equipped with an online sensor system (MQC-C, Lely Industries N.V., The Netherlands), which measures electrical conductivity in-line for each separate udder quarter, abnormalities in milk color in composite milk and composite milk SCC at the cow level. Electrical conductivity and color were measured every milking and SCC was measured almost every milking and expressed in cells/mL. Detection of clinical mastitis was based on the estimated SCC, in combination with electrical conductivity as well as the color of milk. Cows suspected to have clinical mastitis, based upon the system's algorithm, were listed on an alert list. Cows on the alert list were further examined by visually inspecting the appearance of milk, udder and cow by the farmer to confirm clinical mastitis. Cases of clinical mastitis were treated with intramammary antibiotics. History of mastitis was recorded in the disease treatment recording system.

A pilot study was conducted to evaluate the sampling scheme and the processing of samples prior to the start of data collection for transmission modelling on September 26th, 2017. Every two weeks from October 10th, 2017 through February 27th, 2018 all cows in the study group were locked on the headlocks to collect quarter milk samples and were released immediately upon completion. Cows were sampled regardless the time since their last milking.

Sampling and culturing of milk samples were performed according to NMC guidelines (National Mastitis Council, 2017). In brief, first three squirts of milk were discarded, udders and teats were cleaned with paper and teat ends were subsequently disinfected with cotton soaked in 70 % ethanol. For each quarter, 1–2 mL milk was collected aseptically, after which milk samples were cooled in an ice box and transported to the laboratory at Utrecht University to be processed immediately after arrival.

### 2.3. Laboratory analysis

In the laboratory, milk samples were mixed by vortex of samples during 5 s and 10  $\mu$ L milk was inoculated on a sheep blood agar plate and incubated at 37 °C. Results were recorded at 24 and 48 h. A negative culture result was defined as a sample with no bacterial growth. Samples with  $\geq 1$  colony forming units (CFU) were considered culture positive, while samples with  $> 2$  morphologically different colony types were considered to be contaminated. Colony forming units were counted for each colony type based on morphology in culture positive samples and were transformed into  $\log_2(\text{CFU})$ . One isolate of every morphologically different colony type of non-contaminated cultures was subjected to matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS (Bizzini and Greub, 2010) in duplicate. Isolates with identification score  $\geq 2.0$  were considered as successful identifications and used in the analysis. Isolates of *Staph. aureus* were genotyped by sequencing of the variable region of the surface protein A gene (Harmsen et al., 2003) in order to model the genotype specific transmission.

Bacteriological culture results from quarter milk samples of cows with mastitis within 7 d after calving or within 14 d after treatment for mastitis (which was indicated by farmer's records of treatment history for mastitis in conjunction with milk separation in the farm management system) were excluded from further analysis because of potential misclassification of bacteriological results of samples collected in these

periods due to the earlier events or treatments.

#### 2.4. Data analysis

Because different types of IMI (chronic and transient IMI) may have a different attribution to transmission of pathogens, we estimated the transmission rate, duration of IMI, and  $R_0$  in 3 different scenarios. In each scenario, different definitions for a quarter having an (new) IMI, being infectious and being susceptible for new IMI, were applied. In general, if quarters were found to be culture positive ( $\geq 1$  CFU/0.01 mL) only once in a single isolated sample, these IMI were defined as transient IMI. If quarters were culture positive in subsequent samples for the same pathogen, these IMI were defined as chronic IMI. Definitions were specified for the different scenarios:

(1) scenario 1, using a strict definition of IMI: only chronic IMI (consecutive culture positive samples) are considered infectious and only new chronic IMI are considered as new IMI, transient IMI (single culture positive samples) are ignored and interpreted as culture negative in this scenario. In this scenario an IMI was thus defined as a quarter with  $\geq 2$  out of 3 consecutive samples being culture positive for the same pathogen species. A quarter was defined as susceptible for new IMI if  $\geq 2$  consecutive samplings were culture negative.

