A mathematical model of *Staphylococcus aureus* control in dairy herds

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SUMMARY

An ordinary differential equation model was developed to simulate dynamics of *Staphylococcus aureus* mastitis. Data to estimate model parameters were obtained from an 18-month observational study in three commercial dairy herds. A deterministic simulation model was constructed to estimate values of the basic (ℛ₀) and effective (ℛₜ) reproductive number in each herd, and to examine the effect of management on mastitis control. In all herds ℛ₀ was below the threshold value 1, indicating control of contagious transmission. ℛₜ was higher than ℛ₀ because recovered individuals were more susceptible to infection than individuals without prior infection history. Disease dynamics in two herds were well described by the model. Treatment of subclinical mastitis and prevention of influx of infected individuals contributed to decrease of *S. aureus* prevalence. For one herd, the model failed to mimic field observations. Explanations for the discrepancy are given in a discussion of current knowledge and model assumptions.

INTRODUCTION

*Staphylococcus aureus* is an important cause of udder infections in dairy herds [1, 2]. Infections with *S. aureus* can result in clinical or subclinical disease and are usually associated with increase in somatic cell count (SCC) [2]. *Staphylococcus aureus* is contagious and spreads easily within dairy herds [3, 4]. When multiple cows in a herd are infected, bulk milk SCC (BMSCC) increases and legal limits for BMSCC may be violated, or thresholds for premium bonus may not be met [5, 6]. Hence, control of *S. aureus* mastitis is necessary and important. The feasibility of *S. aureus* control is a matter of debate. Some authors state that *S. aureus* mastitis can be controlled [7, 8] or even eradicated [9, 10]. Goodger and Ferguson [11] showed the economic benefit of a control programme. However, others contend that it is difficult to control *S. aureus* mastitis and impossible to eradicate the disease [12, 13].

Control programmes include post-milking teat disinfection (PMTD), antibiotic treatment of all cows at dry-off (dry cow therapy, DCT), culling of chronically infected animals, and segregation of infected and non-infected animals [7, 10, 14, 15]. In addition, antibiotic treatment of cows with clinical
mastitis is routine practice. For cows with subclinical infections, the usefulness of treatment is disputed. In a cost–benefit analysis of treatment for the individual cow, Craven [16] claimed that treatment of subclinical mastitis is economically unjustified. Other authors look beyond the individual cow and comment on the importance of treatment of subclinical cases to prevent spread of infection in the population [8, 17, 18]. Dodd et al. [19] state that the only practical way of increasing the rate of elimination of infections is by using antibiotics more effectively, i.e. treating clinical as well as subclinical infections.

To be successful, a control programme must reduce the number of new infections and the duration of existing infections [20]. Several control programmes have proven their effectiveness in field trials, but it is impractical to test all possible control scenarios. The cost of examining different combinations of control measures would be prohibitive. However, the merits of different control programmes can be examined with simulation models [6, 19, 21]. Models are simplified representations of real systems that are presented as a set of computational rules or assumptions. They characterize the system in terms of mathematical, logical and temporal relationships [22]. Models of mastitis control were reviewed by Allore and Erb [23].

Allore and Erb [23] developed a system of ordinary differential equations (ODE) that describes a herd of dairy cows as a population of uninfected, subclinically infected, clinically infected, and recovered animals. This system of ODEs can be used to calculate a value of $R_n$, the basic reproductive number, for mastitis. $R_n$ is defined as the expected number of secondary cases produced by a primary infectious case in a wholly susceptible population [24]. When $R_n$ is less than 1, between-animal transmission cannot maintain a disease in a population. The main input components of $R_n$ are the rate of new infections and the duration of infections. Thus, $R_n$ is a summary indicator of the efficacy of mastitis control schemes as proposed by Neave and co-workers [20]. Calculation of $R_n$ has been used to estimate the efficacy of PMTD during an outbreak of $S. aureus$ mastitis in a dairy herd [4]. The ODE model that Allore and Erb [23] developed could not be run, because no estimates were available for many of the model parameters.

The purpose of the current paper is to describe the dynamics of $S. aureus$ infection in three endemically infected commercial dairy herds, elaborating on the ODE model of Allore and Erb [23]. Values for model parameters and $R_n$ are estimated from observational data, and assumptions underlying the model and the parameter estimation procedures are discussed. The model is used in a deterministic manner to evaluate whether $R_n$ can be reduced to a value below 1, as would be necessary to make a control programme successful in the long term.

**MATERIALS AND METHODS**

**Data collection**

Data were obtained from a longitudinal observational study (from May 1997 to December 1998) in three commercial dairy herds (A, B and C) in The Netherlands. The herds were known to be endemically infected with $S. aureus$, and were considered to be illustrative for the level of management in such herds in The Netherlands [25]. Herd A consisted of $67 \pm 3$ lactating animals that belonged predominantly to the Meuse–Rhine–Yssel and Red Holstein breeds with 305-day milk production of $7187 \pm 149$ kg. Herd B consisted of $95 \pm 5$ lactating animals that belonged predominantly to the Holstein–Friesian and Dutch Friesian breeds, with some Meuse–Rhine–Yssel cross-breeds. The 305-day milk production was $8166 \pm 459$ kg. Herd C consisted of $41 \pm 2$ lactating Holstein Friesian and Dutch Friesian animals, with 305-day milk production of $8508 \pm 165$ kg.

Herd were housed in free stall barns with cubicles and concrete slatted floors in winter, and mostly grazed on pasture in summer (May to October). Animals were milked twice a day. Dry udder preparation was used in all herds. In herd A, cotton towels were used for udder preparation of one or multiple cows. In herds B and C, paper towels were used and per towel only one cow was treated. At every milking, the cows, the udders and the first streams of milk from each quarter were checked for signs of clinical mastitis (any visual abnormality of milk and/or udder, with or without systemic signs of disease). During the study, farmers received information on infection status of their animals. Farmers were free to make changes in herd management using such information, as they would be if they did not participate in a study. In herd C, milking clusters were flushed with hot water (90°C) after milking of $S. aureus$-infected cows, to prevent transmission of bacteria via the milking machine. Farmers supplied information on dates of calving, clinical mastitis, antibiotic treatments, dry-off and culling. For each farm, records on the infection status of cows with subclinical or clinical mastitis in the year(s) preceding
the study (2, 1 and 1.5 years for herds A, B and C, respectively) were available through the Animal Health Service, Deventer, The Netherlands.

To determine the infection status of udder quarters, foremilk samples (approximately 15 ml) were collected at 3-week intervals from all lactating quarters in each herd. Samples were taken after the first streams of milk were discarded, and after teat ends had been disinfected with cotton swabs drenched in methylated spirits [26]. At the start of the study, duplicate samples were taken on two consecutive days to determine the initial infection status of all lactating quarters. Additional quarter milk samples (approximately 5 ml) were collected by farmers at calving (prior to first contact with the milking machine), dry-off, culling and in the case of clinical mastitis. Milk samples that were used for bacteriological culture were stored at 20 °C until processing.

