Animal 17 (2023) 100840

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Assessment of control measures against livestock-associated methicillin-resistant *Staphylococcus aureus* in a farrow-to-finish pig herd using infectious disease modelling

K.S. Tuominen [a,*], S. Sternberg Lewerin [a], S. Widgren [b], T. Rosendal [b]

[a] Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences (SLU), Box 7036, SE-750 07 Uppsala, Sweden
[b] Department of Disease Control and Epidemiology, National Veterinary Institute (SVA), SE-751 89 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 5 January 2023
Revised 18 April 2023
Accepted 21 April 2023
Available online 28 April 2023

Keywords:
Biosecurity
Disease eradication
Environmental transmission
Swine
Veterinary epidemiology

ABSTRACT

Pigs are considered to be the main reservoir for livestock-associated methicillin-resistant *Staphylococcus aureus* (**LA-MRSA**), which is a zoonotic opportunistic pathogen. As LA-MRSA is an occupational hazard, there is an incentive to control its spread in pig herds. Currently, knowledge about effective control measures which do not require culling the whole herd are limited, and the control strategies against LA-MRSA vary between countries. This study uses a stochastic compartment model to simulate possible control measures for LA-MRSA in a farrow-to-finish pig herd. The aims of the study were to (1) extend a previously published disease spread model with additional management and control measures; (2) use the extended model to study the effect of the individual LA-MRSA control measures on the within-herd LA-MRSA prevalence; (3) evaluate the effect of control measures when they are implemented in combinations. From the individual control measures tested in the study, thorough cleaning was found to be most effective in reducing the LA-MRSA prevalence in the herd. When the different control measures were combined, cleaning together with disease surveillance had the largest impact on reducing the LA-MRSA and a higher chance of causing disease elimination. The results of the study showed that achieving disease elimination once LA-MRSA had been introduced in the herd was challenging but was more likely when control measures were introduced early during the outbreak. This emphasises the importance of early detection of the pathogen and subsequent rapid implementation of LA-MRSA control measures.

Implications

Pigs are common carriers of livestock-associated methicillin-resistant *Staphylococcus aureus*. These bacteria can be transmitted from animals and cause disease in humans. This simulation study indicated that vigorous cleaning of the pig herd environment was the most effective control measure to reduce the within-herd prevalence of the bacteria. When different control measures were combined, cleaning the environment and regular disease surveillance were the most effective measures to reduce the prevalence. The study confirms that eradication of livestock-associated methicillin-resistant *Staphylococcus aureus* from a pig herd is challenging, but the best results are obtained when control measures are introduced early in an outbreak.

Introduction

Livestock-associated methicillin-resistant *Staphylococcus aureus* (**LA-MRSA**) is a commensal and opportunistic pathogen that is resistant to most beta-lactam antibiotics and often to other antimicrobial substances such as tetracycline (European Food Safety Authority and European Centre for Disease Prevention and Control [EFSA and ECDC], 2022; Rao et al., 2022). While the LA-MRSA strains belonging to the clonal complex 398 (**CC398**) are predominant in Europe, the distribution of different strains varies globally (Smith, 2015; Goerge et al., 2017; EFSA and ECDC, 2022). Although LA-MRSA is capable of colonising several species including cattle, poultry and horses (Verkade and Kluytmans, 2014), pigs are considered to be the main reservoir (EFSA and ECDC, 2022). While pigs are usually asymptomatic carriers of LA-MRSA (Verkade and Kluytmans, 2014), LA-MRSA is zoonotic and colonisation through occupational exposure is common (Goerge et al., 2017; Chen and Wu, 2021). Spillover of LA-MRSA

* Corresponding author.
E-mail address: krista.tuominen@slu.se (K.S. Tuominen).

https://doi.org/10.1016/j.animal.2023.100840

to the non-farming community as well as nosocomial spread have also been reported (Larsen et al., 2015; Bosch et al., 2016; Sieber et al., 2019). In humans, both methicillin-susceptible and methicillin-resistant CC398 have been reported to cause various health problems including skin infections and life-threatening infections (Smith and Wardyn, 2015; Goerge et al., 2017; Slott Jensen et al., 2020). Resistance to antimicrobials which are reserved for human use has also been reported in some LA-MRSA isolates, which is a public health concern (EFSA and ECDC, 2022; Leão et al., 2022). As people working with livestock are at significantly higher risk to become colonised by LA-MRSA (Chen and Wu, 2021), finding control measures in the pig farm environment that would reduce or eradicate LA-MRSA might result in reduced occupational exposure.

In Europe, the approaches to monitoring methicillin-resistant *S. aureus* in animals vary between countries (EFSA and ECDC, 2022). This leads to insufficient information about the prevalence in different regions, with subsequent challenges for risk assessment and risk management. In some countries, the lack of effective evidence-based LA-MRSA control strategies that do not involve culling the herd could contribute to the low level of surveillance in livestock. Disease modelling is a cost-effective way to study disease dynamics and control measures when experimental studies are not feasible for practical, ethical or economic reasons. Previously, Sørensen et al. (2018) have used an individual-based model to study the effect of reducing antimicrobial consumption, number of animals and mixing of pigs as well as improved biosecurity on within-herd LA-MRSA prevalence. The simulation indicated that eradication of the bacteria was difficult to achieve, but concluded that changing antimicrobial consumption patterns might be important in reducing the prevalence (Sørensen et al., 2018). Similarly, Schulz et al. (2019) concluded in their simulation study that combinations of different intervention measures, such as reducing the usage of high-risk antimicrobials and the probability of indirect transmission via humans, restricting movements from LA-MRSA-positive herds and using a voluntary eradication process in some of the positive herds, led to a larger reduction in the LA-MRSA herd prevalence than applying each intervention separately. However, the intervention combinations did not fully clear LA-MRSA from all the herds (Schulz et al., 2019). Control measures targeting the between-farm trade network have also been studied by Bastard et al. (2020). The study concluded that targeting control measures to farms with the highest outward trade of pigs had the biggest impact in reducing the LA-MRSA prevalence in the network.

To provide support for effective decision-making when choosing control measures against LA-MRSA CC398 (later "LA-MRSA"), the aims of the study were first (1) to extend a previously published LA-MRSA transmission model of a farrow-to-finish pig herd (Tuominen et al., 2022) with additional management practices and control measures. Secondly (2) to investigate the effect of environmental and biosecurity-related control measures and disease surveillance on within-herd LA-MRSA prevalence. And finally (3), to evaluate the efficacy of novel combinations of control measures for LA-MRSA.