(2) scenario 2, using an intermediate definition of IMI: chronic and transient IMI are differentiated with respect to infectiousness, with only chronic IMI being considered as infectious. New chronic IMI as well as new transient IMI are, however, considered as new IMI. In this scenario transient IMI do not contribute to the infection pressure. A chronic IMI was defined as  $\geq 2$  out of 3 consecutive samples being culture positive for the same pathogen species. A transient IMI was defined as a single culture positive sample, not preceded or followed by a culture positive sample with the same pathogen and not being part of a chronic IMI. A quarter was defined as susceptible for new IMI, either chronic or transient, if  $\geq 2$  consecutive samplings were culture negative.

(3) scenario 3, using a lenient definition of IMI: no effect of type of IMI on infectivity is assumed and there is no differentiation between chronic IMI and transient IMI in this scenario. Any culture positive sample was considered as an IMI, being infected and infectious, until the quarter was found negative for that pathogen (Dohoo et al., 2011). A quarter was defined as susceptible for IMI if  $\geq 1$  consecutive samplings were culture negative.

**Transmission Rate** At any given timepoint during the study, quarters were defined to be either susceptible or infected. A stochastic Susceptible-Infectious-Susceptible (SIS) model as described by Zadoks et al. (2002) was used with the aforementioned definitions for IMI status for chronic and transient IMI in each of the three scenarios to estimate the transmission rate and  $R_0$  of each pathogen. The stochastic SIS-model assumed homogeneous mixing, random contacts, equal infectivity of infectious quarters and equal susceptibility of susceptible quarters as well as independence among quarters of the same cow during transmission of IMI as described by Zadoks et al. (2002).

We estimated the transmission rate of the stochastic SIS model by a generalized linear model. Maximum likelihood estimation (equations are given in Supplementary Material S1) under the assumption of a binomial distribution of the number of new IMI occurring during a time step was used to estimate transmission rate (Van der Goot et al., 2003) by using the function `mle2` from the `bbmle` package version 1.0.20 (Bolker, 2020). Because of the limited number of new IMI cases, the 95 % confidence interval (CI) for estimates of transmission rate was calculated using the profile likelihood (Stryhn and Christensen, 2003). For *Staph. aureus* in scenario 1, this method could not be used, because only 1 new IMI was observed, and the 95 % CI was calculated assuming a normal distribution (estimate  $\pm 1.96 \times$  SE).

The number of new IMI during a sample interval  $\Delta t$  is given by:

$$E(C) = S \left( 1 - \exp \left( -\beta \times \frac{I}{N} \times \Delta t \right) \right) \quad (1)$$

where  $E(C)$  is the expected number of new IMI,  $\beta$  is the transmission rate per day,  $I$  is the number of infectious quarters at the beginning of the sampling interval,  $N$  is the total number of quarters present at the beginning of the sampling interval (i.e. the total number of all infected and susceptible quarters at the beginning of each sampling interval),  $S$  is the number of susceptible quarters at the beginning of the sampling interval and  $\Delta t$  is the duration of the sampling interval (14 days).

**Duration of IMI** Duration of IMI was calculated using the midpoint between samplings (Zadoks et al., 2003). The start of the IMI was defined as the midpoint between the last sampling at which the quarter was considered susceptible and the first sampling at which it was considered infected. The end of the IMI was either one week after the last sampling moment of the whole study or when a quarter became susceptible again, at the midpoint between the last sampling at which the quarter was considered infected and the first sampling at which it was considered susceptible. We assumed that a quarter with a single missing culture result maintained its infection status from the previous sampling. The association between outcome of the infection (chronic or transient IMI) and  $\log_2(\text{CFU})$  in samples with chronic and transient IMI at the first sampling for each pathogen was tested using Welch's *t*-test to correct for the unequal sample sizes in samples with chronic and transient IMI. The median duration of IMI was estimated by using the `survreg` function with Weibull distribution in the survival package version 2.44–1.1 in R (Therneau, 2020). Both left truncation and right censoring, were ignored in the analysis due to the fact that the `Surv` function in the survival package of R could not model both left truncation and right censoring. For quarters with right censoring, the week after the last sampling was considered as the end of infection. Duration of transient IMI was considered as 14 d based on the definition described above. In addition to estimating the median duration of IMI, we tested the association (indicated by Pearson's correlation coefficient) between the observed duration of chronic IMI and  $\log_2(\text{CFU})$  in chronic IMI of both pathogens.