Within 3 weeks of collection, 0–0.1 ml of milk was cultured and bacterial species were identified according to National Mastitis Council standards [27]. A quarter was considered to have an intramammary infection (IMI) with *S. aureus* when ≥1000 c.f.u./ml of the pathogen were cultured from a single sample, when ≥500 c.f.u./ml of the pathogen were cultured from two out of three consecutive milk samples, when ≥100 c.f.u./ml were cultured from three consecutive milk samples, or when ≥100 c.f.u./ml were cultured from a single sample. Samples that contained more than three bacterial species were considered contaminated, and were not used to determine IMI status. Samples that were culture negative during antibiotic treatment for udder disease were not used either. A previously infected quarter was considered recovered from infection if none of the above definitions were met and the sample was free of the pathogen [28]. For statistical analysis, *S. aureus* content of milk samples was treated as a categorical variables with four levels, (0–9, 10–49, 50–199 or ≥200 c.f.u./plate).

**Model formulation**

The lactating herd was described as a population of individuals that were uninfected (U) subclinically infected (S), i.e. infected with *S. aureus* but not showing any visible signs, clinically infected (C), i.e. infected with *S. aureus* and showing signs of disease, or recovered-uninfected (R). In traditional SIR models, where ‘S’ indicates the susceptible compartment, ‘I’ indicates the infected compartment, and ‘R’ indicates the recovered compartment, recovered individuals are often considered to be resistant or removed from the susceptible population. In our study, cure and reinfection of individuals was observed, showing that recovery did not confer absolute resistance to reinfecion. This could be described by an SIS model, where ‘S’ indicates ‘susceptible’ and ‘I’ indicates ‘infected’, assuming that susceptibility does not differ between naive individuals and recovered individuals. We preferred to model uninfected individuals (U) and recovered uninfected individuals (R) separately, because for some pathogens, susceptibility may differ between individuals that have not experienced infection before and individuals that have recovered from infection [28].

Individuals entered the lactating herd after purchase or at calving, and left the lactating herd at dry-off or culling. Individuals that were dried off usually re-entered the lactating population after the next calving. Entry into the population could be into any compartment. Culling or dry-off could take place for non-mastitis related reasons, e.g. infertility, lameness, or low production, or it could be because of mastitis. Uninfected and recovered uninfected individuals could get infected (primo-infection and reinfection, respectively), and infections could be subclinical or clinical. Flare-up of subclinical infection to clinical infection, i.e. occurrence of clinical signs in a previously asymptomatic infection, and remission of clinical infection to subclinical infection, i.e. disappearance of clinical signs without disappearance of infection, were also observed. Finally, cure of subclinical and clinical infections occurred, with or without preceding antibiotic treatment. The model, a modification of the model by Allore and Erb [23], is graphically represented in Figure 1. Entry, exit and transition rates are given in Appendix 1.

**Model assumptions**

In the analysis, udder quarters of cows were treated as individuals. This approach was preferred above taking cows as individual units for the following reasons. When looking at contagious mastitis, such as *S. aureus* mastitis, exposure to herd mates is a major factor in the spread of disease [3, 4, 14]. A cow with three infected quarters shedding *S. aureus* causes more exposure to herd mates than a cow with one infected quarter that sheds *S. aureus*. Also, each exposed quarter is a unit at risk for infection [4]. Therefore, the individual of interest for infectious disease modelling...
is quarter rather than cow. In addition, classification of individuals is clear-cut when quarters are considered as individuals. For example, a cow with one subclinically infected quarter would be classified as ‘subclinically infected’ at cow level and would not be at risk for infection, while she has three non-infected quarters that are at risk of new infections and that would justify classification of the same cow as ‘uninfected’. Furthermore, cessation of milking in one quarter of a cow is possible, resulting in a ‘blind’ quarter. In dairy herds, this technique is used out of necessity, or to remove an infected quarter from the population while the remaining healthy quarters of the cow continue to be productive [29]. Thus, culling is an event that may occur at quarter level, specifically for *S. aureus*-infected quarters. Finally, the number of quarters is approximately four times as large as the number of cows, resulting in a larger population of individuals under study and lowest impact of random events. We note that in adopting the udder quarter as the individual unit in a compartmental model, we ignore dependencies among quarters of the same cow [26, 30].

The spread of *S. aureus* in the lactating population was assumed to be the result of quarter-to-quarter transmission, and depended on the size of the susceptible compartment, the prevalence of infection, and the transmission parameter, $\beta$ [4]. The transmission parameter is the probability per unit of time that an infectious quarter will infect a non-infected quarter. Because the definition of infection was based on shedding of the infectious agent, all infected quarters were assumed to be infectious albeit at possibly different levels for clinical and subclinical infections. We chose to model quarters in lactation only. Non-lactating quarters were excluded from the model, because management and contact structure differ between lactating cows and non-lactating cows (dry cows, replacement heifers, and young stock). Such differences may affect pathogen transmission [31].

Separate transmission parameters were calculated for infection of uninfected and recovered uninfected individuals, to model the possible differences in susceptibility between the two compartments. Possible differences in infectiousness between the subclinical...
and clinical compartments were also incorporated in the model:

Rate of new IMI in uninfected individuals
\[
\frac{\beta_u}{U} \times \frac{[(S + bC)/N]}{U} \times R. \tag{1a}
\]

Rate of new IMI in recovered-uninfected individuals
\[
\frac{\beta_u}{R} \times \frac{[(S + bC)/N]}{R}. \tag{1b}
\]

where \(S\) is the size of subclinically infected compartment, \(b\) the relative change in transmission rate for clinical infections compared to subclinical infections, \(C\) the size of clinically infected compartment, \(N\) the total population size, \(U\) the size of uninfected compartment, \(R\) the size of recovered-uninfected compartment, and subscripts refer to the susceptible compartment \((U = \text{uninfected})\), \(R = \text{recovered-uninfected}\). This Reed–Frost transmission function assumes homogeneous mixing of hosts. This assumption, discussed by Lam et al. [4], was considered to be an acceptable approximation of possible contacts between cows. Although contacts between individuals in herd \(C\) may have been affected by flushing of teat cup liners, homogeneous mixing was also assumed for herd \(C\). In addition to homogeneous mixing, the model assumes homogeneity of individuals within compartments with respect to susceptibility and with respect to infectiousness [31].

Rates of entry, exit or transition between compartments were calculated as mean rates (total number of events per total time at risk) based on observational data (see Parameter estimation below). Use of a mean rate assumes that the mean is an adequate measure of the central value for a parameter [22]. The mean was used because a better approximation of the central value was not available. Furthermore, all rates were assumed to be constant over the 18-month observation period.