Material and methods

The model simulations and data analyses were run using the R programming language version 4.2.0 (R Core Team, 2022) and the SimInf package version 9.0.0 (Widgren et al., 2019). In the SimInf framework, the transitions between compartments were modelled as a continuous-time discrete-state Markov chain using the Gillespie stochastic simulation algorithm (Gillespie, 1977). The simulation model consisted of a farrow-to-finish pig herd and it was based on a previously published study by Tuominen et al. (2022), with extensions for the current study.

Model structure

The disease spread model is an SIS_E compartment model, where animals move between susceptible (S) and infected (I) states and E represents the indirect transmission through a contaminated environment. In this context, the word "infected" is used to label the pigs that are carriers/shedders of LA-MRSA and not as an indication of clinical disease. The infected state was assumed to be transient and the animals could be recolonised immediately after moving to the susceptible state. The herd structure and animal flow in the model have been described previously in Tuominen et al. (2022).

Model extensions

To improve the conceptual model validity, the existing model was extended to include disease spread between pens located in the same room as well as between rooms within the same herd, which is referred to as between-pen transmission. Here, "room" refers to the different sections within the herd which, in a farm environment, would be divided by walls. Additionally, the recovery rate parameter used in the model was changed from an exponential distribution to an Erlang-distributed recovery time.

Between-pen transmission

The transmission through the contaminated environment was modelled by including a term (φ_i) that described the pen (i) level LA-MRSA contamination from the infected pigs (Tuominen et al., 2022). In the current study, each pen additionally had a coupling to the infectious pressure in other pens within the same room (φ_r) as well as to the whole farm (φ_f). The φ_i, φ_r and φ_f were recalculated when simulated time had proceeded by one unit (day). The φ_r for each room (r) per time step was determined as:

$$\varphi_r = \sum_{i=1}^{n_{pen}(r)} \varphi_i \quad (1)$$

where φ_i is the within-pen environmental infectious pressure of pen i in room r, and $n_{pen}(r)$ the number of pens in the room. The φ_f for the farm (f) per time step was determined by:

$$\varphi_f = \sum_{j=1}^{n_{room}(f)} \varphi_r \quad (2)$$

where φ_r is the environmental infectious pressure of room r in the farm f and $n_{room}(f)$ the number of rooms in the farm.

The daily decay rate of the environmental infectious pressure was set to 0.871 (Tuominen et al. 2022), which was based on a 5-day half-life of LA-MRSA in dust, as reported by Feld et al. (2018). Currently, there is a lack of studies on the between-pen transmission of LA-MRSA in pigs. For porcine circovirus type 2, the between-pen transmission between adjacent pens was reported to be 10–17% of the within-pen transmission (Andraud et al., 2008). For foot-and-mouth disease, the between-pen transmission has been reported be to approximately 10% of the within-pen transmission (Eblé et al., 2006). Based on this, the rate for between-pen spread within the same room was assumed to be 0.1 of the within-pen transmission. The probability of transmission between rooms was assumed to be lower than the transmission within the room and was therefore set to be 0.01 of the within-pen transmission. The transition functions for transitions between the compartments are described in Supplementary Material S1.

Distribution of the infectious time period

To shift the distribution of the infectious period from an exponential towards a more biologically plausible shape, the previously used single infected compartment was divided into three subcompartments (I_1, I_2, I_3). This resulted in the recovery time following an Erlang distribution [k = 3, λ = 1/(3 * duration of carriage)], where the duration of carriage was 17.4 days based on the study by Broens et al. (2012a).

Model parameters

Similar to the previous study (Tuominen et al., 2022), the current model had different transmission rates for different age groups. These age groups were mature pigs (sows and gilts), suckling piglets, growing pigs and finishing pigs. Due to the change in infected categories (I), the model transmission rates were re-parameterised for the current study by using the Approximate Bayesian Computation (**ABC**) sequential Monte Carlo algorithm (Toni et al., 2009), which is available in the SimInf package. The parameterisation process was similar in both studies; the best-fitting transmission rates were obtained by comparing the simulated within-herd LA-MRSA prevalences to expected prevalences, which were based on a study by Broens et al. (2012b). For each model trajectory run in the parameterisation, the transmission rates were sampled from the accepted fitted values. Each generation of the ABC run was required to have two hundred accepted particles, where the accept condition was specified as described in Supplementary Material S2. In contrast to the previous study, adaptive tolerance selection, as proposed by Simola et al. (2021), was implemented to iteratively decrease the tolerance in each generation. The tolerance was used to determine when the simulated data were sufficiently close to the expected prevalences to accept a parameter proposal. In addition, the adaptive tolerance selection algorithm contained a stopping rule based on the estimated sequential ABC posterior distributions to avoid unnecessary iterations of the algorithm. The adaptive tolerance selection and the stopping rule functionalities were implemented as part of the SimInf package.

Control measures

Different control measures were modelled separately and in combination. To study the effect of the control measures at different stages of disease spread, the measures were applied to the herd at two time points:

- During the outbreak phase – the control measures were applied simultaneously with disease introduction, mimicking a herd management practice that was in place prior to disease introduction or a very early detection and subsequent intervention.
- During the endemic phase – the control measures were applied after the disease prevalence had reached stationarity in the herd. In practice, these measures were set to start at 770 days of burn-in after disease introduction.

In both of the cases, LA-MRSA was first introduced to the herd by infecting 20% of gilts in the growing unit on day 1 at the beginning of every trajectory. This proportion of infected pigs corresponded to approximately 0.4% of all pigs in the herd. The growing gilts were considered to be a reasonable pig group for the introduction because farrow-to-finish herds may replace culled sows with gilts from other herds. The disease was introduced to the herd once. However, multiple LA-MRSA introductions over time (e.g. through repeated gilt purchases) could result in different disease dynamics.

Each control measure or a combination of control measures was run in a total of 10 000 trajectories for both the outbreak phase and endemic phase. From each trajectory, the within-herd prevalence on the last day of the simulation and time required for LA-MRSA to be eliminated from the herd by stochastic extinction were recorded. The prevalence was determined by calculating the proportion of animals in the infected compartment out of all animals (susceptible and infected) in the herd. The control strategies were considered effective if the simulation resulted in reduction in disease prevalence or elimination of disease from the herd. When modelling the control measures, extreme values for each parameter were tested to determine the maximum effect of the measures (e.g. when cleaning the environment, all infectious pressure was removed). Therefore, if a control measure was not effective with the tested values, it was also unlikely to result in a reduction in the within-herd prevalence if partially implemented. The combinations of different control measures were chosen based on what was deemed practically feasible to implement in a Swedish pig herd. Some control measure combinations were excluded from the modelling based on the results that were obtained during the simulation process (e.g., measures that resulted in disease elimination on their own were not run with all possible control measure combinations).