**Basic Reproduction Number ( $R_0$ )** The  $R_0$  is the average number of new IMI caused by one infectious quarter in a naïve susceptible population, and was calculated according to Eq. (2):

$$R_0 = \beta \times (p_{\text{chronic}} \times D_{\text{chronic}} + (1 - p_{\text{chronic}}) \times D_{\text{transient}}) \quad (2)$$

where  $R_0$  is the estimated basic reproduction number,  $p_{\text{chronic}}$  is the proportion of new chronic IMI out of all new IMI across all sampling moments,  $\beta$  is the estimated transmission rate,  $D_{\text{chronic}}$  is the estimated median duration of chronic IMI,  $D_{\text{transient}}$  is the estimated duration of transient IMI (14 days). The 95 % confidence interval for the estimator of  $R_0$  was constructed, assuming independence of the transmission coefficient and duration of the infectious period, such that we could calculate the standard deviation of the estimator:  $\text{SD}(R_0) = \sqrt{\text{Var}(\beta \times D)} = \sqrt{E(D)^2 \times \text{Var}(\beta) + E(\beta)^2 \times \text{Var}(D)}$  (Rao, 1973). The analyses for *Staph. aureus* and *Strep. agalactiae* were performed separately, assuming independent infection dynamics. All analyses were performed in R version 3.6.2 (R Core Team, 2019).

### 3. Results

#### 3.1. Descriptive statistics

During this study in total 317 quarters from 81 cows were sampled at  $\geq 2$  consecutive sampling moments. There were, on average, 61 (ranging from 57 to 67) cows and 240 (ranging from 222 to 261) quarters at each sampling moment, with 99 out of a total of 317 quarters being sampled at all the 11 sampling moments. The arithmetic average of monthly geometric mean SCC of the study group was 117,189 cells/mL (ranging from 73,644 to 145,911 cell/mL).

The summary of the culture results is given in Table 1. The non-*aureus* staphylococci (NAS) and *C. bovis* were the most prevalent groups

**Table 1**

The number of isolates at each sampling moment and the average number of isolates during the study for the indicated mastitis pathogens in one group of cows on a Dutch dairy farm using an automatic milking system. A total of 81 cows were included in this study. Pathogens in the ‘Other pathogens’ category were *Streptococcus* spp., *Enterococcus* spp., *Bacillus* species and *Trueperella pyogenes*.

Culture results	2017-10-10	2017-10-24	2017-11-07	2017-11-21	2017-12-05	2017-12-19	2018-01-02	2018-01-16	2018-01-30	2018-02-13	2018-02-27	Average number of isolates (percentage)
Non-aureus <i>Staphylococcus</i>	51	45	50	39	38	49	34	45	51	44	34	43.6 (16.8 %)
<i>Corynebacterium</i> species	55	41	40	32	36	29	20	30	48	43	18	35.6 (13.7 %)
<i>Streptococcus agalactiae</i>	4	4	5	5	5	7	6	5	6	4	6	5.2 (2.0 %)
<i>Staphylococcus aureus</i>	5	4	4	5	6	6	4	5	7	7	2	5.0 (1.9 %)
<i>Streptococcus dysgalactiae</i>	3	4	4	5	5	1	4	3	4	4	5	3.8 (1.5 %)
<i>Escherichia coli</i>	0	1	1	1	1	3	1	1	0	2	2	1.4 (0.6 %)
<i>Streptococcus uberis</i>	1	0	0	0	1	0	0	0	0	0	0	1.0 (0.4 %)
Other pathogens	57	54	52	73	44	55	36	63	29	62	43	51.6 (19.9 %)
Culture negative	59	100	97	81	99	98	142	85	106	73	135	97.7 (37.9 %)
Contamination	24	22	12	19	15	19	5	18	7	18	2	14.6 (5.6 %)
Total	259	275	265	260	250	267	252	255	258	257	247	26.0 (10.0 %)