Parameter estimation

To estimate rates, transmission parameters and proportions, the size of the compartments \(U\), \(S\), \(C\), and \(R\) had to be known. A quarter was considered to be uninfected when there were no records of infection preceding the study, and no episodes of \(S. aureus\) infection during the study. A quarter was considered subclinically infected when a definition of \(S. aureus\) infection was met, but clinical signs were not recorded. A quarter was clinically infected when a definition of \(S. aureus\) infection was met and clinical signs were recorded. A quarter was considered recovered when it did not meet any of the definitions of \(S. aureus\) infection, and had been positive for \(S. aureus\) prior to the study, as documented by Animal Health Service records, or during the study. For subclinical or clinical infections that were first detected at calving, the calving date was assumed to be the date of onset of infection. For clinical infections that started during lactation, the recorded date of clinical mastitis was used as the starting date for the clinical infection. For subclinical or clinical infections that were last detected at dry-off or at culling, sample date was taken as the endpoint of infection. For other combinations, e.g. infectious episodes starting during lactation or ending between a clinical sample and a consecutive routine sample, the midpoint of the last negative and the first positive sample was taken as starting point of the episode, and the midpoint between last positive and first negative sample was taken as endpoint of the episode. The terms ‘positive’ and ‘negative’ apply to clinical status and to infection status of the sample. From the starting points and end points of lactations and infected episodes, the number of days that quarters contributed to a specific compartment in the population was calculated. The summation of the number of days was used as time at risk in that compartment, or compartment size. When samples were missing at dry-off or cull, the last observation with known IMI status was used as the moment of dry-off or cull.

For each herd, dry-off rates, flare-up rates, remission rates, spontaneous cure rates and cure rates after treatment were calculated as the number of observed events, divided by the time at risk in a compartment. For example, the dry-off rate for uninfected individuals was calculated as the number of dry-offs from the uninfected compartment divided by the total number of quarter days in the uninfected compartment. Reasons for culling were not recorded by farmers. Therefore, it was not possible to calculate mastitis-related culling rates directly from the data. The non-mastitis related rate of culling was assumed to be the same for all compartments. This rate was calculated for each herd based on compartment \(U\), and was assumed to apply to compartments \(S\) and \(C\) as well. The non-mastitis related culling rate was subtracted from the total culling rate for compartments \(S\) and \(C\) to obtain the mastitis-related culling rate. Blind quarters were considered culled.

The fraction of entries for each compartment was calculated per herd based on the status \((U, S, C \text{ or } R)\) of quarters at calving or purchase. For new infections during lactation, the fraction that was subclinical or...
clinical was calculated for infections originating from compartments U and R, respectively, in each herd. Fractions were compared between herds, and between compartments of origin by means of two-tailed Fisher Exact test using SAS (SAS System for Windows, Version 8.01, SAS Institute Inc., Cary, NC, USA). To examine the infectiousness of quarters in compartments S and C, levels of *S. aureus* shedding were compared between milk samples from subclinical infectious episodes and from clinical infectious episodes within each herd by means of $\chi^2$ analysis using SAS (Version 8.01).

For each herd, values for $\beta_U$ (transmission parameter for infections from U) and $\beta_R$ (transmission parameter for infections from R) were calculated from a simplified version of the generalized linear model with log-link and Poisson distributed error that was used [4, 28]:

\[
\varepsilon[\ln(IMI)] = \ln(\beta_U) + \ln[U \times (S + bC)/N], \quad (2a)
\]

\[
\varepsilon[\ln(IMI)] = \ln(\beta_R) + \ln[R \times (S + bC)/N], \quad (2b)
\]

where $\varepsilon$ is the expected value, and IMI the number of new IMI in the observation period. To allow for estimation of $\ln(\beta)$, data on number of new IMI and compartment sizes were entered for each 3-week period, and $\ln(U \times (S + bC)/N)$ or $\ln(R \times (S + bC)/N)$ was used as model offset. The value for $b$ was set at 1 (see Results). Analysis was done using Statistix (Statistix for Windows, Version 1.0, Analytical Software Co., La Jolla, CA, USA). A 95% confidence interval for $\beta$ was calculated, taking into account the limited number of observations (25 observations for each estimation of $\beta$) [32].

### Calculation of reproductive number

In previous ODE models of *S. aureus* mastitis, no distinction was made between clinical and subclinical infections, and $R_0$ was simply given by the product of $\beta$ and the mean duration of the infectious period [4]. In the current model, the end of a clinical or subclinical episode was not necessarily the end of infectiousness, because interchange between the subclinical and clinical compartments occurred. This interchange was quantified by the flare-up rate $\chi$ and the remission rate $\theta$, and needed to be accounted for in the calculation of $R_0$. Furthermore, the basic reproductive number depended on the fraction of new infections that were subclinical and clinical, respectively, as quantified by $f$ and $1-f$, and on the relative infectiousness of subclinical and clinical infections, as quantified by $b$. The mathematical expression for $R_0$ is given in Appendix 2, together with a derivation [33]. Using the parameter estimates from this study, $R_0$ was estimated for each herd.

In addition to the basic reproductive number, an effective reproductive number, $R_e$, was calculated. This is the expected number of secondary cases produced by an infectious case in a population that is not wholly susceptible [24]. The subscript ‘$t$’ is used to indicate the time-point for which the effective reproductive number is calculated, and to differentiate between $R$ (recovered) and $R_t$ (effective reproductive number). $R_t$ depends on $\beta_U$ and $\beta_R$, and on the composition of the herd at time $t$ (Appendix 3).

### Deterministic simulation

Using the transition rates from Appendix 1, the effective reproductive number can be calculated for any time-point $t$, and for any composition of the population at the onset of the simulation (proportion $U$, $S$, $C$ and $R$). To this end, the mathematical model was translated into the C language and compiled with Microsoft C++ version 1.52 as a Windows application. The model was written as a deterministic model.

To assess whether the model reflected the observed dynamics of infection, simulations were run for each herd with the parameter estimates obtained from the field study and the herd composition that was observed at the onset of the study. Next, to simulate the effect of cure of subclinical mastitis on herd dynamics, the highest cure rate that was observed for subclinical infections (cure rate from herd B) was substituted into herds with a lower cure rate of subclinical infections (cure rate from herd B) was substituted into herds with a lower cure rate of subclinical infections (herds A and C). Finally, simulations were performed using the parameter estimates from the field study but assuming zero influx into compartments S and C ($p = q = 0$), as would be the case if all infections in non-lactating animals and herd additions were prevented or cured before entry into the lactating population. To calculate the proportion of influx into the uninfected compartment, $(1-p-q-r)$, and into the recovered compartment ($r$) in this scenario, all infections at calving in primiparous animals were assumed to have been prevented (entry into S or C substituted by entry into U), and all infections in non-lactating multiparous animals were assumed to be cured by DCT before re-entry into the population (entry into S or C substituted by entry into R).
RESULTS

Descriptive results

During the 18-month observation period, 26049 milk samples were collected out of which 96% could be used to determine infection status of quarters. In herd A, 15 infected quarters were present at the start of the study, 23 quarters were infected at calving, and 41 new infections with S. aureus were detected in lactating quarters. In herd B, 8 infected quarters were present at the start of the study, 16 quarters were infected at calving, and 18 new infections were detected in lactating quarters. In herd C, 3 infections were present at the start of the study, 6 infections were detected at calving, and 40 new infections were detected during lactation. Table 1 lists the number of subclinical and clinical infections in lactating quarters per compartment of origin for each herd. The proportion of new infections during lactation that was S or C did not differ between herds ($P = 0.10$), or between compartments of origin ($P = 0.29$).

Number of new infections and prevalence of S. aureus are shown per herd in Figures 2 and 3, respectively. In herds A and C, incidence of new infections in lactating quarters was lower in the second part of the study than during the first part of the study. In herd B, incidence was approximately constant throughout the study, with an average of one new infection per month. The number of events (cull, flare-up, cure, dry-off, remission, and entry) and the number of days at risk within each compartment per herd are listed in Appendix 4.