Improved biosecurity

The effect of improved biosecurity within the herd was modelled by reducing the room and farm level between-pen transmission to 0.

Disease surveillance

Disease surveillance was modelled by implementing the following disease testing scenarios:

- Testing all sows individually in the farrowing unit two days before they were moved to breeding.
- Testing the gilts in the gilt-unit two days before they were moved to breeding. The testing was done as pen-level pooled samples.
- Both of the above scenarios combined.

In a study Agersø et al. (2014), the diagnostic sensitivity for pooled nasal and ear-skin swab samples were estimated to be 78 and 90%, respectively. For this modelling work, the disease testing was modelled by assuming 70% diagnostic sensitivity in both individual and pooled samples. The modelling of the imperfect test was implemented in the model in the same way as described by Rosendal et al. (2020) for the simulation of disease testing. The conservative 70% sensitivity was chosen due to different sample-pooling assumptions than what was described by Agersø et al. (2014). Additionally, testing with 100% diagnostic sensitivity was modelled to compare the results to a perfect test.

To simulate the removal of test-positive individuals as part of a disease surveillance programme, if a sow received a positive test result, the sow and its piglets in the same pen were moved back to the susceptible compartment and the environmental infectious pressure in the pen was removed ($\varphi_i = 0$). Similarly, if the gilts had a positive pooled test, all animals in the same pen were moved back to the susceptible compartment and the environmental infectious pressure in the pen was removed ($\varphi_i = 0$). This approach was considered to be analogous to a scenario where infected pigs are replaced with susceptible ones, and it was chosen from a model functionality perspective to keep the number of animals in the herd unaltered. A similar approach has been previously described in a study by Widgren et al. (2018).

Table 1
Parameterised median transmission rates in pigs and associated 95% credible intervals (in parenthesis) and the model fit values for the final generation of the approximate Bayesian computation.

Item	Value
Parameter estimates	
Mature	1.92×10^{-4} ($1.68 \times 10^{-4} - 2.23 \times 10^{-4}$)
Piglets	28.33×10^{-4} ($24.05 \times 10^{-4} - 34.86 \times 10^{-4}$)
Growing	1.14×10^{-4} ($0.10 \times 10^{-4} - 3.41 \times 10^{-4}$)
Finishing	1.73×10^{-4} ($0.85 \times 10^{-4} - 2.53 \times 10^{-4}$)
Model fit	
Final generation tolerance	1.52
Proposed particles in final generation	8 051

Cleaning the environment

The effect of cleaning was incorporated into the model by removing the environmental infectious pressure in the pen ($\varphi_i = 0$), which corresponds to perfect cleaning where all viable LA-MRSA have been removed from the environment. In the pens that followed the all-in-all-out principle (farrowing, growing and finishing unit), the cleaning was done the day after the pen had been emptied from pigs. For continuous-flow pens (breeding, gestation and gilt units), the cleaning was scheduled to occur in a weekly cycle the day before weaning occurred and the sows were moved to the breeding unit. The cleaning all-in all-out and continuous-flow pens were modelled individually and in combination.

Mixing of pigs

In the baseline configuration of the model (without control measures), 10% of the piglets were mixed with other piglets within the same farrowing room on the day after birth ("cross-fostering"). Additionally, all pigs (100%) arriving to the finishing unit were mixed on the day of arrival. In this study, alternative mixing practices were simulated, where cross-fostering and finisher pig mixing were reduced to 0%. In the model configurations where reduced mixing was combined with other control measures, cross-fostering and mixing of finishing pigs were simultaneously reduced to 0%.

Extended empty period in pens

In this control measure, the length of the time period that the pen was kept empty before the next batch of pigs was increased by seven days. Therefore, the animal movements from one unit to another occurred every other week. To compensate for the reduced number of pens available, the herd size was halved. This control measure was only modelled during the outbreak phase of disease spread.

Data analysis

The mean herd prevalence per day and the associated 95% credible intervals were calculated over the 10 000 trajectories of each different control measure model. Livestock-associated MRSA was considered to have been eliminated when the mean herd prevalence was 0. The mean time to disease elimination for each model was calculated as a mean of the observed first time points when the elimination had been reached. The probability of disease elimination for each model configuration was calculated as $P = n/N$, where n is the number of trajectories where the herd prevalence was 0 on the last day of the trajectory and N is the total number of trajectories run.

Results

Parameterisation of transmission rates and model validation

The transmission rates obtained from parameterisation and the model fit indicators are presented in Table 1. The final generation tolerance presented in Table 1 is a measure of model fit, and it represents how closely the model-predicted within-herd prevalences matched the expected prevalences obtained from literature. Empirical model validity was further assessed by comparing the model-predicted LA-MRSA prevalences to the expected prevalences from the literature (Broens et al., 2012b), which is presented in Supplementary Figure S1.

Control measures

The mean within-herd LA-MRSA prevalence and the corresponding 95% credible intervals for single control measures when the control measures were introduced in the outbreak phase of disease spread are presented in Fig. 1. The corresponding control measures introduced in the endemic phase are presented in Fig. 2. For combined control measures, the mean LA-MRSA within-herd prevalence and the corresponding 95% credible intervals when the control measures were introduced in the outbreak phase of disease spread are presented in Fig. 3. The corresponding combined control measures introduced at the endemic phase are presented in Fig. 4. The mean within-herd prevalences for disease surveillance with 100% test sensitivity are available in Supplementary Figure S2.

Improved biosecurity

Improving the herd biosecurity by fully removing the transmission between pens slowed the progression of disease spread and reduced the mean herd prevalence but was not successful in causing disease elimination (Fig. 1). A reduction in within-herd prevalence could also be observed when improved biosecurity was combined with other control measures, e.g., disease testing of sows and cleaning all-in all-out pens (Figs. 3a, c, 4a and c).