of pathogens, followed by *Staphylococcus aureus* and *Strep. agalactiae*. There were 59 samples from 21 quarters of 15 cows culture positive for *Staph. aureus*, and *Strep. agalactiae* was cultured 60 times from 20 quarters of 14 cows. In total, 1 out of 11 (9%) new *Staph. aureus* IMI and 5 out of 13 (38 %) new *Strep. agalactiae* IMI were chronic. Of the *Staph. aureus* isolates, 56/59 belonged to the same spa type, t529 (95 % of the total number of isolates). The remaining 3/59 isolates belonged to t527 (5% of the total number of isolates), which is unrelated to t529 as it has a completely different repeats succession.

**3.2. Transmission dynamics**

The number of infectious, susceptible and newly infected quarters in each infection status at every sampling moment for the 2 contagious pathogens in the 3 scenarios is provided in Table 2. The estimated

transmission rates for *Staph. aureus* were largely similar to those for *Strep. agalactiae* within each scenario but estimates of transmission rate for both pathogens were substantially lower in scenario 1 compared to scenario’s 2 and 3. The duration of chronic IMI was estimated at 95 (95 % CI: 72–125) d for *Staph. aureus* and 86 (95 % CI: 67–111) d for *Strep. agalactiae* in both scenario 1 and 2 when only the chronic IMI was considered contributing to the transmission process. The duration of IMI regardless of chronic or transient was 30 (95 % CI: 20–45) d and 39 (95 % CI: 27–57) d for *Staph. aureus* and *Strep. agalactiae*, respectively, in scenario 3 (Table 3).

The corresponding  $R_0$  was estimated below 1 in all scenario’s and ranged between 0.16 (95 % CI: 0.05–0.27) (scenario 1) and 0.34 (95 % CI: 0.20–0.48) (scenario 3) and between 0.64 (95 % CI: 0.41–0.87) (scenario 1) and 0.68 (95 % CI: 0.48–0.88) (scenario 3) for *Staph. aureus* and *Strep. agalactiae*, respectively (Table 3).

**Table 2**

Number of quarters susceptible, infectious or newly infected, used to estimate transmission rate in 3 scenarios for *Staphylococcus aureus* and *Streptococcus agalactiae* on a Dutch dairy farm using an automatic milking system. In scenario 1, only chronic IMI were included in the analysis; in scenario 2, only chronic IMI were considered as infectious, while both, new chronic IMI and new transient IMI were defined as new IMI; in scenario 3, single sampling with bacteriological culture positive and negative were considered as infectious and susceptible, respectively.