Parameter estimates

Estimates for herd-specific cull rates, flare-up rates, cure rates, dry-off rates and remission rates are summarized in Table 2. When formulating the model, a mastitis-related cull rate, $\alpha$, was incorporated for the infected compartments S and C. When calculating cull rates per compartment, cull rate from U was lower than cull rates from S and C, but also lower than cull rate from R in each herd (data in Appendix 4). Therefore, an additional mastitis-related cull rate, $\alpha_R$, was introduced for compartment R. Staphylococcus aureus-infected quarters where milking was ceased constituted 2 out of 20, 4 out of 7, and 1 out of 5 quarters that were infected upon cull in herd A, B and C, respectively.

For herd B, the number of new infections in quarters originating from R was low ($n = 2$). Therefore, the estimate of $\beta_R$ for herd B may not be an accurate estimate of the true transmission parameter. Calculation of an overall value for $\beta$, irrespective of compartment of origin, was considered. However, estimates for $\beta_R$ were considerably higher than estimates for $\beta_U$ in each herd (Table 2). This is similar to results obtained for Streptococcus uberis in herd B [28]. Therefore, separate values for $\beta_U$ and $\beta_R$ were used for each herd. The observed and predicted number of infections from compartment U, as predicted by the Poisson regression model for calculation of $\beta_U$, is illustrated per herd in Figure 4.

Cure rates were calculated for treated quarters, $\delta$, and non-treated quarters, $\gamma$. In quarters with clinical infection, cure was never observed without treatment. In quarters with subclinical infection, cure was observed without treatment (spontaneous cure) and after treatment. Dry-off rates were similar between compartments in herds A and B (data in Appendix 4). In herd C, dry-off rate in compartment S was higher than in compartments U or R (5.5, 2.6 and $2.4 \times 10^{-3}$ quarters/day-at-risk, respectively). Because the highest rate was associated with the smallest compartment, i.e. most prone to random effects, and because early dry-off of infected quarters was not consciously used as control strategy, one dry-off rate was used for all compartments within each herd. The overall dry-off rate (i.e. dry-off rate for all compartments combined) was similar between herds.

For calculation of the fraction of new infections in lactation that were subclinical ($f$) or clinical ($1-f$) data from all herds were combined, because proportions did not differ significantly between herds or compartments. Fractions are included in Table 2.

In herd A, the number of bacteria that was shed in milk was lower for clinically infected quarters than for subclinically infected quarters (334 samples, $P < 0.01$). In herd B (91 samples, $P = 0.26$), and herd C (142 samples, $P = 0.87$) bacterial numbers did not differ between samples from subclinically or clinically infected quarters. Higher numbers of bacteria in milk from clinically infected quarters may lead to higher exposure from C than from S individuals, resulting in a value of $b$ higher than 1. During the field study, it was noted that milking clusters were usually rinsed with water when a clinically infected quarter had been milked. Rinsing affects the number of bacteria and may result in lower exposure caused by C individuals, leading to a value of $b$ lower than 1. Because the
Table 1. Number of new infections with Staphylococcus aureus in lactating udder quarters observed over an 18-month period in three dairy herds. Average population size expressed in number of lactating udder quarters was 268, 384 and 164 for herds A, B and C, respectively.

<table>
<thead>
<tr>
<th>Herd</th>
<th>New infections from U</th>
<th>New infections from R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subclinical</td>
<td>Clinical</td>
</tr>
<tr>
<td>A</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>B</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>29</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>12</td>
</tr>
</tbody>
</table>

Fig. 2. Number of new intramammary infections (IMI) with Staphylococcus aureus in lactating udder quarters during an 18-month observation period (27 samplings at 3-week intervals) in three Dutch dairy herds. Average population size expressed in number of lactating udder quarters was 268, 384 and 164 for herds A, B and C, respectively.

combined effect of the two phenomena could not be quantified, \( b \) was set at 1 for each herd.

Reproductive number and model output

Based on the parameter estimates from Table 2 and equation (A 2.1) from Appendix 2, values for \( R_0 \) were calculated. For each herd, the value of \( R_0 \) was below one (Table 2). Results for \( R_t \) are not tabulated because \( R_t \) changes over time. Using the observed herd composition at onset of the study and the transmission parameters calculated from the data, the value of \( R_t \) ranged from 0.53 to 0.67, from 0.40 to 0.44, and from 0.75 to 0.89 for herds, A, B and C, respectively, from the start to the end of the first simulated year. \( R_t \) was higher than \( R_0 \) in each herd, and increased with the proportion of recovered individuals in the simulated population.

Simulated dynamics for three scenarios (observed, higher cure rate of subclinical infections, zero influx of infections) are exemplified for herd A in Figure 5. In the simulation of the observed dynamics, prevalence of subclinical infection levels off at 9.4 infected udder quarters (Fig. 5). During the field study, observed prevalence of infection hovered around a constant level from interval 7–21 (Fig. 3), and observed average prevalence of subclinical infection in that time period was 191 infected quarter days per 3-week period. This is equivalent to 9.1 infected udder quarter on any day. Thus, the simulated number of infected quarters reflected the field data. Observed prevalence dropped to the stable level faster than simulated prevalence. Similarly, the prevalence of subclinical infections in herd B levelled off at 1.5 infected udder quarter when observed dynamics were simulated (Fig. 6), while field data showed an average prevalence of subclinical...
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Sampling interval

Prevalence (quarterdays with IMI)

Herd A

Herd B

Herd C

50

100

150

200

250

300

350

Fig. 3. Prevalence of *Staphylococcus aureus* infection in lactating udder quarters observed over an 18-month period (26 intervals of 3 weeks) in three dairy herds. Average population size expressed in number of lactating udder quarters was 268, 384 and 164 for herds A, B and C, respectively.