Disease surveillance

With 70% diagnostic sensitivity, testing of sows was more effective in lowering the mean within-herd prevalence than testing gilts, in both the outbreak and the endemic phase of disease spread (Fig. 1 and 2). Testing gilts had a low chance of causing disease elimination in the herd when the testing was applied in the endemic phase of the disease spread (Table 2). Combining the two testing protocols did not have an additional impact on the within-herd prevalence, but combining the gilt and/or sow testing with cleaning all-in all-out pens resulted in an additional reduction in the prevalence (Fig. 3 and Fig. 4). When the diagnostic sensitivity was assumed to be 100%, the disease prevalence was lower when testing gilts or testing both gilts and sows (Supplementary

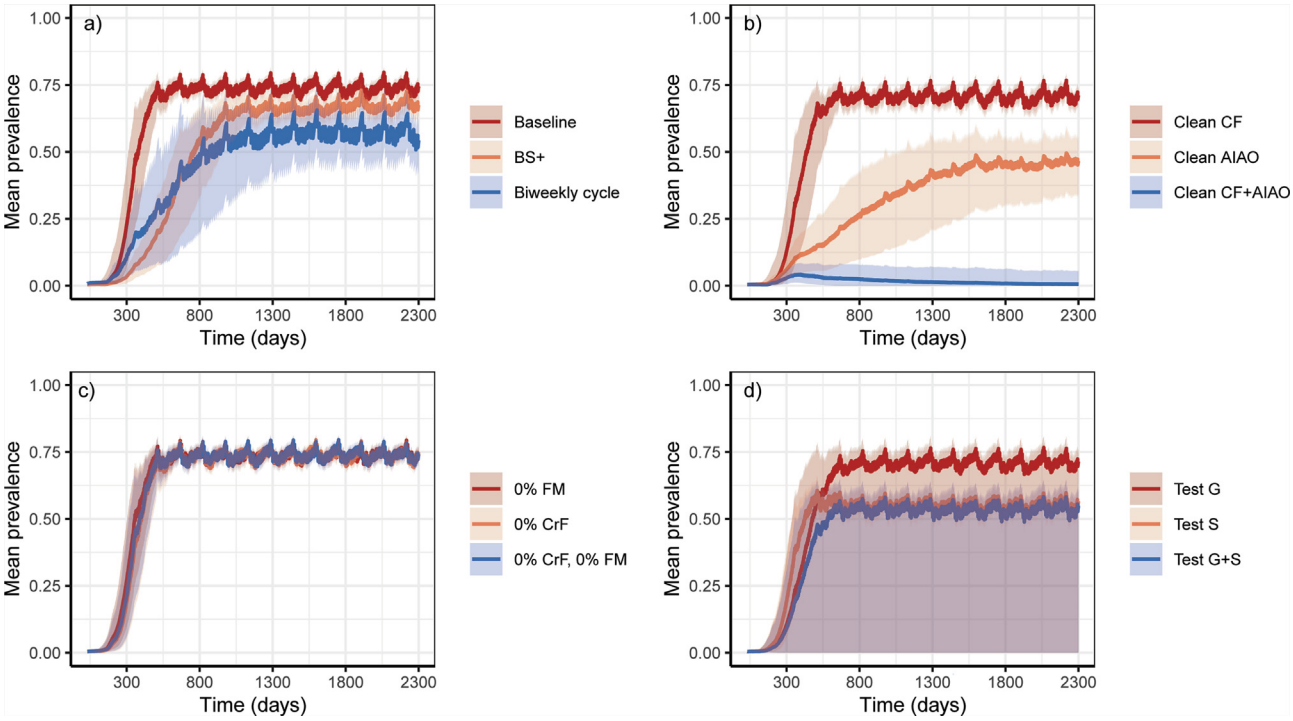

Fig. 1. The model-predicted mean prevalence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in a pig herd when single control measures were introduced in the disease outbreak phase. The lines represent the within-herd prevalence and the ribbons the corresponding 95% credible intervals. The disease was introduced in 20% of the new gilts on model day 1. The control measures were introduced at the same time as the disease introduction. Each model was run for 10 000 trajectories. (a) Prevalence without control measures (Baseline), with improved biosecurity (BS+) and when the animals were moved between units only every other week (Biweekly). (b) Prevalence when the environmental infectious pressure was removed with the weekly cleaning routine either in continuous-flow (CF) pens, all-in all-out pens (AIAO) or simultaneously in both pen types. (c) Prevalence when either mixing of finisher pigs (FM) or cross-fostering (CrF) 1 day after birth was reduced to 0% and the combination of both measures. (d) Prevalence when new gilts (G), sows (S) or both new gilts and sows (G + S) were tested (diagnostic sensitivity 70%) for LA-MRSA.

Figure S2), but for testing only sows, the mean within-herd prevalence remained almost the same as with 70% diagnostic sensitivity.

Cleaning the environment

Cleaning the continuous-flow pens had a very limited effect on the mean within-herd prevalence when it was introduced as the only control measure (Figs. 1 and 2). However, disease elimination was observed when the measure was paired with cleaning all-in all-out pens at the outbreak phase of disease spread (Fig. 3, Table 2). In the endemic phase, the combined cleaning measures did not cause disease elimination, but the prevalence was reduced to low levels (Fig. 4).

Mixing of pigs

Reducing the cross-fostering of piglets or the mixing of the finishing pigs did not have an observable effect on the within-herd prevalence in any of the tested interventions (Figs. 1–4).

Extended empty period in pens

Extending the period where pens were kept empty between batches of pigs resulted in slower progression of the disease spread and reduced the within-herd prevalence (Fig. 1).

Probability of disease elimination

The probability for LA-MRSA to be eliminated from the herd and the mean time to elimination are presented in Table 2 (outbreak phase) and Supplementary Table S1 (endemic phase). The probability of elimination for disease testing measures with 100% test sensitivity are presented in Supplementary Table S2.

Discussion

This study presents potential scenarios for LA-MRSA spread in a pig herd and studies how several control measures could be used to mitigate spread. The focus was on control measures that were deemed to be feasible to implement in a Swedish context on either a voluntary basis or enforced through changes in legislation. This modelling work can provide a basis for strategic planning of control strategies. Based on the results of the study, achieving a complete disease elimination is challenging when LA-MRSA has been established in the herd, which supports the results of previous studies (Sørensen et al., 2018; Schulz et al., 2019; Bastard et al., 2020). In the current study, the highest probability of elimination during the outbreak phase of the disease spread was observed when all pens in the herd were cleaned weekly, or when cleaning all-in all-out pens was combined with disease surveillance in both gilts and sows (Table 2). Achieving elimination was less likely if LA-MRSA had reached an endemic state in the herd and the effective control measure combinations took a longer time to cause elimination than the corresponding measures in the outbreak phase (Table 2, Supplementary Table S1).