Pathogen	Scenario	Infection status	10/10/2017	10/24/2017	11/7/2017	11/21/2017	12/5/2017	12/19/2017	1/2/2018	1/16/2018	1/30/2018	2/13/2018	2/27/2018
<i>Staphylococcus aureus</i>	1	Susceptible	121	231	233	235	234	232	216	220	222	216	210
		Infectious	2	4	4	5	6	5	4	4	5	4	2
		New IMI	NA <sup>1</sup>	0	0	0	0	0	0	0	0	1	0
	2	Susceptible	119	231	233	235	234	232	216	218	222	216	209
		Infectious	2	4	4	5	6	5	4	4	5	4	2
		New IMI	NA	1	0	0	0	2	2	2	3	1	0
	3	Susceptible	122	232	233	235	234	234	216	220	222	216	218
		Infectious	3	5	4	5	6	7	6	6	7	5	2
		New IMI	NA	1	0	0	0	2	2	2	3	1	0
<i>Streptococcus agalactiae</i>	1	Susceptible	209	232	234	238	238	235	217	222	224	218	211
		Infectious	3	5	5	5	5	6	5	4	6	4	2
		New IMI	NA	0	0	0	1	1	1	0	2	0	0
	2	Susceptible	208	232	234	238	238	235	217	222	224	218	211
		Infectious	3	5	5	5	5	6	6	4	6	4	2
		New IMI	NA	0	0	1	1	2	2	1	2	0	4
	3	Susceptible	213	233	234	238	238	236	217	222	224	218	220
		Infectious	4	5	5	6	5	7	6	5	6	4	6
		New IMI	NA	0	0	1	1	2	2	1	2	0	4

<sup>1</sup> Not applicable.

**Table 3**

Transmission rate (number of transmission events per quarter-day), median duration of intramammary infection (IMI) that are considered as infectious and the corresponding basic reproduction number ( $R_0$ ) estimated for *Staphylococcus aureus* and *Streptococcus agalactiae* on a Dutch dairy farm using an automatic milking system according to 3 different modelling scenarios. In scenario 1, only chronic IMI were included in the analysis; in scenario 2, only chronic IMI was considered as infectious and new chronic IMI and new transient IMI were defined as new IMI; in scenario 3, single sampling with bacteriological culture positive and negative were considered as infectious and susceptible, respectively. Only quarters with  $\geq 2$  consecutive samples were included. The 95 % confidence intervals (95 % CI) for estimates of transmission rates were profile likelihood based confidence intervals (except the 95 % CI for transmission rate of *Staph. aureus* in scenario 1 which was calculated using the normal distribution because of only one new IMI case), while the 95 % CI for median duration of IMI and  $R_0$  were normal distribution confidence intervals.

	Scenario	Transmission rate (cases/quarter-day, 95 % CI)	Median duration of IMI (expressed as days, 95 % CI)	$R_0$ (95% CI)
<i>Staph. aureus</i>	Scenario 1	0.002 (0–0.005)	95 (72–125)	0.16 (0.05–0.27)
	Scenario 2	0.019 (0.010–0.032)	95 (72–125)	0.16 (0–0.32)
	Scenario 3	0.016 (0.008–0.027)	30 (20–45)	0.34 (0.20–0.48)
<i>Strep. agalactiae</i>	Scenario 1	0.007 (0.005–0.010)	86 (67–111)	0.64 (0.41–0.87)
	Scenario 2	0.019 (0.011–0.032)	86 (67–111)	0.64 (0.35–0.94)
	Scenario 3	0.016 (0.009–0.027)	39 (27–57)	0.68 (0.48–0.88)

In chronic IMI, the duration of IMI was positively associated with the  $\log_2$ (CFU) in quarter milk samples during the whole study period for *Strep. agalactiae*, while for *Staph. aureus* no significant association between these two variables was found. For each of the pathogens, the  $\log_2$ (CFU) at the first culture positive sampling was not significantly associated with the type of IMI (transient or chronic).

#### 4. Discussion

With this study, it was our aim to get more insight in the transmission rate, the duration of IMI and the  $R_0$  of *Staph. aureus* and *Strep. agalactiae* in a Dutch dairy herd using an AMS. We found that transmission of these two contagious pathogens was limited. The  $R_0$  of both pathogens were  $< 1$ , and the  $R_0$  values were comparable to those in well-performing CMS herds (Zadoks et al., 2002; Kirkeby et al., 2019). Our results suggest that the different definitions of IMI are not largely affecting the  $R_0$  even though the estimations of the transmission rates and median duration of IMI differed substantially in the different definition scenarios. By removing chronic IMI cows from the herd, the  $R_0$  could be significantly decreased, which means that early intervention on mastitis cases infected by contagious pathogens would largely limit the transmission of contagious pathogens in the herd. Because of the existence of chronic IMI cases with both pathogens, spanning the whole study period, we were not able to distinguish the transmission between cow to cow and environment to cow transmission routes.