**Table 2.** Parameter estimates for transitions rates, proportions and transmission parameters in a compartmental model that represents the dynamics of *Staphylococcus aureus* infections in a population of lactating udder quarters. Rates and transmission parameters are expressed as number of events per 10$^3$ quarter days at risk.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Herd A</th>
<th>Herd B</th>
<th>Herd C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra culling rate from clinical compartment</td>
<td>$\alpha_c$</td>
<td>53.5</td>
<td>35.8</td>
<td>12.7</td>
</tr>
<tr>
<td>Extra culling rate from subclinical compartment</td>
<td>$\alpha_s$</td>
<td>2.4</td>
<td>2.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Extra culling rate from recovered compartment</td>
<td>$\alpha_r$</td>
<td>1.4</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Transmission parameter for new infections from U*</td>
<td>$\beta_U$</td>
<td>5.1†</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Transmission parameter for new infections from R</td>
<td>$\beta_R$</td>
<td>42</td>
<td>52</td>
<td>41</td>
</tr>
<tr>
<td>Flare-up rate (S to C)</td>
<td>$\chi$</td>
<td>2.1</td>
<td>4.6</td>
<td>4.1</td>
</tr>
<tr>
<td>Cure rate for treated clinical infections</td>
<td>$\delta_c$</td>
<td>32.6</td>
<td>36.5</td>
<td>13.3</td>
</tr>
<tr>
<td>Cure rate for treated subclinical infections</td>
<td>$\delta_s$</td>
<td>1.3</td>
<td>10.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Spontaneous cure rate for clinical infections</td>
<td>$\gamma_c$</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Spontaneous cure rate for subclinical infections</td>
<td>$\gamma_s$</td>
<td>5.2</td>
<td>15.7</td>
<td>11.9</td>
</tr>
<tr>
<td>Exit rate due to culling (non-mastitis related)</td>
<td>$\mu_{CU}$</td>
<td>0.8</td>
<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Exit rate due to dry-off (non-mastitis related)</td>
<td>$\mu_{DR}$</td>
<td>2.5</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Remission rate (C to S)</td>
<td>$\theta$</td>
<td>163</td>
<td>61</td>
<td>120</td>
</tr>
<tr>
<td>Fraction of new entries that is S</td>
<td>$p$</td>
<td>0.033</td>
<td>0.020</td>
<td>0.022</td>
</tr>
<tr>
<td>Fraction of new entries that is C</td>
<td>$q$</td>
<td>0.012</td>
<td>0.003</td>
<td>0.000</td>
</tr>
<tr>
<td>Fraction of new entries that is R</td>
<td>$r$</td>
<td>0.049</td>
<td>0.023</td>
<td>0.074</td>
</tr>
<tr>
<td>Fraction of new infections from U that goes to S</td>
<td>$f_U$</td>
<td>0.87</td>
<td>0.87</td>
<td>0.87</td>
</tr>
<tr>
<td>Fraction of new infections from R that goes to S</td>
<td>$f_R$</td>
<td>0.87</td>
<td>0.87</td>
<td>0.87</td>
</tr>
<tr>
<td>Change in infectiousness for C relative to S</td>
<td>$b$</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Basic reproductive number</td>
<td>$R_0$</td>
<td>0.53</td>
<td>0.40</td>
<td>0.75</td>
</tr>
</tbody>
</table>

* U = uninfected, R = recovered-uninfected, S = subclinically infected, C = clinically infected.

† 95% confidence intervals are (5; 10), (8; 23), and (9; 21) for herds A, B and C, respectively.

‡ 95% confidence intervals are (22; 80), (12; 223), and (21; 81) for herds A, B and C, respectively.

infections of 1-3 udder quarter from interval 7–21 (Fig. 3). Decline of infection prevalence after onset of the study was faster in herd B than in herd A, both for observed and simulated dynamics. For herd C, simulated dynamics showed an increase of infection prevalence over time (Fig. 6), in disagreement with
Fig. 4. Number of new infections with *Staphylococcus aureus* in lactating udder quarters as observed in three dairy herds over an 18-month period (26 intervals of 3 weeks), and as predicted by a Poisson regression model, using \( d[\ln(\text{IMI})] = \ln(\beta_c) + \ln(S + C/N)U \) where \( \varepsilon \) = expected value, IMI = number of new intramammary infections in the current time interval, \( \beta_c \) = transmission parameter for new infections in quarters that have not experienced *S. aureus* infection before, \( S \) = number of quarterdays with subclinical *S. aureus* infection in the preceding time interval, \( C \) = number of quarterdays with clinical *S. aureus* infection in the preceding time interval, \( N \) = total number of quarter days in the population, and \( U \) = number of uninfected quarterdays at risk in the current time interval for quarters that have not experienced *S. aureus* infection before.
Mathematical model of *S. aureus* control

### Fig. 5
Simulated number of quarters with subclinical *Staphylococcus aureus* mastitis in a population of 268 lactating udder quarters (herd A), based on composition of the population at onset of the observational study, and on parameter estimates as calculated from the data (thick black line), or with substitution of a higher cure rate for subclinical infections (grey line), or assuming zero influx of infected individuals into the population (thin black line). The number of clinically infected quarters is close to zero throughout the simulated period and is not displayed.

### Fig. 6
Simulated number of quarters with subclinical *Staphylococcus aureus* infection based on composition of the population at onset of the observational study, and on parameter estimates as calculated from the data for herds A (thick black line), B (grey line) and C (thin black line). Population size is 268, 384 and 164 quarters, respectively.

Field observations. The deterministic model did not simulate changes in infection prevalence that were observed in each herd at the end of the field study.

**DISCUSSION**

*Staphylococcus aureus* is a contagious pathogen that spreads easily in dairy herds unless adequate control measures are taken. Successful control of contagious spread is achieved if $R_0$ is reduced to a value below 1. One of the aims of this paper was to estimate the value of $R_0$ for *S. aureus* mastitis in lactating populations under field conditions, and to use simulation to show how changes in management may contribute to reduction of $R_0$ to a value below 1. Even without simulation of changes, the combined effect of control measures in the study herds was that $R_0$ was below 1. Thus, if all transmission of *S. aureus* were the result of cow-to-cow or quarter-to-quarter transmission, it should be possible to eliminate *S. aureus* mastitis from the study populations. Farmers in the study herds used control measures that are used by many farmers.
During the study, the farmers were informed of the infection status of all quarters every 3 weeks. This led to well-informed treatment, segregation or cull decisions, and may have contributed to the successful control of contagious transmission in the participating herds.

Prevalence of *S. aureus* infection was highest in herd A, while the probability per time unit that an infected quarter would cause a new infection, i.e. the transmission parameter, was lowest in herd A. High prevalence and low number of new infections in the lactating population are not necessarily contradictory. It can occur when the pool of susceptible individuals in the population is exhausted, when there is entry of infected individuals into the population, or when infections have long duration. However, none of those situations explain the low transmission parameter. The low transmission parameter in herd A was unexpected, because herd A was the only herd that did not use individual towels, PMTD or disinfection of teat cup liners. Those measures were used in the other two herds and reduce transmission of *S. aureus* [4, 14, 15]. Because of the small number of herds involved in the study, it is not possible to determine the cause of the difference in transmission parameter with certainty. Potential contributing factors include milk production, breed, and bacterial strains. Production was similar for herds B and C, and much lower for herd A. At herd level, high production is associated with a higher risk of *S. aureus* mastitis [34]. Thus, a lower risk in herd A could be expected based on production. Herd A had the largest proportion of Meuse–Rhine–Yssel cows, and this breed is associated with an increased risk of *S. aureus* [35]. Based on breed, a higher risk could be expected in herd A. Preliminary evidence suggests that differences between bacterial strains may be associated with differences in spread of *S. aureus* in a population [29]. In a small scale study, the predominant *S. aureus* strain in lactating quarters in herd A was different from the predominant *S. aureus* strain in lactating quarters from herds B and C [36]. Summarizing, management, cow and pathogen characteristics may have contributed to differences in *S. aureus* spread.