Control measures

When assessing individual control measures, cleaning of all-in all-out pens was most effective in reducing the mean within-herd prevalence. Cleaning continuous-flow pens as the only control measure had a smaller impact on the prevalence. This could be explained by the relative proportion of the two pen types. In the model, the proportion of continuous-flow pens was only 8.9% of all pens and they contained approximately 10% of all pigs in the

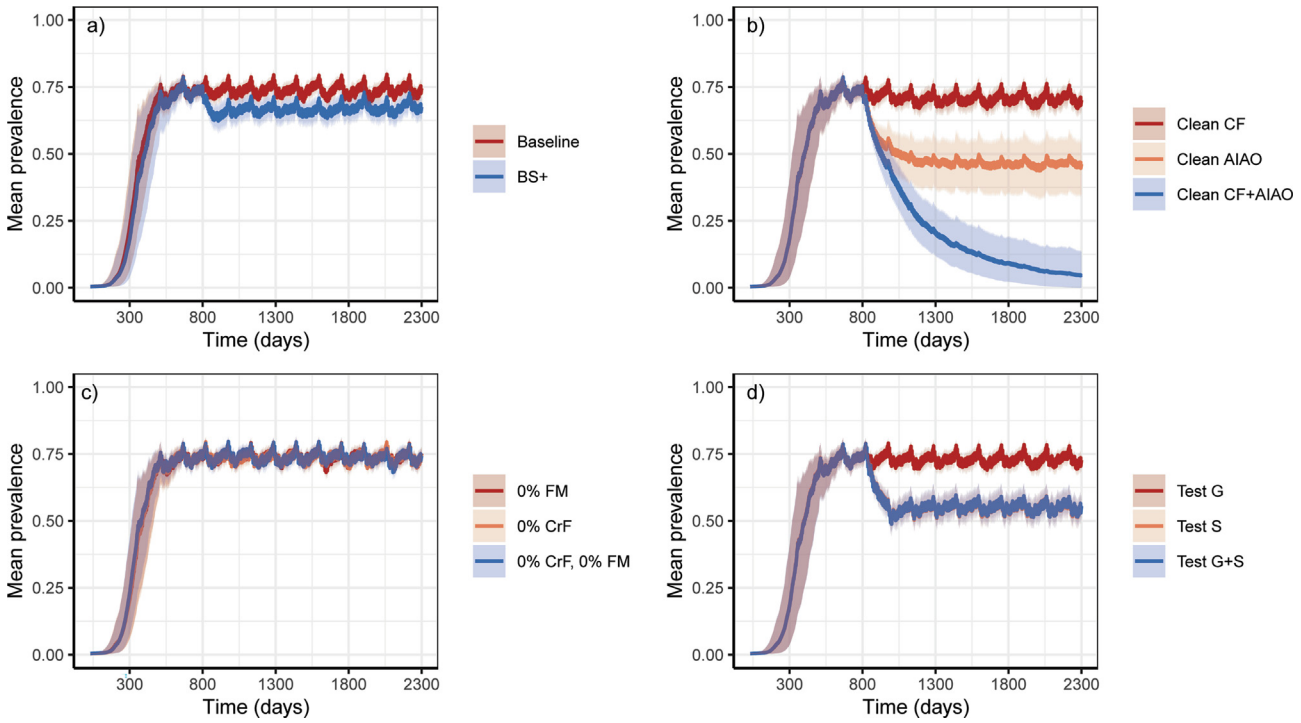

Fig. 2. The model-predicted mean prevalence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in a pig herd when single control measures were introduced in the endemic phase of disease spread. The lines represent the within-herd prevalence and the ribbons the corresponding 95% credible intervals. The disease was introduced to 20% of new gilts on model day 1. The control measures were introduced on day 770. Each model was run for 10 000 trajectories. (a) Prevalence without control measures (Baseline) and with improved biosecurity (BS+). (b) Prevalence when the environmental infectious pressure was removed with the weekly cleaning routine either in continuous-flow (CF) pens, all-in all-out pens (AIAO) or simultaneously in both pen types. (c) Prevalence when either mixing of finisher pigs (FM) or cross-fostering (CrF) 1 day after birth was reduced to 0% and the combination of both measures. (d) Prevalence when new gilts (G), sows (S) or both new gilts and sows (G + S) were tested (diagnostic sensitivity 70%) for LA-MRSA.