The number of farms using AMS worldwide is steadily increasing (Barkema et al., 2015) and as the contact dynamics between cows and milking machine in AMS herds are different from cows in CMS herds, contagious mastitis may behave differently in AMS herds as compared to CMS herds. In both systems, reduction of the transmission rate or duration of infection of contagious pathogens are key parts in a mastitis control program. Our study, therefore, aimed to estimate the transmission rate, duration of IMI and the corresponding  $R_0$  of these two pathogens and we modelled possible differences in infectivity of transient and chronic IMI using 3 scenarios. Because it is costly and time consuming to conduct transmission studies, this type of studies are generally limited to a few herds. We do have to realize that before findings in this type of studies can be generalized, validation in a larger number of herds is required.

We found only 2 genotypes of *Staph. aureus* (the majority of isolates being *spa* type t529) in this herd, which is in line with other studies that found also only one or very few *Staph. aureus* genotypes in a herd (Anderson et al., 2012; Boss et al., 2016; Leuenberger et al., 2019). The genotyping of isolates enabled modelling of genotype specific

transmission processes, therefore, the quarters solely infected with *spa* type t527 were treated as potential susceptibles for quarters infected with *spa* type t529 in the modelling. A highly predominant farm specific genotype of *Strep. agalactiae* has been reported in CMS herds (Radtke et al., 2012). Meanwhile, Holmøy et al. (2019) found all *Strep. agalactiae* isolates from the same herd belonged to the same multi-locus sequence types for both, AMS herds ( $n = 25$ ) and CMS herds ( $n = 61$ ) on 86 Norwegian dairy farms, which suggests that the *Strep. agalactiae* isolates in our study could be from the same genotype. Thus, we assumed in our analyses that the *Strep. agalactiae* isolates were of the same genotype when estimating the transmission rate in our study.

##### 4.1. Transmission dynamics

In our study, the average quarter prevalence of *Staph. aureus* per sampling for chronic IMI was 1.9 % (ranging from 0.94 % to 2.5 %). The estimated transmission rate was comparable to results from previous studies in CMS herds that also had a low *Staph. aureus* prevalence. Zadoks et al. (2002) described the transmission rate in 3 herds, being 0.014 (0.008–0.023) for naïve susceptible quarters, while excluding quarters that could be considered more susceptible after being recovered from an IMI. Kirkeby et al. (2019) described the transmission rate to vary from 0.007 (95 % CI: 0–0.0175) to 0.009 (95 % CI: 0.006–0.015) in a herd with a low *Staph. aureus* prevalence. In the only comparable study on transmission dynamics of *Staph. aureus* in an AMS herd (Dalen et al., 2019), transmission rate was found to be 0.009 (95 % CI: 0.006–0.014) on cow level. We considered modelling at the quarter level more appropriate because in an AMS quarters are milked independent with separate milklines and no mixing of milk until the milk is far away from the teats. Thus we considered cross infection among different quarters of the same cow negligible. Additionally, this approach delivers more precise data on the infection status of individual units of infection, and thus a more reliable estimated transmission rate and  $R_0$ . All these studies did not differentiate between IMI with different duration and considered transient IMI as “true IMI” as long as they were from a single sample with clinical mastitis and/or the CFU in the sample exceeded a certain threshold. The estimated transmission rates from these studies were in the range of our estimates, which suggests that the transmission of *Staph. aureus* is comparable in AMS and CMS herds. However, our estimates for  $R_0$  were lower than in previous studies in CMS herds that had a comparable prevalence of *Staph. aureus*. Zadoks et al. (2002) described that untreated subclinical *Staph. aureus* mastitis with a mean duration of 64 d had an  $R_0$  of 0.42 (95 % CI: 0.24–0.68). Kirkeby et al. (2019) described that *Staph. aureus* IMI with a median duration of infection of