For all three herds, the value of $\beta_R$ was higher than the value of $\beta_V$. Recovered quarters were not immune to reinfection, but, on the contrary, had increased susceptibility to reinfection. This result at population level is in agreement with results at quarter level from a risk factor study in the observed herds [25]. The observed differences in susceptibility between uninfected and recovered-uninfected quarters justified the choice to model uninfected quarters and recovered-uninfected quarters as separate compartments. Farmers may be aware that recovered quarters are at higher risk of infection. Such awareness could explain why culling from compartment R took place at a higher rate than culling from compartment U. Other reasons include decreased milk production and elevated somatic cell counts in quarters that suffered intramammary infection [2, 16]. An interesting consequence of $\beta_R$ being higher than $\beta_V$ is that $R_t$ can be higher than $R_v$, as observed in this study. As a result, measures that are sufficient to prevent spread of *S. aureus* in a wholly susceptible population may not suffice to prevent spread of *S. aureus* in a population with recovered individuals.

Despite the fact that $R_v$ and $R_t$ were below one, indicating that contagious transmission of *S. aureus* was controlled in each herd, new infections in lactating individuals were observed. $R_v$ and $R_t$ indicate the average number of new infections caused by an existing infection. Hence, a number of new infections should be expected on theoretical grounds, as some existing infections will not cause new infections, while other existing infections cause more than zero new infections. In models, the number of new infections can be fractional. In reality, an individual becomes infected, or it does not. Fractional infections do not occur. Over the total observation period, the average number of predicted new infections was equal to the average number of observed new infections. However, for each time interval the observed number of new infections differed from the number of new infections that was predicted based on prevalence. The discrepancy is partly the result of random variability in the number of new infections under field conditions. A deterministic model cannot capture random variability. Similarly, temporary changes in management may affect transmission. Such changes were not reflected in the constant transition rates in the model. Small outbreaks of mastitis that are not predicted based on prevalence, as in herd A during intervals 15 and 16, or in herd C during intervals 7–9 (Fig. 4), may be the result of temporary ‘breakdowns’ in mastitis control. Such breakdowns can occur at farm level, or at national level. Examples include reduced culling because of Bovine Spongiform Encephalitis in the United Kingdom [37], leading to increased incidence of *S. aureus* mastitis, and Food and Mouth Disease in The Netherlands, which was followed by an increase in national BMSCC in 2001. The BMSCC increase is
partly attributed to reduced culling of infected cows due to restrictions on animal movements, and partly to reduced availability of information because herd health visits by veterinarians and routine cow milk SCC testing by the Royal Dutch Cattle Syndicate were suspended. Other reasons for lack of agreement between observed and predicted numbers of new infections are discussed below.

In the model, homogeneity of compartments was assumed. This implies homogeneity of susceptibility among individuals in the susceptible compartments and homogeneity of infectiousness among individuals in the infectious compartments. The homogeneity assumption may have been violated, as cows and udder quarters differ with respect to susceptibility to *S. aureus* infection [25, 30], and with respect to numbers of bacteria shed in milk from infected quarters [38]. Similarly, homogeneity of infected compartments with respect to cure rates is assumed, while differences in probability of cure of *S. aureus* infections exist, depending on host factors and pathogen factors [39, 40]. To account for all possible combinations of susceptibility, infectivity, and ‘cureability’ levels, the number of compartments in the model would need to be increased dramatically, leading to an intractable model. Discrete event stochastic simulation models [21] are better suited to incorporate heterogeneity of susceptible and infected individuals.

Discrepancy between observed and predicted numbers of new infections can be expected if infections in lactating individuals are not the result of contagious transmission. Evidence that *S. aureus* infections can be of environmental rather than contagious origin is growing. Infections in non-lactating animals are generally considered to be the result of infection from an environmental source [41]. New infections with *S. aureus* in non-lactating animals were observed in this study (Appendix 4) and others [41]. Many environmental sources of *S. aureus* have been identified [41], and environmental factors such as disinfection procedures, bedding replacement and hygienic status of stalls, are associated with the risk of *S. aureus* mastitis in dairy herds [35]. Isolation of multiple pathogen strains from a herd [36], and failure to find a persistent strain may also be used as evidence that sustained transmission has been eliminated [42]. A stable incidence of new infections irrespective of prevalence, as observed in herd B, would be consistent with a base line infection rate with *S. aureus* from environmental sources. Infections from environmental sources could also explain why *S. aureus* continued to be the third-most occurring cause of clinical mastitis in a herd with excellent control of contagious transmission of mastitis pathogens [7, 37]. Identification and elimination of sources of *S. aureus*, other than infected quarters, may be crucial for the success of a control programme [8].

Despite shortcomings of the deterministic model used in this study, and the failure to describe the dynamics of mastitis in herd C, prevalence of infection in herds A and B was simulated at a realistic level, suggesting that the model could be used to study the effect of changes in control measures. Changes in cure rate and changes in influx of infections were examined. It must be noted that ‘cure rate’ in this paper is defined as rate, i.e. number of occurrences over time at risk. This is different from cure probabilities that are often reported in literature, i.e. number of cases cured out of number of cases treated. Cure probabilities are commonly called ‘cure rates’ in every day language, which may lead to confusion. In our study, clinically infected quarters never cured spontaneously. Cure was only achieved after treatment, but cure rate for clinically infected quarters, δ, was lower than the rate of cure, α, or the rate of remission, θ. This implies that most clinically infected quarters were lost for production, or contributed to the pool of subclinical infections. Cure rate of subclinically infected quarters was lower at treated quarters (δ) than in quarters that cured spontaneously (γ). This should not be interpreted as delayed cure as a result of treatment, but rather as infrequent occurrence of treatment of subclinical mastitis or low cure proportions in treated cases. Usually, farmers would not decide to treat subclinically infected quarters unless they were *S. aureus* positive at two or more consecutive samplings, i.e. for at least 3 weeks. Because farmers were informed of IMI status 3 weeks after samples had been collected, bacteria had often been present for 6 weeks or more when treatment of subclinical infections was initiated. Thus, subclinical infections were usually chronic when they were treated and it is known that long duration of infection is associated with a low probability of cure [39]. In herd B, treatment was usually initiated shortly after diagnosis of subclinical infections, and cure probability after treatment was high (data not shown), resulting in a high cure rate for treated subclinical infections compared to the other two herds. Simulations for herd A showed that increase of the cure rate for subclinical infections would result in faster decline of the prevalence of
infection than observed in the field study, and in a lower *S. aureus* prevalence in the long term. To assess the economic benefit of treatment of subclinical mastitis, such effects at population level should be taken into account, and not just effects at cow level.

Influx of infected individuals into the lactating population, either as a result of infections in non-lactating individuals or after purchase of infected animals, does not contribute to incidence in the lactating population directly. Indirectly, influx does contribute to incidence, because influx increases the exposure to *S. aureus* in the milking herd. In herds B and C, the majority of infections in non-lactating animals were detected in heifers at first calving (Appendix 4). This indicates the importance of mastitis control in young stock [41]. For multiparous animals, the number of infected quarters at calving depends on cure of existing infections and prevention of new infections by DCT [14]. In herd A, the success of DCT was limited, and persistent and new infections in dry cows were observed (Appendix 4). This indicates a need to re-evaluate treatment and cull strategies. When animals are introduced into a herd as replacements or for herd expansion, *S. aureus* infections may be introduced with them. For newly introduced animals, screening of udders for infection should be an element of mastitis control and biosecurity on dairy farms [43]. The combination of fully successful mastitis control in heifers, DCT and biosecurity is simulated by zero influx of infected individuals. In combination with a reproductive number below 1 and the assumption that all new infections in lactating individuals are the result of contagious transmission, zero influx results in a prevalence of *S. aureus* infection that approaches zero in the deterministic model, as shown by simulation for herd A.

