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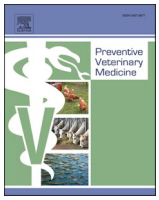

A stochastic modelling approach to determine the effect of diverse *Staphylococcus aureus* strains on the economic and epidemiological outcomes of mastitis intervention strategies in dairy cattle

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ABSTRACT

Staphylococcus aureus (*S. aureus*) strains with considerable genetic and phenotypic differences have previously been identified. The economic and epidemiologic impact of *S. aureus* mastitis has been investigated, but none of these studies took differences between strains into account. Here we aimed to investigate how differences between *S. aureus* strains affect the economic and epidemiologic outcome of various intervention strategies against clinical and subclinical intramammary infections. Five *S. aureus* strains were modelled using a stochastic bio-economic model simulating a dairy herd of 200 cows using single-day time steps. The strain characteristics of the five simulated *S. aureus* strains (general, contagious, spill-over, clinical and persistent) were based on divergent phenotypes as described in literature. Outcomes of the model included incidence (both clinical and subclinical), number of antibiotic treatment days, number of culled cows, and net income. Intervention strategies against clinical and subclinical intramammary infections were based on (variations of) intramammary antibiotic treatment, testing, and culling. Both single and multiple pathogen (intramammary infection caused by *S. aureus*, *Escherichia coli,* and non-aureus staphylococci) scenarios were simulated to determine the effect of the five *S. aureus* strains on the impact of 19 different intervention strategies. The results showed that the incidence (both clinical and subclinical), number of treatment days, number of culled cows, and net income varied considerably for the different *S. aureus* strains. Comparison of the model outcomes within and between strains showed that for most intervention strategies the relative impact differed per strain. However, the intervention strategy with the best outcome for most variables and strains was the culling of cows with a recovery probability lower than 50%. This shows that the relative economic and epidemiologic impact of most of the modelled intervention strategies were strain-dependent, while some intervention strategies were not strain-dependent. From this, we conclude that, depending on the intervention strategy applied on a farm, it could be advantageous to type *S. aureus* to determine whether it would be economically and epidemiologically beneficial for the existing intervention strategy to be changed.

1. Introduction

Mastitis, resulting from intramammary infection (IMI), has detrimental effects on the health and wellbeing of dairy cows and has a considerable economic impact on dairy farms worldwide (Halasa et al., 2007). Costs for mastitis arise from treatment costs, replacement of culled animals, and production losses (Halasa et al., 2007).

While there is a vast number of pathogens that can cause mastitis, mastitis control programs are often generic, only tailoring intervention strategies towards contagious or environmental pathogens. *Staphylococcus aureus* (*S. aureus*) is one of the pathogens causing IMI and it is difficult to eradicate due to its contagious nature, poor cure rates with

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current therapies and hence high risk of persistency (Rainard et al., 2018). Research has shown that different *S. aureus* strains exist and that there is considerable genetic variation between strains, particularly in virulence genes (Graber et al., 2009; Hoekstra et al., 2018; Klaas and Zadoks, 2018; Pichette-Jolette et al., 2019; van den Borne et al., 2010). These genetic differences cause variation in the phenotypic properties of the strains. Fournier et al. (2008), for example, showed that the 16S-23S rRNA intergenic spacer PCR (RS-PCR) genotype B was highly contagious and virulent, while genotype C was only found sporadically in IMI in cows. Genetic variation does not only cause differences in transmission but it is also associated with variation in persistence or clinical manifestation (Haveri et al., 2005; Pichette-Jolette et al., 2019). Pichette--Jolette et al. (2019) found that certain strains were more persistent compared to other *S. aureus* strains. Using *Spa* typing, a method to distinguish between different *S. aureus* strains by determining the number and succession of tandem repeats in the highly variable Staphylococcal protein A (*Spa*) gene, they showed that strains from *Spa* type t359 and t529 were around 3–4 times more likely to be eliminated from infected quarters than strains from *Spa* type t13401. Haveri et al. (2005) found that pulsotype B was associated more with clinical mastitis compared to the other strains found in that study. These strain differences affect mastitis characteristics at the farm level (e.g. prevalence, the ratio of clinical to subclinical cases, cure rate) and therefore there is a need to investigate how differences between *S. aureus* strains change the impact of mastitis intervention strategies.

Sommerhäuser et al. (2003) and Leuenberger et al. (2019) proposed that strain-specific intervention strategies could improve the control and prevention of different *S. aureus* strains. Although several studies have investigated the economic and epidemiologic impact of *S. aureus* mastitis (e.g. Gussmann et al., 2019; Swinkels et al., 2005), none of these studies took differences between *S. aureus* strains into account and there are currently no experimental or epidemiological data to support strain-specific interventions.

The aim of this study, therefore, was to investigate how phenotypic, clinical, and epidemiological characteristics of *S. aureus* strains affect the economic and epidemiologic outcome of various intervention strategies against clinical and subclinical mastitis.

2. Material & methods

2.1. Herd and transmission model

A stochastic mechanistic bio-economic simulation model called Mastitis-iCull (MiCull) model version 4.2 was used in this study. In the model a Danish Dairy herd consisting of 200 cows, divided over five production steps (calves, heifers, lactating cows, dry cows), is simulated. This herd is simulated in daily time steps, and each cow spend a stochastically determined period in each production step before moving to the next step or being culled. Lactation and somatic cell count (SCC) curves are estimated on individual cow basis and adjusted for IMI and milk production (Gussmann et al., 2018). MiCull version 4.1 was previously described and used in Gussmann et al. (2020). MiCull model version 4.2 differs from the original version as additional pathogen groups and strains were added, and the starting point of the intervention strategies was changed to after the burn-in time. The original version of this model was described in detail in Gussmann et al. (2018). All simulations were run in the statistical computing software R (version 3.5.2, R Core Team, 2019).