64 d had an  $R_0$  varying from 0.48 (95 % CI: 0–1.23) to 0.59 (95 % CI: 0.35 to 0.94) in their herd with a low *Staph. aureus* prevalence. Dalen et al. (2019) described *Staph. aureus* IMI with a mean duration of 128 days in an AMS herd to have an  $R_0$  of 0.76 (95 % CI: 0.41–1.42). To estimate the duration in an unbiased way was not possible in our study, because we were dealing with data with both left truncation and right censoring, essentially leading to insufficient information to make a good estimation of the duration of infection. As a consequence, it is likely that we underestimated the duration of IMI and therefore, of  $R_0$ , by ignoring the left truncation and right censoring in our study. This may be an explanation of the somewhat lower  $R_0$  we found in an AMS herd in comparison to studies in CMS herds that have been carried out so far.

In CMS herds with a higher prevalence of *Staph. aureus*, both the estimated transmission rate and  $R_0$  were higher than our estimates. Barlow et al. (2013), using the definition of IMI from Zadoks et al. (2002), estimated transmission rate at 0.008 (95 % CI: 0.006–0.012) with the corresponding  $R_0$  as 1.08 (95 % CI: 0.81–1.62) for *Staph. aureus* IMI in a group of cows without antimicrobial treatment. Schukken et al. (2014) calculated the transmission rate ranging from 0.008 (95 % CI: 0.007–0.010) to 0.010 (95 % CI: 0.008–0.012) and the corresponding  $R_0$  as 1.72 (95 % CI: 1.06–3.17) for unvaccinated cows while van den Borne et al. (2017) reported transmission rate as 0.023 (95 % CI: 0.020–0.027) in Swiss dairy herds. Finally, Kirkeby et al. (2019) reported transmission rate as 0.013 (95 % CI: 0.010–0.016) and the  $R_0$  as 1.16 (95 % CI: 0.93–1.45) in a herd with a high *Staph. aureus* prevalence. In these herds with a higher prevalence of *Staph. aureus*, all  $R_0$  were higher than 1 and outbreaks of *Staph. aureus* mastitis were highly likely or occurring. The AMS herd of our study could maintain  $R_0 < 1$ , which is comparable to well-performing CMS herds, and is therefore able to prevent transmission and outbreaks of contagious pathogens. This is likely a result of the AMS functioning well in terms of teat cleaning, teat disinfection as well as steaming of milking clusters in between cows.

In all scenarios evaluated, we found the estimated transmission rate for *Strep. agalactiae* to be no less than 0.007 (95 % CI: 0.001–0.060), which was estimated in a CMS herd by Leelahapongsathon et al. (2016), which indicates *Strep. agalactiae* may transmit easier in this AMS herd. The estimated  $R_0$  in our study herd, however, was lower than the 1.86 (95 % CI: 0.21–16.61) estimated by Leelahapongsathon et al. (2016). Our study, however, only lasted for 6 mo, which again potentially underestimated the duration of IMI, as compared to the 10 mo study of Leelahapongsathon et al. (2016) and likely resulted in an underestimation of  $R_0$  in our study. In addition, the homogeneity assumption for susceptibility may have been violated, which may also result in an underestimation of the transmission rate of both pathogens. Zadoks et al. (2002) found that quarters recovered from infection (regardless of being the result of treatment or natural cure) seemed to be more susceptible to new IMI than naïve susceptible quarters, which would contribute to higher estimates of transmission rate. In our study, we did not distinguish between the susceptibility of naïve susceptible quarters and recovered quarters, which might have resulted in an underestimation of the transmission rate.