The key question is: can *S. aureus* mastitis be eliminated? If the reproductive number is below 1, if all infections are the result of contagious transmission, and if there is no influx of infected individuals, the prevalence of *S. aureus* infection would asymptotically decrease to zero in a deterministic model. In a stochastic model, prevalence would fall to zero as soon as an absorbing state is reached. This implies that elimination of infection would occur under the conditions listed above, if elimination of infection is understood to mean total absence of cases in the population. In the study herds, elimination of infection *sensu stricto* was not achieved during the study period. A more lenient definition of elimination is a situation in which sustained transmission cannot occur, i.e. $R_0 < 0$ [42]. Under this lenient definition, elimination had been achieved in each herd in this study. To a farmer, the lenient definition of elimination will be of little meaning when new cases of mastitis may continue to occur. ‘Control’ or ‘keeping the prevalence and incidence of mastitis at an acceptable level’ would be more meaningful terminology to describe this situation in practice. The merit of $R_0$ is that it allows us to quantify such control.

*Staphylococcus aureus* mastitis was not eradicated, but reduced to acceptable levels in all three herds in this study. Well known mastitis control measures, in combination with knowledge of infection status and regular and frequent attention for mastitis control, were sufficient to attain this. Similar results have been achieved in other studies [7, 8, 11]. Treatment of subclinical infections and prevention of entry of infected individuals into the lactating herd, through DCT, fly control, or screening of replacement animals, contribute to the reduction of *S. aureus* prevalence and hence to the reduction of contagious transmission. When control of contagious transmission of *S. aureus* is achieved, research and management can focus on ways to prevent infections from environmental sources. Prevention of environmental *S. aureus* infections could be approached through detection and removal of sources, through limitation of contact between sources and susceptible individuals, or through improved resistance of individuals to mastitis. The economic feasibility of the control measures discussed in this paper remains to be established.

**ACKNOWLEDGEMENTS**

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APPENDIX 1. Transition rates describing dynamics of *Staphylococcus aureus* mastitis in a population of lactating individuals. Identification numbers of equations correspond to identification numbers of transitions in Figure 1

Elaborating on the differential equation model for the dynamics of contagious mastitis that was developed by Allore and Erb [23], transitions rates were defined to describe the dynamics of *S. aureus* mastitis in lactating dairy herds:

rate of influx into compartment $U = (1-p-q-r)\left[(\mu_{CU} + \mu_{DR})N + x_cC + x_sS\right]$, \hspace{1cm} (A 1.1)
rate of outflow from compartment $U = (\mu_{CU} + \mu_{DR})U$, \hspace{1cm} (A 1.2)
rate of subclinical infection from compartment $U = f_C\beta_C U(bC+S)/N$, \hspace{1cm} (A 1.3)
rate of clinical infection from compartment $U = (1-f_C)\beta_C U(bC+S)/N$, \hspace{1cm} (A 1.4)
rate of influx into compartment $S = p[(\mu_{CU} + \mu_{DR})N + x_cC + x_sS]$, \hspace{1cm} (A 1.5)
rate of clinical flare-up of subclinical infections $= \chi S$, \hspace{1cm} (A 1.6)
rate of influx into compartment $C = q[(\mu_{CU} + \mu_{DR})N + x_cC + x_sS]$, \hspace{1cm} (A 1.7)
rate of outflow from compartment $S = (\mu_{CU} + \mu_{DR} + x_s)S$, \hspace{1cm} (A 1.8)
rate of subclinical infection from compartment $R = f_R\beta_R R(bC+S)/N$, \hspace{1cm} (A 1.9)
rate of recovery from compartment $S = (\gamma_s + \delta_s)S$, \hspace{1cm} (A 1.10)
rate of remission of clinical infections $= \theta C$, \hspace{1cm} (A 1.11)
rate of recovery from compartment $C = (\gamma_C + \delta_C)C$, \hspace{1cm} (A 1.12)
rate of clinical infection from compartment $R = (1-f_R)\beta_R R(bC+S)/N$, \hspace{1cm} (A 1.13)
rate of outflow from compartment $C = (\mu_{CU} + \mu_{DR} + x_c)C$, \hspace{1cm} (A 1.14)
rate of influx into compartment $R = r[(\mu_{CU} + \mu_{DR})N + x_cC + x_sS]$, \hspace{1cm} (A 1.15)
rate of outflow from compartment $R = (\mu_{CU} + \mu_{DR} + x_R)R$, \hspace{1cm} (A 1.16)

where the symbols represent the following (in alphabetical order):

$\alpha_{c,8}$ = extra culling because of mastitis from $C$ ($x_c$) or $S$ ($x_s$),
$\beta_{C,R}$ = transmission parameter for new infections from $U$ ($\beta_C$) or $R$ ($\beta_R$),
$b$ = relative change in transmission rate for $C$ compared to $S$,
$C$ = size of clinically infected compartment,
$\gamma_{C,R}$ = spontaneous cure rate from $C$ ($\gamma_C$) or $S$ ($\gamma_s$),
$\delta_{C,R}$ = cure rate after treatment from $C$ ($\delta_C$) or $S$ ($\delta_s$),
$\theta$ = remission rate ($C$ to $S$),
$f_{U,R}$ = fraction of subclinicals among infections coming from $U$ ($f_C$) or $R$ ($f_R$),
$\mu_{CU,DR}$ = non-mastitis related exit due to culling ($\mu_{CU}$) or dry-off ($\mu_{DR}$),
$N$ = total population size,
$p$ = fraction of entries that enters into $S$,
$q$ = fraction of entries that enters into $C$,
$r$ = fraction of entries that enters into $R$,
$R$ = size of recovered uninfected compartment,
$S$ = size of subclinically infected compartment,
$U$ = size of uninfected compartment,
$\chi$ = flare-up rate ($S$ to $C$).

When an additional culling rate because of recovery from mastitis is added, equations (A 1.1), (A 1.5), (A 1.7), (A 1.15) (A 1.16) must be adapted as shown below:

rate of influx into compartment $U = (1-p-q-r)\left[(\mu_{CU} + \mu_{DR})N + x_cC + x_sS + x_R\right]$, \hspace{1cm} (A 1.1a)
rate of influx into compartment $S = p[(\mu_{CU} + \mu_{DR})N + x_cC + x_sS + x_R]$, \hspace{1cm} (A 1.5a)
rate of influx into compartment $C = q[(\mu_{CU} + \mu_{DR})N + x_cC + x_sS + x_R]$, \hspace{1cm} (A 1.7a)
rate of influx into compartment $R = r[(\mu_{CU} + \mu_{DR})N + x_cC + x_sS + x_R]$, \hspace{1cm} (A 1.15a)
rate of outflow from compartment $R = (\mu_{CU} + \mu_{DR} + x_R)R$, \hspace{1cm} (A 1.16a)

where $x_R$ is the extra culling because of recovery from mastitis.
APPENDIX 2. The basic reproductive number \( R_0 \) for *Staphylococcus aureus* mastitis

The basic reproductive number is in our model given by

\[
R_0 = \frac{\beta U (f_c (\mu + \alpha_c + \gamma_s + \delta_c + \theta + b \chi) + (1 - f_c) (\theta + b (\mu + \alpha_s + \gamma_s + \delta_s + \chi)))}{(\mu + \alpha_s + \gamma_s + \delta_s + \chi)(\mu + \alpha_c + \gamma_c + \delta_c + \theta) - \chi \theta}.
\]  
(A 2.1)

For simplicity, \( \mu \) is used to represent \( (\mu_{C_{\text{U}}} + \mu_{\text{BR}}) \).