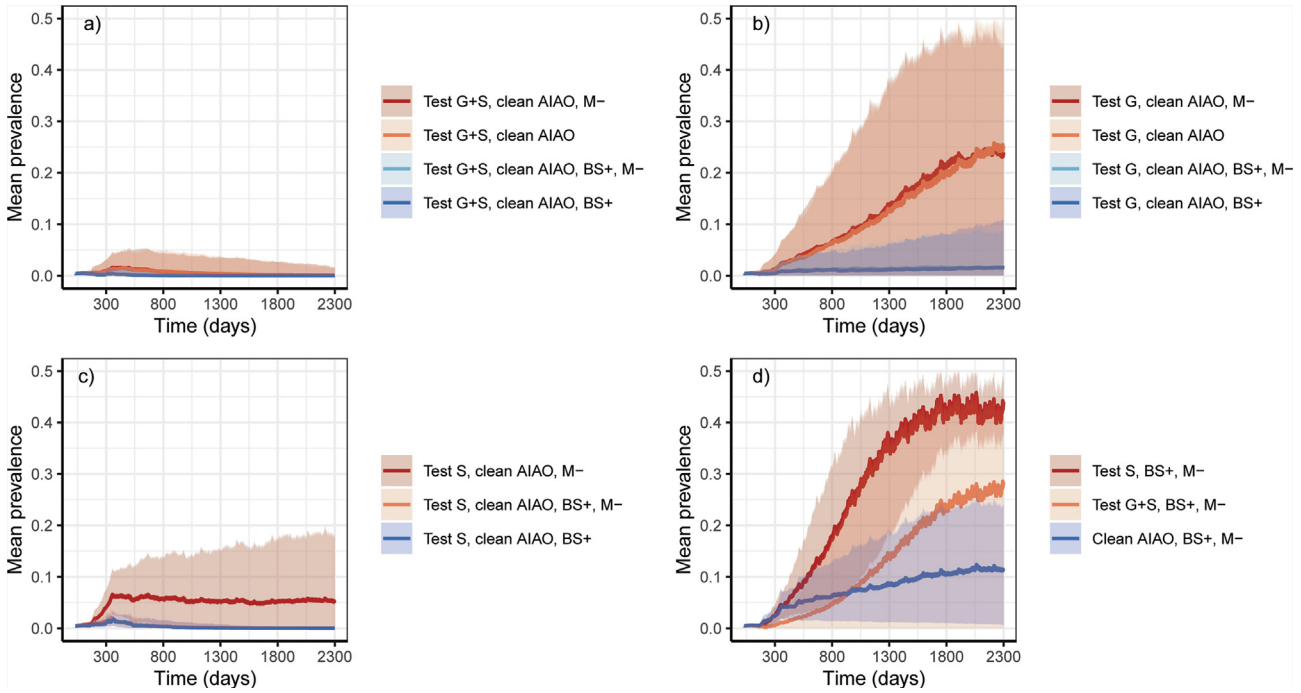

Fig. 3. The model-predicted mean prevalence of livestock-associated methicillin-resistant *Staphylococcus aureus* in a pig herd when combined control measures were introduced in the outbreak phase of disease spread. The lines represent the within-herd prevalence and the ribbons the corresponding 95% credible interval. The disease was introduced to 20% of new gilts on model day 1. The control measures were applied at the same time as the disease introduction. Each model was run for 10 000 trajectories. The possible control measures used in different combinations were: testing gilts (test G) or sows (test S) or testing both gilts and sows (test G + S), cleaning all-in all-out (AIAO) pens when the pens were empty, improving biosecurity by removing between-pen disease transmission (BS+) and reducing cross-fostering piglets and mixing of finishing pigs to 0% (M−).

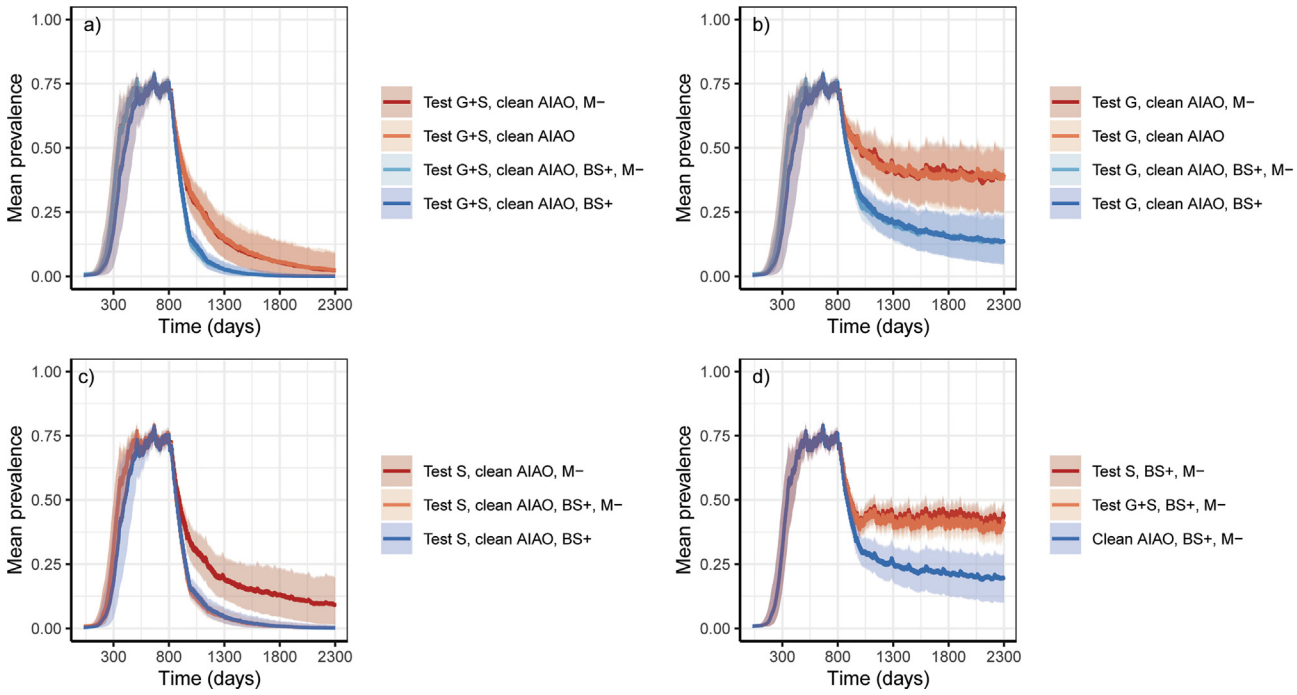

Fig. 4. The model-predicted mean prevalence of livestock-associated methicillin-resistant *Staphylococcus aureus* in a pig herd when combined control measures were introduced in the endemic phase of disease spread. The lines represent the within-herd prevalence and the ribbons the corresponding 95% credible interval. The disease was introduced to 20% of new gilts on model day 1. The control measures were applied on day 770. Each model was run for 10 000 trajectories. The possible control measures used in different combinations were: testing gilts (test G) or sows (test S) or testing both gilts and sows (test G + S), cleaning all-in all-out (AIAO) pens when the pens were empty, improving biosecurity by removing between-pen disease transmission (BS+) and reducing cross-fostering piglets and mixing of finishing pigs to 0% (M−).

herd, while the rest of the population were in all-in all-out pens. Therefore, the proportion of pens and animals affected when cleaning the continuous-flow pens was much smaller than when cleaning all-in all-out pens. However, combining the cleaning of continuous-flow pens with all-in all-out pens resulted in a larger prevalence reduction than what was observed in the individual cleaning measures, indicating that there is an interaction between the two cleaning protocols. This is likely the result of successful removal of the pathogen reservoir: when both all-in all-out and continuous-flow pens are cleaned, the mature animals in the breeding cycle are less likely to maintain and disseminate LA-MRSA to other parts of the herd. The approach to model cleaning that removes all infectious pressure from the environment was chosen because it represents the best possible effect of cleaning. Based on studies by Schmithausen et al. (2015) and Elstrøm et al. (2019), it was considered reasonable to assume that it is possible to remove viable LA-MRSA below the infectious dose with diligent cleaning and disinfection. However, in these studies, the farms were also emptied before cleaning and disinfection. In a field study in a German farm, Kobusch et al. (2020) showed that standard cleaning and disinfection are effective against environmental contamination with LA-MRSA but when implemented in a situation where the prevalence was already high it was not sufficient for elimination. While the results of the modelled cleaning measures might not be fully achievable in practical setting, they show that efficient cleaning and disinfection can have a major impact in reducing LA-MRSA in a pig herd. However, further studies are needed to assess the efficacy of less than perfect cleaning measures.