2.1.1. Transmission framework

The transmission parameters for each strain are shown in Tables S1 and S2. The IMI transmission framework itself was the same as used in Gussmann et al. (2018) and models a dairy herd using costs, income, and practices which are common for Danish dairy herds (Table S3). Infection probabilities were calculated and simulated differently for lactating cows, dry cows, and heifers. Heifers do not have a dynamic transmission, but they had a certain probability of being infected before entering the lactational period. For lactating cows, the infection probability for non-infected quarters was calculated each day, and this probability depended on the active pathogens, the susceptibility, and the number of infected quarters (Gussmann et al., 2018). When infected, the probability of clinical state gave the probability of becoming clinical, if the susceptible quarter did not become clinical after infection it would be subclinically infected. Quarters infected with clinical IMI were treated for 3 days with intramammary antibiotics (in the default scenario) after which they are cured and become susceptible again or return to subclinical (remission) as determined by the recovery probability. Each day a subclinical quarter had a certain probability to recover spontaneously or to become clinical (flare-up). If a high SCC was observed during lactation and a decision needed to be made about whether or not the cow would receive treatment for subclinical IMI, bacterial culture was performed. This was only performed when subclinical IMI was included in the intervention strategy. The sensitivity and specificity of PCR and bacterial culture can be found in Table S3. New IMI and spontaneous recovery could occur during the whole dry period, but flare-up to clinical mastitis could only occur in the first or last week of the dry period.

Cows were assessed for culling once a week, however this only occurred when the total number of cows exceeded 200. Different factors were used to weigh and prioritize cows for culling, and this list included parity, reproduction status, milk yield, SCC, and previous cases of clinical IMI (Gussmann et al., 2018). Cows with the highest priority for these combined parameters were culled. Involuntary culling was prioritized over other cases. Culling costs (Table S3) included the market value of a new heifer subtracted by the slaughter value of the culled cow.

The aim of this study was to model different strains with 'phenotypic' characteristics as observed in literature. It has previously been observed and explained by Gussmann et al. (2018) that parameter estimates for the transmission framework calculated from field observations are overestimated. Therefore, calibration of the parameter estimates was necessary and focused on obtaining those outcome values as observed in literature. For all scenarios the incidence was taken into account and, depending on the specific strain, calibration was performed for the duration of IMI (persistent strain), and clinical to subclinical ratio (clinical strain). For the *S. aureus* strains modelled in this study, calibration was only performed on those parameters changing for that strain as calibration for the other parameter values was performed in previous studies (Gussmann et al., 2019, 2018). The calibrated values can be found in Tables S1 and S2.

2.2. S.aureus strains

For this study, five *S. aureus* strains with different characteristics were modelled based on divergent phenotypes as described in literature (detailed below). The strains were named as follows: "general", "contagious", "spill-over", "clinical", and "persistent" *S. aureus*. The naming of the strains was based on the deviating phenotypic trait of that strain.

2.2.1. General S.aureus strain

The general *S. aureus* strain was modelled previously in Gussmann et al. (2019). This strain was the reference for the other strain values. The reference parameter values of this strain were the same as used in Gussmann et al. (2019), only the calibrated value of the transmission rate was different because single pathogen scenarios were simulated, compared to multiple pathogen scenarios in previous studies. Calibration was performed so that an IMI incidence of 100 cases per 200 cows per year was reached, this value was calculated using the incidence from Santman-Berends et al. (2016).

2.2.2. Contagious S.aureus strain

Fournier et al. (2008) observed that *S. aureus* genotype B (RS-PCR genotype) was contagious with a high within herd prevalence and associated with farms that had an *S. aureus* IMI problem, and van den

Borne et al. (2017) determined that this strain had a higher transmission rate. Other studies have made similar observations (Cosandey et al., 2016; Graber et al., 2009). Van den Borne et al. (2017) determined the transmission rate for genotype B and this value was used as a starting point for the calibration of the transmission rate for this strain. Calibration was performed so that an IMI incidence of 200 cases per 200 cows per year was reached. This incidence was estimated using the increase in prevalence described by Voelk et al. (2014) which was combined with the incidence described by van den Borne et al. (2017).

2.2.3. Spill-over S.aureus strain

Fournier et al. (2008) and Leuenberger et al. (2019) both showed that *S. aureus* genotype C (using RS-PCR) only caused IMI sporadically and Leuenberger et al. (2019) also showed that genotype C often colonized body sites, other than the mammary gland, of cattle. The main reservoir for this strain appeared to be non-mammary gland niches and, although contagious transmission of IMI to other mammary glands occurred, the main transmission was spill-over from those non-mammary gland niches to the mammary gland. This strain therefore had a deviating transmission route compared to the other strains and a novel formula for transmission was added to the model (Spill-over, Eq. 1). This formula contained the spill-over from non-mammary gland niches into the mammary gland (ρ) and transmission from mammary gland to mammary gland (I/N) as for normal *S. aureus*.

$$1 - \exp\left(- \beta * \left(\frac{I}{N} + \rho \right) * susc_q \right) \quad (1)$$

Where: β = transmission rate, I = number of already infected quarters, N = number of quarters, ρ = the environmental share in the opportunistic transmission, the probability that transmission from a non-mammary gland niche into the mammary gland occurs, $susc_q$ = the number of susceptible quarters.

Although it was described that some strains caused IMI sporadically, transmission rates for these strains have not been determined. To estimate a plausible transmission rate, the first quantile for the range of transmission rates determined for *S. aureus* (Barlow et al., 2013; Down et al., 2013; Kirkeby et al., 2019; Lam et al., 1996; Schukken et al., 2014; Zadoks et al., 2002) was used as the starting value for the calibration of the transmission rate for this strain. There was no published data on what the proportion of contagious and non-contagious transmission could be, and therefore different values and proportions of transmission rate and ρ were simulated. Calibration was performed to reach an IMI incidence of 20 cases per 200 cows per year, this incidence was calculated using the prevalence estimate for a sporadic strain by Graber et al. (2009).

2.2.4. Persistent S.aureus strain

In a large field study, Pichette-Jolette et al. (2019) showed that *Spa* type t3401 was more persistent compared to other strains. Several other studies also showed that some *S. aureus* strains resulted in more persistent IMI than other strains (Veh et al., 2015; Zadoks et al., 2000). A lower spontaneous recovery rate of this strain caused the IMI to persist for a longer period, therefore this parameter was adjusted for the persistent strain. The spontaneous recovery probability was estimated using the mean duration of IMI for the persistent strain and the general strain given by Pichette-Jolette et al. (2019). Calibration of the spontaneous recovery probability was performed so that the difference in duration of IMI between the general and persistent strain in the scenario was similar to that found by Pichette-Jolette et al. (2019), in this case, a difference of 11 days.