The estimated median duration of IMI for chronic IMI of *Staph. aureus* and *Strep. agalactiae* were in-between the estimates from previous studies. Previous studies estimated the duration of IMI for *Staph. aureus* at 135.8–177.9 days (mean duration of IMI; Lam et al., 1996), 29 days (mean duration of IMI; Zadoks et al., 2002), and 64–91 days (median duration of IMI; Kirkeby et al., 2019). There are two factors contributing to the differences between duration of IMI in these studies and our current study: the definition of IMI and the length of the study period that could capture the potential full length of the duration of IMI. Lam et al. (1996) excluded short subclinical infections by the definition of IMI, while both Zadoks et al. (2002) and Kirkeby et al. (2019) included transient IMI in their analysis. By taking transient IMI into account, the estimated average duration of IMI obviously is shortened, and in addition a shorter study period might have contributed to a shorter duration of IMI and thus an underestimation of  $R_0$  in our study.

The duration of chronic IMI was significantly and positively associated with the  $\log_2(\text{CFU})$  for *Strep. agalactiae*, which suggests that higher bacterial loads in milk samples are likely to be persistent infections for IMI caused by *Strep. agalactiae*. However, the duration of chronic IMI caused by *Staph. aureus* could not be forecasted by the bacterial load in quarter milk samples. We did not find a significant association between the  $\log_2(\text{CFU})$  at the first positive sampling and whether this infection became chronic or transient. This suggests that the bacterial load in the first culture positive sample does not predict the outcome of the infection.

*Staphylococcus aureus* and *Strep. agalactiae* are generally considered to be contagious pathogens that transmit between cows during milking. However, there are several studies that describe environmental transmission of *Staph. aureus* (Fox and Gay, 1993; Barlow et al., 2013; Cobo-Ángel et al., 2018) and *Strep. agalactiae* (Jørgensen et al., 2016; Klaas and Zadoks, 2018). The contribution of environmental transmission may be underestimated in low *Staph. aureus* IMI prevalence herds (Barlow et al., 2013). We were unable to estimate the transmission rate of environmental transmission of both pathogens in this study because there were chronic IMI that spanning the whole study period, and new IMI resulting from environmental transmission could not be distinguished from those derived from infectious quarters. Isolates of spa type t527 have been found in bulk tank milk samples (Boss et al., 2016; Patel, 2018), but we are not aware of reports of environmental sources of spa type t527. Further studies are needed to elucidate the role of AMS and of the environment in the transmission of these contagious pathogens.

The estimated  $R_0$  for both pathogens was below 1 in all scenarios, which theoretically would lead to fading out of the infection in this herd. However, both pathogens seemed to be persistently present in the herd during our study. This has been seen in CMS herds as well (Zadoks et al., 2002; Leelahapongsathon et al., 2016). Possible explanations could be more heterogeneity in infectivity and susceptibility of IMI in the population than we were able to evaluate, with a subgroup of cows maintaining the IMI throughout the course of our study. Zadoks et al. (2002) found that quarters recovered from IMI were more susceptible to new IMI than naïve susceptible quarters. When recovered quarters are more susceptible than naïve quarters this might lead to an endemic situation even when  $R_0$  is below the threshold of 1 (Greenhalgh et al., 2000), thus eradication of the infection will take more effort than prevention of entrance of new infected animals into the herd (Zadoks et al., 2002).

## 5. Conclusion

Transmission of *Staph. aureus* and *Strep. agalactiae* between quarters in this AMS herd was limited and  $R_0$  of both pathogens was estimated to be below 1, although some underestimation of  $R_0$  is likely. Future studies are needed to quantify transmission in more AMS herds and get more insight in the transmission routes of these pathogens in AMS herds to better facilitate further prevention of transmission.

## Submission declaration

This study has not been published anywhere else previously.

## Declaration of Competing Interest

None.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.prevetmed.2021.105384>.

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