As cleaning the continuous-flow pens weekly may not be a feasible control measure from a practical point of view, cleaning all-in all-out pens was combined with other control measures to study if effective results could be obtained with other approaches. In general, cleaning of all-in all-out pens seems to be one of the key components in achieving disease elimination, but the largest reduction of the mean within-herd prevalence was obtained when cleaning was combined with disease surveillance. Based on the results in Fig. 3 and Table 2, disease surveillance by testing both gilts and sows together with all-in all-out pen cleaning resulted in disease elimination, especially when improved biosecurity was added. Testing only sows together with all-in all-out cleaning also had a high probability in causing elimination when it was combined with improved herd biosecurity, whereas testing only gilts was not as effective as the corresponding sow-testing scenarios. A possible explanation is that testing gilts is mostly effective in early detection and disease eradication, but if LA-MRSA has already spread to the rest of the herd, testing sows is more effective in limiting the spread to the offspring which will become the majority of the herd population. In both sow and gilt surveillance measures, the test results were available after one day and the positive animals were removed after another day. This may affect the reliability of the test results as the previously negative animals might become positive during this period but is consistent with the time passing between testing and receiving laboratory results.

Improving the herd biosecurity by removing the between-pen transmission route had only a minor impact on the within-herd LA-MRSA prevalence when used as the only control measure. However, the between-pen transmission rates used in this study were based on assumptions. In practice, the herds that have poorer biosecurity might benefit more from the improved biosecurity than what has been presented in this study. Similar to the improved biosecurity measure, disease surveillance as the only control measure was not enough to substantially reduce the herd prevalence when a test with 70% diagnostic sensitivity was used. However, a more sensitive test had a bigger impact on reducing the prevalence when only gilts or both gilts and sows were tested. As improving the diagnostic sensitivity did not decrease the within-herd prevalence when testing only sows, the reduction in prevalence when testing gilts was most likely affected by the increased probability of disease elimination, which was probably

Table 2
The probability of elimination of livestock-associated methicillin-resistant *Staphylococcus aureus* and the mean time to extinction in the pig herd model when different control measures were applied at the outbreak phase of disease spread. Only control measures that had >0% probability of elimination are included in the table. Each control measure was run for 10 000 trajectories per scenario. The mean time to elimination was calculated from the day of disease introduction.

Control measure	Mean time (days) to elimination	Probability of elimination (%)
Single control measures		
BS+	559	0.01
Biweekly[1]	587	0.07
Test[2] gilts	300	2.96
Clean AIAO	1 158	0.02
Combined control measures		
Test G + S, clean CF and AIAO, BS+, M−	365	100.00
Test G + S, clean CF and AIAO, M−	536	100.00
Test G + S, clean AIAO, BS+, M−	533	99.99
Test G + S, clean AIAO, BS+	492	99.98
Test G + S, clean AIAO, M−	946	94.04
Test G + S, clean AIAO	920	94.33
Test G + S, BS+, M−	565	23.7
Test G + S	291	3.26
Test gilts, clean AIAO, BS+, M−	868	54.39
Test gilts, clean AIAO, BS+	780	63.31
Test gilts, clean AIAO, M−	660	18.92
Test gilts, clean AIAO	648	23.63
Test sows, clean AIAO, BS+, M−	977	99.1
Test sows, clean AIAO, BS+	931	99.39
Test sows, clean AIAO, M−	1 600	18.67
Test sows, BS+, M−	1 109	0.02
Clean AIAO, BS+, M−	1 510	1.46
Clean CF and AIAO	1 370	73.02

Abbreviations: BS+ = improved biosecurity (between-pen transmission reduced to 0); M− = cross-fostering and finishing mixing reduced to 0; AIAO = all-in all-out pens; G + S = gilts and sows; CF = continuous-flow pens.
[1] In the biweekly model, animal movements occurred every other week instead of every week as in other model scenarios.
[2] The diagnostic test sensitivity for the surveillance control measures (testing) was 70%.

a consequence of improved early disease detection. These results may indicate that, if several tests with different diagnostic sensitivities are available, investing in more sensitive testing methods could be beneficial for maximising the chance of detecting LA-MRSA carriers early in an outbreak and consequently improving the chances of eradicating the disease before it spreads widely in the herd.

Ceasing the mixing of pigs in the farrowing unit (cross-fostering) and in the finishing unit did not have an impact on LA-MRSA within-herd prevalence when it was used as the only control measure nor when combining with other control measures (Figs. 1–4). Similar findings have also been reported in the modelling study by Sørensen et al. (2018). The reason for the observed lack of effectiveness in the current study remains largely unknown, but one explanation could be that the infectious pressure within the farrowing and finishing rooms is fairly evenly disseminated within each room and therefore moving animals between pens in the same room does not influence the prevalence.

This study also investigated the effect of extending the time the pens were held empty between batches of pigs on the within-herd prevalence of LA-MRSA. To be able to achieve this, the number of animals in the herd needed to be halved to be able to fit animals in pens that were free for use. This control measure slowed the disease spread and reduced the steady-state disease prevalence. As this control strategy required major changes to the base model structure, it was only modelled as a single control strategy at the outbreak phase of the disease spread. Moreover, in a farm environment, it would induce a major economic setback and would be only implemented when there was a sense of urgency and hope of eradication, i.e., in the outbreak phase of disease spread. Combining this control measure with other measures such as cleaning could have a bigger impact on the prevalence, but reducing the number of pigs in the herd could be difficult and costly in practice.

Time to disease elimination

In addition to the low probability of achieving disease elimination, the mean number of days required to reach elimination was high. Even the most effective combination of control measures (cleaning of all pens, surveillance of both gilts and sows, improved biosecurity and no mixing practices) took at least one year to reach elimination when the measures were introduced immediately at the outbreak phase of the spread. This may seem discouraging from a practical perspective, but it should be noted that, in herds with different management practices, the results might be better than those described in the current study. The transmission parameters used in the study were parameterised against values obtained from the study by Broens et al. (2012b), and therefore, these modelling results reflect the conditions of the herds sampled in that study. Additionally, the proportion of pigs that were infected to introduce the disease to the herd was fairly high. If the disease was introduced via fewer individuals, eradicating LA-MRSA might have been more likely and occurred sooner. In a country where the number of LA-MRSA-positive herds is low, the likelihood of LA-MRSA introduction to the herd and the intensity of the introduction may be smaller than what was modelled in this study. Therefore, the control measures presented in this study may still be an attractive alternative to whole-herd culling in low-prevalence countries.