2.2.5. Clinical S.aureus strain

Case-control studies have shown that specific *S. aureus* strains are associated with a higher odds to be isolated from clinical rather than from subclinical IMI (Haveri et al., 2005 and Hoekstra et al., 2018). To model the clinical *S. aureus* strain only the probability of clinical state and flare-up probability were changed since there was no evidence that strains with a higher tendency towards clinical IMI differed for any other epidemiological parameter (Haveri et al., 2005; Hoekstra et al., 2018). Together with the clinical to subclinical ratio for the general strain and the odds ratio calculated from the data of Haveri et al. (2005) and Hoekstra et al. (2018), the clinical to subclinical ratio for this strain (2.33) could be calculated, which was then used for calibration of the clinical strain. The highest ratio of clinical to subclinical cases is reached when the probability of clinical state is set to 100% in the model and this resulted in a ratio of 1.24. This showed that in the model the ratio according to the data from Hoekstra et al. (2018) and Haveri et al. (2005) cannot be reached. To be able to model a clinical *S. aureus* strain with a similar phenotypic divergence from the general *S. aureus* strain as described by Hoekstra et al. (2018) and Haveri et al. (2005), we decided to calibrate towards a clinical to subclinical ratio of 1 with an IMI incidence comparable to the general *S. aureus* strain (100 cases per 200 cows per year).

2.2.6. Intervention strategies

Intervention strategies in this study were based on (variation and combinations of) intramammary antibiotic treatment, testing, and culling. Table 1 gives a summary of all intervention strategies (previously described by Gussmann et al. (2019)).

2.2.7. Simulations and model output

The simulations were run for 10 years, starting with a five-year burn-in time and each scenario was run for 500 iterations. During the burn-in time the model is run, but data is not collected and the intervention

Table 1
Description of the intervention strategies simulated in this study. All intervention strategies except for Cullheifers were previously described in Gussmann et al. (2019).

Intervention strategy	Description
Basic3 (default)	All clinical cases receive three days of intramammary antibiotic treatment.
Basic5	All clinical cases receive five days of intramammary antibiotic treatment.
Treattoplonger	The future average milk production is calculated and used to determine the future milk production. Cows in the top 25% receive five days of antibiotic treatment, all other cows receive three days of antibiotic treatment.
Before50	From each new clinical quarter, a milk sample is taken and a PCR test is performed to determine the recovery probability. According to the causative pathogen, history of IMI, parity, days in milk, and SCC at the last milk recording the recovery probability is calculated. If the recovery probability is lower than 50% the cow will be culled, otherwise the cow will receive three days of intramammary antibiotic treatment.
Cullheifers	Heifers with a recovery probability below 50% are culled, all other heifers are treated.
Notculltop	Cows in the top 25% according to the expected future average milk production are not culled, but directly treated with intramammary antibiotic treatment.
Notcullpregnant	Cows that are pregnant for four months or longer will not be culled.
After	Seven days after treatment is finished, milk samples from treated quarters are tested by bacterial culture to determine if the cow is still infected. If still infected, the cow will be culled.
Cullbottom	Cows in the lowest 25% of the expected future average milk production will be culled directly instead of treated.
Test SCM	If a high SCC is observed at two subsequent monthly milk recordings, a milk sample of the affected quarter is tested by bacterial culture. If the bacterial culture comes back positive, the cow will be treated with three days of intramammary antibiotic treatment.
Cull SCM	Treated subclinical cases will be tested using bacterial culture a month after treatment is finished. If still positive, the cow will be culled.

strategies are not implemented. The burn-in time ensures that the model has stabilized so that the observed changes following implementation of the intervention are a result of the intervention strategy rather than being influenced by the initial parameter values. After the five-year burn-in time the intervention strategies were implemented and data was collected. Different economic and epidemiologic output data were collected over simulation years five to ten. The following economic output was collected: income from milk, IMI related costs (treatment costs, testing of infected animals, and opportunity costs), other costs such as feed, and culling. The net income for the farm was calculated by subtracting the costs from the income from milk, additional costs were not considered. The collected epidemiologic output included the number of clinical cases (quarter level), number of subclinical cases (quarter level), number of treatment days, and the number of culled animals. The output was presented as a rounded median value (with the 5th and 95th percentile) of the annual average over the 5 simulated years. Additionally, each month the true prevalence of all mastitis pathogens was recorded. Prevalence graphs were made using the R package ggplot2 (Wickham, 2016).

To evaluate and compare the impact of the different intervention strategies on the model output between all the strains, heatmaps of the normalized outcome values were generated using the heatmap.2 function of the R package gplots (Warnes et al., 2020). Normalization of the outcome values was performed within strains, which enabled comparison of the relative impact of intervention strategies between strains. Min-max normalization (Eq. 2) was used to normalize the values so that the values of each strain varied between 0 (Xminimum) and 1 (Xmaximum). The normalized outcome, visualized using color in the heatmap, shows the relative impact of the intervention strategies for each strain. The light yellow color indicates the best outcome. For net income, this was the highest income (normalized value 1) and for total incidence, treatment days and culled cows this was the lowest number (normalized value 0). The dark purple color indicated the worst outcome. For net income, this was the lowest income (normalized value 0) and for total incidence, treatment days and culled cows this was the highest number (normalized value 1).

$$X_{normalized} = \frac{(X - X_{minimum})}{(X_{maximum} - X_{minimum})} \quad (2)$$

2.2.8. Multiple pathogen scenario

Multiple pathogen scenarios were run for all strains to simulate field conditions where multiple mastitis pathogens may be present on a dairy farm. In the multiple pathogen scenarios, the particular *S. aureus* strain was present together with *Escherichia coli (E. coli)* and non-aureus staphylococci (NAS). Calibration was performed so that the IMI incidence of *E. coli* was around 40 cases (per 200 cows per year) and the incidence of NAS around 25 cases (per 200 cows per year), and the characteristics of the *S. aureus* strains was the same as for the single pathogen scenarios. The transmission rates and ρ were re-calibrated for the multiple pathogen scenarios and are presented in Tables S1 and S2.