There are several ways to derive the above expression. Perhaps the most efficient way is by using an elegant scheme introduced by Diekman and Heesterbeek [33]. Below we present a derivation along a more intuitive line.

**Derivation**

We start by noting that \( R_0 \) is defined as the expected number of secondary infections caused by a single primary infection in an otherwise wholly susceptible population. The primary infection might (initially) be either a subclinical (with probability \( f_c \)) or a clinical infection (with probability \( 1 - f_c \)), thus giving rise to two contributions (\( R_s \) and \( R_c \)) to \( R_0 \)

\[ R_0 = f_c R_s + (1 - f_c) R_c. \]  
(A 2.2)

\( R_s \) is the expected number of secondary infections caused by a single primary infection starting out in compartment \( S \). Clearly, this primary infection can cause secondary infections while being in \( S \) (we denote these secondary infections with \((R_{s})_s\)), but it can also move from \( S \) to \( C \) and cause secondary infections from there \((R_{s})_C \). The probability of moving from \( S \) to \( C \) is the ratio of the per capita rate \( \chi \) from \( S \) to \( C \) to the per capita rate from \( S \) to anywhere, so splitting into two contributions again we get

\[ R_s = (R_{s})_s + \frac{\chi}{\mu + \alpha_s + \gamma_s + \delta_s + \chi} (R_{s})_C. \]  
(A 2.3)

For \( R_c \) we find analogously

\[ R_c = (R_{c})_s + \frac{\theta}{\mu + \alpha_c + \gamma_c + \delta_c + \theta} (R_{c})_s. \]  
(A 2.4)

\((R_{s})_s\) and \((R_{c})_s\) can be obtained by multiplying the expected time of presence in the compartment (denoted with \( T_s \) and \( T_c \) respectively) with the respective rates of new infections arising from the primary infection in a totally susceptible population: \((R_{s})_s = \beta U T_s\) and \((R_{c})_c = b \beta U T_c\).

This yields the overall expression

\[ R_0 = f_c \beta U \left( T_s + \frac{\chi}{\mu + \alpha_s + \gamma_s + \delta_s + \chi} b T_c \right) + (1 - f_c) \beta U \left( b T_c + \frac{\theta}{\mu + \alpha_c + \gamma_c + \delta_c + \theta} T_s \right). \]  
(A 2.5)

To complete the derivation, we need to express \( T_s \) and \( T_c \) in terms of model parameters. Since infections can make back-and-forth movements between \( S \) and \( C \), we can write

\[
T_s = \frac{1}{\mu + \alpha_s + \gamma_s + \delta_s + \chi} (1 + p_t),
\]  
(A 2.6)

\[
T_c = \frac{1}{\mu + \alpha_c + \gamma_c + \delta_c + \theta} (1 + p_t),
\]  
(A 2.7)

where \( p_t \) is the probability of re-entering compartment \( S \) or \( C \) (by coming back from \( C \) to \( S \), summing over all multiple back-and-forth moving processes)

\[
p_t = \sum_{n=1}^{\infty} x^n
\]
with
\[
x = \frac{\chi}{(\mu + \alpha_s + \gamma_s + \delta_s + \chi)(\mu + \alpha_c + \gamma_c + \delta_c + \theta)}.
\]
(A 2.8)

where \(x\) is the probability of moving back and forth once.

The sum \(1 + p_e\) is a geometric series with result
\[
1 + p_e = \sum_{n=0}^{\infty} x^n = \frac{1}{1 - x} = \frac{(\mu + \alpha_s + \gamma_s + \delta_s + \chi)(\mu + \alpha_c + \gamma_c + \delta_c + \theta)}{(\mu + \alpha_s + \gamma_s + \delta_s + \chi)(\mu + \alpha_c + \gamma_c + \delta_c + \theta) - \chi \theta}.
\]
(A 2.9)

**APPENDIX 3. The effective reproductive number \(R_t\) for *Staphylococcus aureus* mastitis**

The effective reproductive number \(R_t\) can be derived in manner comparable to the derivation of the basic reproductive number \(R_0\) that was described in Appendix 2. The resulting expression for \(R_t\) has a form similar to the right-hand side of (A 2.1), the difference being that \(\beta_v\) is replaced by \((\beta_v U N + \beta_R R N)\) and \(f_v\) by \((f_v \beta_v + f_R \beta_R) / (\beta_v U + \beta_R)\).
**APPENDIX 4. Observational data on number (and percentage) of events and number (and percentage) of quarter days at risk in three dairy herds (A, B, C) during an 18-month period.**

The population of lactating udder quarters in each herd is considered to consist of an uninfected compartment (U), a recovered-uninfected compartment (R), a subclinically infected compartment (S), and a clinically infected compartment (C).

<table>
<thead>
<tr>
<th>Event</th>
<th>U n (%)</th>
<th>R n (%)</th>
<th>S n (%)</th>
<th>C n (%)</th>
<th>Total (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cull</strong></td>
<td></td>
<td></td>
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<tr>
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<td>4 (6.2)</td>
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<td>n.a.</td>
<td>9 n.a.</td>
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<tr>
<td><strong>Cure from C after treatment†</strong></td>
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<td>n.a.</td>
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<tr>
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<td>n.a.</td>
<td>3 n.a.</td>
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<td>1 n.a.</td>
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<td><strong>Cure from S after treatment</strong></td>
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<td>6 n.a.</td>
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<td>11 n.a.</td>
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<td>n.a.</td>
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<td><strong>Spontaneous cure from S</strong></td>
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<td>Herd A</td>
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<td><strong>Remission (C to S)</strong></td>
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<td>n.a.</td>
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<td>n.a.</td>
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<td>n.a.</td>
<td>n.a.</td>
<td>9 n.a.</td>
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<td>25 (4.9)</td>
<td>17 (3.3)</td>
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<td>Herd B</td>
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<td>14 (2.0)</td>
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<td><strong>Quarter days at risk</strong></td>
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<td>Herd A</td>
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<td>2180 (2.5)</td>
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<td>870440</td>
</tr>
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</table>

* n.a. = not applicable.
† Spontaneous cure from C was never observed.
‡ Samples were missing at calving for five quarters. Ten infections were detected in quarters from heifers, and 13 infections were detected in quarters from multiparous animals.
§ Samples were missing at calving for 32 quarters. Ten infections were detected in quarters from heifers, and six infections were detected in quarters from multiparous animals.
‖ 271 samples were taken at calving, and 8 samples were taken from quarters that entered the population after purchase of animals. One sample at entry was missing. Six infections were detected in quarters from heifers, and no infections were detected in quarters from multiparous animals.
REFERENCES