2.2.9. Sensitivity analysis

Sensitivity analyses were performed to assess how variation in specific transmission framework parameters affected the outcome of the model and relative impact of different intervention strategies. A selection of intervention strategies were simulated for the sensitivity analyses. These were Basic3, After, Test SCM, and Before50. Variations in the following parameters were modelled: transmission rate (general, contagious and spill-over strain), ρ (spill-over strain), probability of clinical state (clinical strain), flare-up probability (clinical strain), and spontaneous recovery probability (persistent strain). Variation in the parameter estimates was based on the difference between the calibrated parameter estimate of the strain of interest and the general *S. aureus* strain, where this difference was divided by two or multiplied by two and added to the parameter estimate for the general *S. aureus* to obtain a low and high parameter estimate in the sensitivity analysis. For example for the contagious strain, the calibrated transmission rate was 0.00737 and subtraction of the general transmission rate of 0.00716 gives a difference of 0.00021, resulting in a low and high parameter estimate for the sensitivity analysis of 0.00727 and 0.00758. For the transmission rate of the general strain and ρ of the spill-over strain, a different approach was used. For the general strain, the transmission rate was calibrated towards a scenario with a high IMI incidence of 180 cases per 200 cows per year and a low incidence of 45 cases per 200 cows per year. For ρ there was no reference value in the general strain, therefore the same value (0.001) was subtracted and added to the calibrated estimate for ρ to obtain a low and a high value for the sensitivity analysis. Additionally, a very high value ($\rho = 0.01$) was modelled to test how extreme values for ρ affected the characteristics of the spill-over strain. For the transmission rate of the spill-over strain, the difference for the lowest value was halved, because, otherwise the transmission rate would be 0. For the high value of the probability of clinical state for the clinical strain, only half the difference was added to the parameter estimate, because, otherwise the value would be higher than 1.

3. Results

3.1. Single pathogen scenarios

Table 2 shows the results for the single pathogen scenarios modelling a general, contagious, spill-over, persistent and clinical type of *S. aureus* strain in combination with the default intervention strategy (Basic3, standard 3 days of antibiotic treatment). The phenotypic differences between strains had an effect on the on-farm *S. aureus* epidemiology as there were considerable differences in the model outcomes for the different strains. For example, the longer duration of IMI for the persistent strain resulted in a higher total IMI incidence, more treatment days, and more culled cows. However, the net income was higher than for the general strain. The high IMI incidence for the contagious strain resulted in more treatment days and number of culled cows, but the effect on net income was minimal. The spill-over strain resulted in the highest net income. Supplementary Table S4 gives the results for all single pathogen scenarios. The incidence of the spill-over strain is affected less by the intervention strategies compared to the other strains.

Fig. 1 shows the heatmaps of the normalized outcome within strain for the single pathogen scenarios. Comparing the effect of the intervention strategies of the different strains show that for most of the strategies the relative impact on net income, IMI incidence, treatment days, and number of culled cows varied between strains. For example for

Table 2
The median yearly output of the default intervention strategy for the single pathogen scenarios (with 5th and 95th percentiles) for a herd with 200 dairy cows, averaged over the 5 simulated years after starting the intervention strategies.

Strain	Clinical IMI cases	Subclinical IMI cases	Treatment days	Culled cows	Net income in Euro
General	35 (0; 87)	70 (0; 156)	98 (0; 252)	16 (1; 23)	207,746 (181,930; 224,791)
Contagious	83 (34; 266)	149 (66; 457)	237 (94; 767)	25 (15; 41)	207,019 (182,242; 220,902)
Spillover	10 (6; 14)	21 (16; 28)	26 (17; 37)	7 (5; 9)	220,997 (209,521; 231,509)
Clinical	50 (0; 93)	61 (0; 106)	138 (0; 265)	17 (0; 24)	213,851 (202,106; 227,674)
Persistent	62 (28; 158)	116 (56; 270)	179 (80; 447)	22 (14; 34)	210,361 (193,986; 222,644)

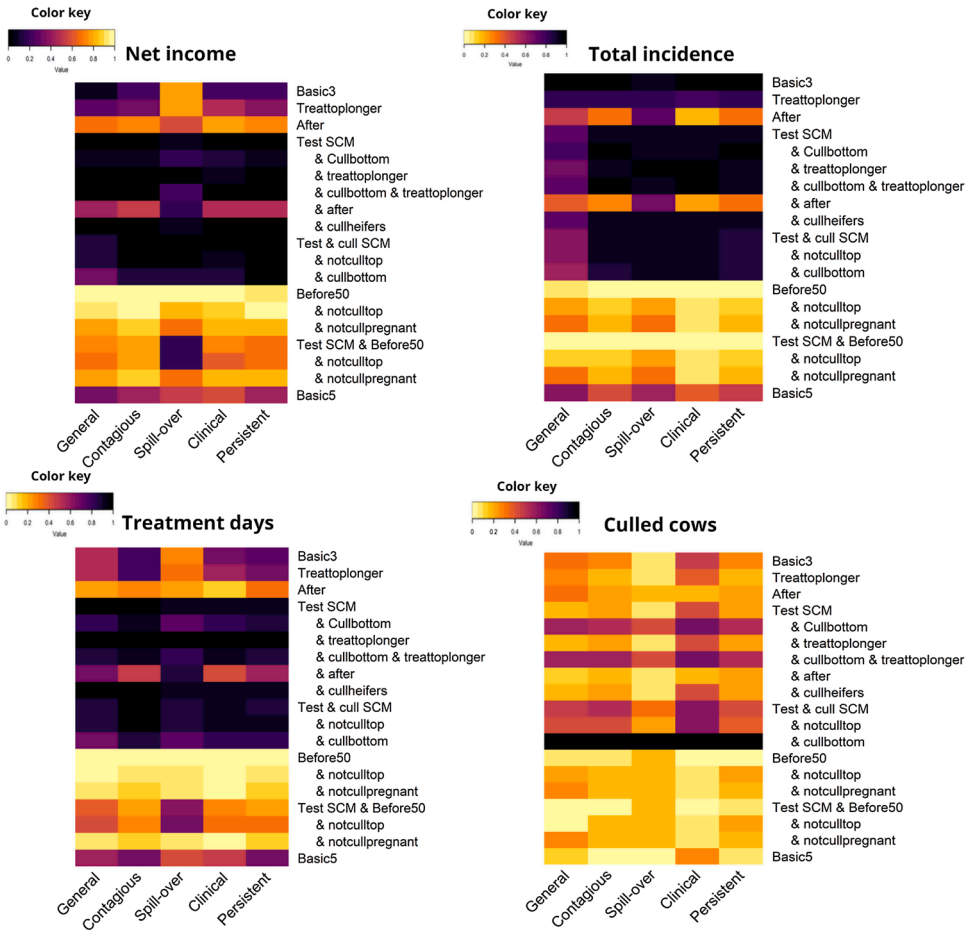

Fig. 1. Heatmaps of the normalized model output of the single pathogen scenarios. The values are normalized within strain for all the intervention strategies. For each of the outcome variables light yellow color indicates the best outcome and dark purple the worst outcome, where best is either the highest or lowest value depending on the outcome variable (normalized value of 1 for net income and 0 for total incidence, treatment days and culled cows). The '&' symbol indicates that the intervention is combined with the intervention above without the '&'.

net income, with Test SCM & Cullbottom the clinical strain had a worse outcome (dark purple color) compared to the spill-over strain (lighter purple color), while for Basic5 (standard 5 days antibiotic treatment) the outcome was better for the clinical strain (dark orange color) compared to the spill-over strain (darker orange color). When looking at the absolute differences in net income, the difference between the intervention strategy with the highest net income and with the lowest income differed per strain as well. For example the difference (€12,998) was larger for the general strain compared to that for the spill-over strain (€4962). In contrast the difference was larger for the contagious strain, as this was €15,602. For total incidence, Basic3 had the same normalized outcome for the general and contagious strain (black), while for After (testing after treatment) the general strain (dark pink) had a better relative impact compared to the contagious strain (purple). For treatment days, Before50 (culling all cows with a recovery probability <50%) & Notculltop had the same normalized outcome for the spill-over strain and the contagious strain (same yellow color), while for Before50 & Notcullpregnant the relative impact of the spill-over strain was better (light yellow color) than the contagious strain (dark yellow color). For culled cows, with Treattoplonger the clinical strain had a worse outcome (dark orange color) compared to the general strain (lighter orange color), while for After the outcome for the clinical strain (dark yellow color) was better compared to the general strain (orange color). Such differences were observed for several outcome variables and intervention strategies (Basic3, Basic5, After, and Test SCM & After). Within the spill-over strain, the relative outcome of the different intervention strategies was most divergent from the other strains as the color difference was larger between the spill-over strain and the other strains, especially for the net income and treatment days (Fig. 1).

Comparing the prevalence of *S. aureus* IMI over time (Supplementary Figure S1) shows that following implementation of the Before50 strategy, *S. aureus* can be eradicated from the farm completely, but the median time it takes to eradicate *S. aureus* is about 2 ½ years after implementation of the intervention strategy for the general strain and longer for the persistent and contagious *S. aureus* strains. The prevalence graph of the contagious and persistent *S. aureus* shows that the prevalence increased over time when the Basic3 or Test SCM (testing and treating of subclinical cases) strategy were applied. For all other strains and intervention combinations, the prevalence remains the same or decreases.

Overall, for some of the intervention strategies the relative impact was similar for all strains and most outcome variables. For example, Before50 had the best outcome for all strains for the outcome variables net income, total incidence, and treatment days. Although the intervention strategy with the worst outcome was different for each outcome variable, the intervention strategy with the worst outcome was the same for all strains within each outcome variable. For example, Basic3 had the worst outcome for total incidence, and Test SCM & Treattoplonger for treatment days.

3.2. Multiple pathogen scenarios

Table 3 gives the results of the multiple pathogen scenarios for the default intervention strategy (Basic3). The clinical and subclinical incidence of the five *S. aureus* strains were similar to the incidence of the single pathogen scenarios. The incidence of NAS and *E. coli* was similar for all scenarios. Generally, the number of treatment days, number of culled cows, and net income were negatively affected by including the additional mastitis pathogens *E. coli* and NAS. Supplementary Table S5 shows the results of the different intervention strategies for the multiple

Table 3
The median yearly output of the default intervention strategy for the multiple pathogen scenarios (with 5th and 95th percentiles) for a herd with 200 dairy cows, averaged over the 5 simulated years after starting the intervention strategies. S. aureus – Staphylococcus aureus, E. coli – Escherichia coli, NAS – non aureus staphylococci.

	all pathogens		S. aureus		E. coli		NAS				
Strain	Clinical IMI cases	Subclinical IMI cases	Clinical IMI cases	Subclinical IMI cases	Clinical IMI cases	Subclinical IMI cases	Clinical IMI cases	Subclinical IMI cases	Treatment days	Culled cows	Net income in Euro
General	53 (17; 93)	114 (43; 180)	34 (0; 73)	69 (0; 136)	18 (14; 21)	19 (16; 22)	1 (0; 2)	25 (21; 29)	153 (49; 271)	18 (10; 24)	204,035 (182,104; 220,886)
Contagious	89 (52; 173)	172 (108; 323)	70 (34; 154)	127 (65; 275)	18 (15; 21)	19 (16; 23)	1 (0; 2)	25 (21; 29)	256 (150; 500)	25 (17; 35)	204,661 (190,626; 218,598)
Spill-over	26 (21; 32)	62 (54; 70)	8 (5; 12)	18 (13; 24)	17 (14; 21)	18 (16; 22)	1 (0; 2)	25 (21; 29)	74 (59; 91)	14 (11; 17)	215,795 (205,032; 227,267)
Clinical	72 (16; 122)	108 (42; 158)	53 (0; 101)	65 (0; 114)	18 (14; 21)	19 (16; 22)	1 (0; 2)	25 (21; 29)	205 (46; 349)	20 (9; 26)	209,864 (196,650; 223,758)
Persistent	80 (48; 142)	156 (103; 261)	61 (30; 121)	112 (59; 214)	18 (15; 22)	19 (16; 23)	1 (0; 2)	25 (21; 29)	230 (138; 415)	24 (17; 32)	206,371 (192,593; 218,068)

pathogen scenarios. For most of the intervention strategies and strains, more cows were culled (Table S5). Interestingly, the incidence of *E. coli* and NAS was only minimally affected by the intervention strategies (Table S5). As a result, the IMI prevalence in the multiple pathogen scenarios was more stable over time (Figure S2). Contrary to the single pathogen scenario, none of the intervention strategies resulted in eradicating mastitis (Figure S2), but Before50 still resulted in very low *S. aureus* incidences for all strain scenarios (Table S5). Overall, the pattern of the IMI prevalence for the other intervention strategies over time was similar to the single pathogen scenario, including the increase in prevalence over time in the contagious and persistent *S. aureus* scenario when the intervention strategies Basic3 and Test SCM were applied.

Fig. 2 shows the heatmaps of the normalized outcome variables of

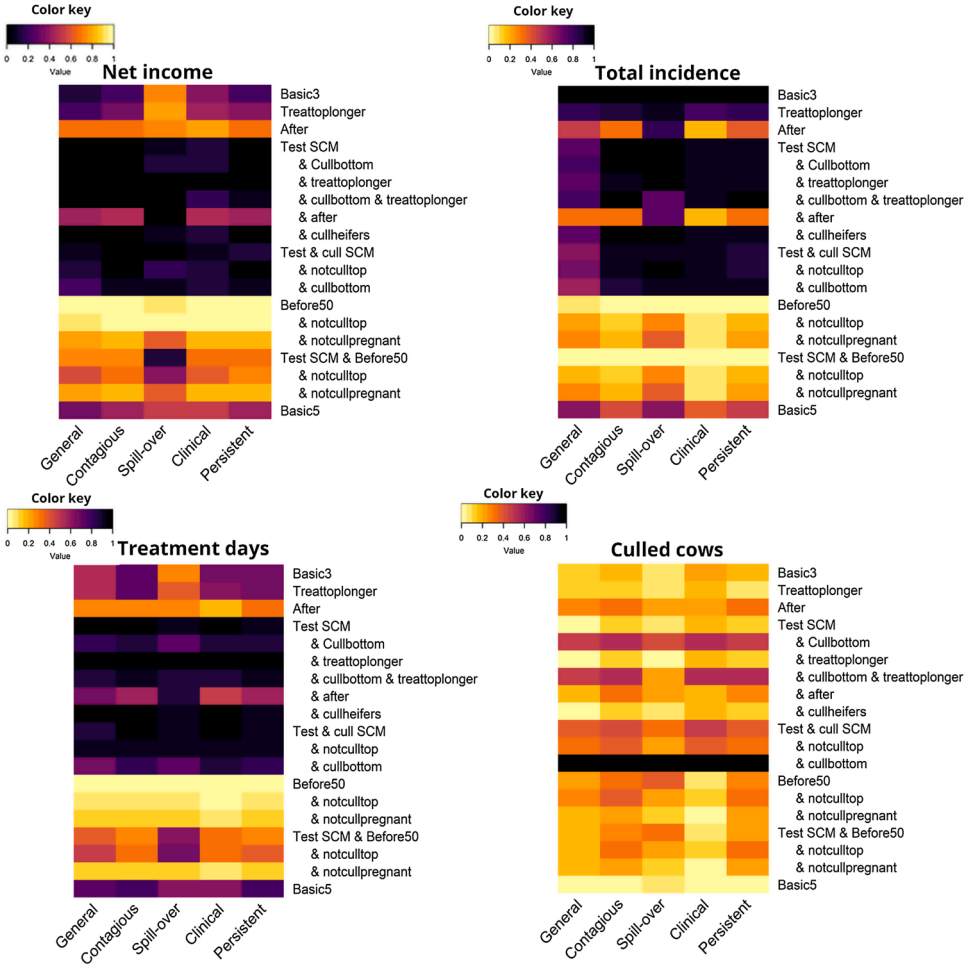

Fig. 2. Heatmaps of the normalized model output of the multiple pathogen scenarios. The values are normalized within strain for all the intervention strategies. For each of the outcome variables light yellow color indicates the best outcome and dark purple the worst outcome, where best is either the highest or lowest value depending on the outcome variable (normalized value of 1 for net income and 0 for total incidence, treatment days and culled cows). The '&' symbol indicates that the intervention is combined with the intervention above without the '&'.

the multiple pathogen scenarios. The relative impact of the different intervention strategies on the outcome variables differed between strains, similar to what was observed for the single pathogen scenarios. For example for net income (Fig. 2), with Treattoplonger (cows in the top 25% according to the future milk production receive 5 days of antibiotic treatment) the spill-over strain had a better outcome (light oranger) compared to the clinical strain (light purple), while for After the spill-over strain had a worse outcome (dark orange) compared to the clinical strain (light orange). The absolute difference in net income between the intervention strategy with the highest income and lowest income was similar as that for the single pathogen scenarios (general strain single pathogen scenario €12,998 and multiple pathogen scenario €12,948). Again, a small difference in net income was observed for the spill-over strain (€5,085) and a large difference for the contagious strain (€14,281). For total incidence, the contagious strain and spill-over strain had a similar normalized outcome (light yellow) with Before50, while for Before50 & Notculltop the contagious strain had a better outcome (dark yellow) compared to the spill-over strain (orange). For treatment days, the contagious strain and the clinical strain had a similar normalized outcome (dark purple) for Test SCM & Cullbottom, while for Test SCM & After the contagious strain had a worse outcome (light purple) compared to the clinical strain (pink). And for culled cows, with Basic3 the general strain had a better outcome (dark yellow) compared to the persistent strain (orange), while for Treattoplonger the general strain had a worse outcome (dark yellow) compared to the persistent strain (light yellow) For the multiple pathogen scenarios the intervention strategies with the best outcome also varied per outcome variable. For instance, Basic5 had the best outcome for culled cows and Before50 for treatment days and total incidence.

3.3. Sensitivity analysis

The results of the sensitivity analyses for the different strains are summarized in Figures S3 – S9. The transmission rate affected all outcome variables of both the general, contagious, and spill-over strain (Figures S3, S4, and S5), but in general, it did not or minimally (single rank change) affect the ranking of the relative impact of the intervention strategies (Tables S6, S7, and S8). The sensitivity analysis of ρ (spill-over from non-mammary gland sites into the mammary gland) showed that small variations in this parameter had minimal effect on all outcome variables (Figure S6). The extreme value (0.01) had the most effect on all outcome variables, nevertheless there were no substantial differences in the ranking of the relative impact of the intervention strategies (Table S9). For the clinical strain, variation in the probability of clinical state had minimal effect on all outcome variables (Figure S7) and did not affect the ranking of the relative impact of the intervention strategies (Table S10). In contrast, both a higher and lower flare-up probability resulted in higher net income and lower incidence, treatment days, and number of culled cows (Figure S8), although the effect of the lower flare-up probability was less pronounced. However, this effect had a minor influence on the ranking of the intervention strategies (Table S11). Variation in the spontaneous recovery probability of the persistent strain had limited impact on the outcome variables (Figure S9, Table S12). Generally, varying the strain-specific parameters within ranges relevant for the simulated strains had limited impact on the outcome of the model and ranking of the tested intervention strategies (Tables S6 - S12). Differences in ranking occurred when the outcome values of the intervention strategies were comparable (i.e. small differences in absolute outcomes). For example for the general strain, the absolute difference for rank 1 and 2 of the number of culled cows was 1 culled cow (16 cows for rank 1 (Test SCM) and 17 cows for rank 2 (Before50)) (Table S6).

4. Discussion

This paper aimed to investigate how differences in epidemiological and clinical characteristics of *S. aureus* strains affected the economic and epidemiological outcomes of intervention strategies. Five *S. aureus* strains with deviating phenotypic, clinical and epidemiological characteristics were simulated in a dairy herd of 200 cows using a stochastic bio-economic model. We showed that there are considerable differences in the model outcome for the strains when implementing the default intervention strategy. However part of these differences in the output are a direct result of the model calibration, for example the ratio of clinical to subclinical for the clinical strain or the calibrated incidence. The results of the single pathogen scenarios showed that the relative within strain impact of most intervention strategies differed between strains (Fig. 1), which suggests that for these intervention strategies the economic and epidemiological effects were strain-dependent. This indicates that it can be beneficial to characterize the *S. aureus* strain in a herd to determine whether the currently implemented intervention strategy should be adapted.

Comparison of the single and multiple pathogen scenarios showed that the main differences between the scenarios were the incidence of the total IMI and the number of culled cows. This was caused by the inclusion of the two environmental mastitis pathogens *E. coli* and NAS. The intervention strategies simulated in the model were based on (variation and combinations of) intramammary antibiotic treatment, testing, and culling and are particularly effective for contagious mastitis pathogens as they remove the reservoir of these pathogens. However, as they do not remove the environmental reservoirs, the intervention strategies simulated in the model had only minimal impact on the incidence of mastitis caused by environmental pathogens. For environmental pathogens, intervention strategies should be aimed at preventing transmission from environmental reservoirs and hence they should focus on hygiene (Garcia, 2004; Hillerton and Berry, 2003; Hogan and Smith, 2012). Such intervention strategies are currently not implemented in the MiCull model, due to the lack of epidemiological knowledge about the impact of such measures on the transmission of pathogens and the risk of IMI. Although the ranking of the intervention strategies was slightly different compared to the single pathogen scenarios, most intervention strategies were still strain-dependent, as the relative impact of the intervention strategies on the outcome variables was different between strains (Fig. 2). These results indicate that, depending on the intervention strategy, characterizing the *S. aureus* strain causing mastitis and changing the intervention strategy accordingly could also be beneficial in a scenario in which multiple pathogens are causing IMI on a farm.

In both single and multiple pathogen scenarios, the intervention strategy Before50 (culling of cows with a recovery probability <50%) had the best outcome for most strains and outcome variables (net income, total incidence, and treatment days). If perfectly implemented, as in the model, this is an effective intervention strategy as it removes the reservoir of most *S. aureus*, except the spill-over strain, and resulted in eradication after a certain period following implementation (Figure S1). However, the beneficial effect of the Before50 strategy may be overestimated in the model. First of all, in this study there was no re-introduction of *S. aureus*, something which may occur in practice (Keefe, 2012). However, the spill-over strain has a constant non-mammary gland reservoir that mimics the re-introduction of *S. aureus* (ρ) and in Figure S1 it can be seen that Before50 was effective in reducing the prevalence when re-introduction from these non-mammary gland niches occurred. Therefore, we expect that even if re-introduction occurs with other strains that Before50 would still be an effective intervention strategy. Secondly, the model assumes perfect implementation. In practice, farmers may not be willing to cull every cow that has mastitis (e.g. cows with high milk yield, unique genetics, or with which there is an emotional bond), and this may affect the effectiveness of the Before50 strategy. To evaluate how the economic and epidemiological outcome of this strategy was affected by not implementing it perfectly, variations of the Before50 strategy were simulated in which certain groups of cows were not culled (Before50 & Notcullpregnant and Before50 & Notculltop). These strategies were still better than intervention strategies in which cows are only treated with antibiotics

(Basic3, Treattoplonger, and Basic5), but these variations of Before50 were strain-dependent as the relative impact differed between strains (Figs 1 and 2). Even when the potential limitations of the Before50 strategy in practice are considered, this strategy is still likely to be effective against *S. aureus* and other contagious pathogens, as shown in these simulations, as it effectively removes the reservoir for new IMI (Middleton et al., 2001; Stott et al., 2002). Nevertheless, it is unlikely that the Before50 intervention strategy will be widely implemented for several reasons as discussed above and therefore it is expected that for most farms it will still be beneficial to characterize the *S. aureus* strain and determine if it is advantageous to implement a different intervention strategy, as for most other intervention strategies the outcome is strain-dependent.

Data on phenotypical, clinical and epidemiological characteristics of *S. aureus* strains are limited. Because of this, we had to make assumptions concerning the parameter estimates. For most strains, parameters were only changed if evidence was found in literature. For the other strains, we only changed the parameters that were linked to the phenotypic trait of that strain using quantitative data from literature. To evaluate the effect of uncertainty in the parameter estimate, sensitivity analyses were performed. The sensitivity analyses showed that the uncertainty in most parameters had minimal effect on the outcome variables, only the extreme value of ρ changed the outcome variables considerably. However, based on the limited data available for spill-over type strains (Leuenberger et al., 2019), the transmission from non-mammary gland niches into the mammary gland is expected to be low and therefore ρ is unlikely to be that high. Besides, the ranking of the tested intervention strategies was only minimally affected (Table S9). Therefore, the general conclusion of this study is unlikely to be affected by the uncertainty in the parameter estimates. Another uncertainty is the possibility that additional parameters are different for these strains, for instance, the recovery probability of the persistent strain. However, if additional parameters are changed this is likely to increase differences between strains and, therefore, we expect that the impact of the intervention strategies will still be strain-dependent. Importantly, the aim of this study was not to perfectly simulate *S. aureus* strains with different characteristics, but rather to study whether strain variation affects the impact of intervention strategies. For this, the exact characteristics and (combinations of) changes in parameter estimates are not essential as long as the variation in the phenotypic characteristics of different *S. aureus* strains are realistically estimated.

For the clinical strain, the desired ratio of clinical to subclinical mastitis could not be reached in the model since remission (entering subclinical state following treatment of clinical IMI) is counted both as a clinical (when the quarter entered clinical state) and a subclinical case (upon remission). This method is comparable to the definition of the total incidence of subclinical and clinical cases in practice. However, an important difference is that the MiCull model detects all subclinical cases, but in practice some subclinical cases are missed. This causes the incidence of subclinical cases to be underestimated in practice compared to that of the model. A lower number of subclinical cases causes the ratio clinical to subclinical IMI to be higher, therefore the ratio clinical to subclinical found in practice is likely an overestimation. Because of the theoretical upper limit of the clinical to subclinical ratio in the MiCull model and the difference between practice and the model, we decided to calibrate towards a clinical to subclinical ratio of 1. This allowed us to model the economic and epidemiologic impact of a strain that causes clinical mastitis more often, despite the incongruencies between data obtained from the model and field conditions.

Leuenberger et al. (2019) and Fournier et al. (2008) identified *S. aureus* strains that colonized body sites of cattle (hocks and teat skin) and only caused IMI sporadically. Their data indicated that the contagious transmission of these strains was limited and that niches other than the mammary gland were the reservoir for these *S. aureus* IMI. We, therefore, modelled such a strain using a new transmission formula, to account for the limited capacity to transmit contagiously (from one mammary gland to another) and sporadic transmission from a non-mammary gland reservoir to the mammary gland. Although information regarding the duration and prevalence of colonization was not known, the most important characteristic of this strain was the different transmission route and non-mammary gland reservoirs. The sensitivity analysis of the transmission rate and ρ showed that altering these values had a limited effect on the outcome of the model and no substantial difference in the ranking of intervention strategies was observed (Tables S8 and S9). Due to the alternative non-mammary gland reservoirs, this strain was affected differently by the intervention strategies, which were aimed at contagious mastitis pathogens, compared to the other simulated *S. aureus* strains.

This is the first study to consider *S. aureus* strain differences when looking at the economic and epidemiological impact of intervention strategies. We showed that for most intervention strategies the economic and epidemiological outcome was strain-dependent. This suggests that, depending on the existing on-farm intervention strategy, it can be beneficial to type the *S. aureus* strain and adjust the intervention strategy accordingly. Further research is required to confirm our results (in practice) and determine how this could be implemented in practice. First of all, knowledge regarding strain characteristics is limited while detailed data on phenotypic and epidemiological characteristics of *S. aureus* strains are needed to implement strain-specific intervention strategies. Secondly, the impact of mastitis intervention strategies in the context of different *S. aureus* strains has to be studied under field conditions. Thirdly, the typing methods and strategy to characterize *S. aureus* strains on a farm need to be studied. Questions that have to be addressed include the typing resolution needed, suitable techniques (MALDI-TOF, sequence-based typing, biochemical) and the sampling source, scheme and costs (individual cows or bulk tank milk, number of samples and selection criteria). We estimate that the costs for characterizing *S. aureus* with *Spa* typing in a commercial lab are around €90 per sample. Total costs depend on the typing strategy (as costs depend on the amount of samples typed), but even if 10–20 samples need to be typed the costs are well below the potential increase in net income estimated in the current study. Considering the limited variation in *S. aureus* strains within a region (Hoekstra et al., 2020), cheaper PCR based typing methods may also be feasible. While there is often a dominant strain on farms with *S. aureus* mastitis, sometimes there are multiple strains with a similar prevalence within a herd (Larsen et al., 2000; Mørk et al., 2012; Sommerhäuser et al., 2003). Therefore, the effect of multiple *S. aureus* strains with different phenotypic properties causing IMI within a herd on the impact of strain-specific intervention strategies should also be studied.

5. Conclusion

Using a stochastic bio-economic model of intramammary infections within a dairy herd, we investigated the effect of five *S. aureus* strains with different phenotypic characteristics on the economic and epidemiologic outcome of different intervention strategies. Our results indicate that the outcome of most intervention strategies were strain-dependent. Therefore, when such intervention strategies are applied on a farm it could be advantageous to characterize the *S. aureus* strains causing mastitis to tailor the intervention strategy toward the main *S. aureus* strain. Further research into the characteristics of various *S. aureus* strains and interaction with intervention strategies in practice are needed to validate the results from our model.

Data availability

Data will be made available on request.

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Declaration of Competing Interest

TL is employed by GD, a national leading organization in animal health diagnostics and advice. CE, TH, GK, WS, LB, and MG do not have any competing interests.

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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.2021.105566.

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