Limitations

This study adapted a model where both the direct and indirect transmission of LA-MRSA were combined into single transmission term. In this approach, all transmission took place indirectly through the environment and it allowed studying the effect of cleaning on the disease prevalence. However, as discussed in Tuominen et al. (2022), separating the direct and indirect transmission could be a more accurate representation of the disease dynamics, but this was not possible with the limited within-herd prevalence observations available in the literature. Due to the environmentally mediated transmission, it is possible that the modelled prevalence reduction obtained with the cleaning measures may have been larger than if the direct transmission would have been separated from the indirect transmission. However, although the model might slightly overestimate the effect of cleaning the environment, it still takes into account the infected animals and their contribution to the environmental load.

The baseline configuration of the model did not include any cleaning routines which most of the pig farms are likely to have. Additionally, no cleaning routines were included when parameterising the transmission rates because the extent of the cleaning measures in the herds represented in the target data were unknown. It is reasonable to assume that a certain baseline cleaning practice was used in these herds. This could result in an underestimation of the transmission rates and consequently an overestimation of the difference between model trajectories with and without cleaning. The model used in this study also assumed that pigs could be recolonised with LA-MRSA immediately after recovering from infected state. While studies have found it difficult to induce immunity against *S. aureus* (Crombé et al., 2013), it is not possible to fully exclude the possibility of pigs obtaining immunity against LA-MRSA. If pigs are capable of developing immunity after

encountering LA-MRSA, this could change the model transmission dynamics.

The disease surveillance modelled in this study was an adaptation of what could be a feasible surveillance strategy in practice. In the model, the test-positive pigs and the pigs sharing the same pen were moved into the susceptible category and the pen environment was fully cleaned. Therefore, this approach assumed the availability of LA-MRSA-negative animals to replace infected ones. In practice, replacement gilts may themselves be a source of disease or not be available immediately to replace those that are culled. If animals were not replaced, the population size would decrease, and this would affect disease transmission dynamics. Also, using more extensive removal strategies, e.g., removing all the animals in the same room and thorough cleaning, could improve the chances of achieving disease elimination in the herd.

Overall aspects

Assessing the practical importance of the individual control measures presented in this study is dependent on the desired goal to be achieved (e.g., reduction below certain within-herd prevalence or disease elimination) as well as how easy the measures are to implement in practice. Ideally, control measures should be cost-effective, feasible to implement and cause quick elimination of the disease. But, as demonstrated in the current and previous studies (Sørensen et al., 2018; Schulz et al., 2019), eradicating LA-MRSA from a pig herd is difficult and requires combining several control measures which may be labour-intensive and costly. The tested control measures were seen as feasible, based on discussions with Swedish pig veterinarians and pig farmers. However, the success relies heavily on full implementation, which might require a legal obligation. To limit the between-herd spread of LA-MRSA, it would also be beneficial to use network models to study the between-herd dynamics and their impact on the introduction of LA-MRSA to individual herds. In addition, a cost-benefit analysis including public health benefits would most likely be required as a basis for discussions about cost-sharing.

This study modelled possible control measures against LA-MRSA in a farrow-to-finish pig herd. The results show that thorough cleaning of the environment may be one of the key factors in reducing the within-herd LA-MRSA prevalence. However, combining cleaning with disease surveillance results in a larger reduction in LA-MRSA prevalence and higher chance of disease elimination. The results highlight that achieving disease elimination can be challenging once LA-MRSA has been introduced to the herd, but more likely if LA-MRSA is detected early in the outbreak.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.100840.

Ethics approval

Not applicable.

Data and model availability statement

The model code is publicly available in a GitHub repository: https://github.com/KSTuominen/LAMRSAControl. The data that support the study findings are available upon request from the corresponding author.

Author ORCIDs

K.S. Tuominen: https://orcid.org/0000-0002-2223-9376.
S. Sternberg Lewerin: https://orcid.org/0000-0001-7907-8377.
S. Widgren: https://orcid.org/0000-0001-5745-2284.
T. Rosendal: https://orcid.org/0000-0002-6576-9668.

Author contributions

K.S. Tuominen: data curation, investigation, methodology, software, formal analysis, validation, visualisation, writing - original draft.

S. Sternberg Lewerin: conceptualisation, validation, funding acquisition, supervision, writing - review and editing.

S. Widgren: methodology, software, formal analysis, resources, writing - review and editing.

T. Rosendal: conceptualisation, data curation, methodology, software, formal analysis, validation, supervision, writing - original draft.

Declaration of interest

None.

Acknowledgements

We thank the pig farmers and the pig veterinarians who took part in the focus group discussions of feasible control measures. We also thank Hedvig Gröndal (Swedish University of Agricultural Sciences) for planning and leading these discussions.

Financial support statement

This work was funded by the Swedish Farmers' Foundation for Agricultural Research grant O-18-20-033 (K.S. Tuominen, S. Sternberg Lewerin, T. Rosendal). Additionally, it was supported by the EJP Full-Force project, which is funded by the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830 (S. Widgren). The funding bodies had no role in the design of the study, analysis and interpretation of the data or in writing the manuscript.

References

Agersø, Y., Vigre, H., Cavaco, L.M., Josefsen, M.H., 2014. Comparison of air samples, nasal swabs, ear-skin swabs and environmental dust samples for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in pig herds. Epidemiology and Infection 142, 1727–1736. https://doi.org/10.1017/s095026881300280x.

Andraud, M., Grasland, B., Durand, B., Cariolet, R., Jestin, A., Madec, F., Rose, N., 2008. Quantification of porcine circovirus type 2 (PCV-2) within- and between-pen transmission in pigs. Veterinary Research 39, 43. https://doi.org/10.1051/vetres:2008020.

Bastard, J., Andraud, M., Chauvin, C., Glaser, P., Opatowski, L., Temime, L., 2020. Dynamics of livestock-associated methicillin resistant *Staphylococcus aureus* in pig movement networks: Insight from mathematical modeling and French data. Epidemics 31,. https://doi.org/10.1016/j.epidem.2020.100389 100389.

Bosch, T., van Luit, M., Pluister, G.N., Frentz, D., Haenen, A., Landman, F., Witteveen, S., van Marm-Wattimena, N., van der Heide, H.G., Schouls, L.M., 2016. Changing characteristics of livestock-associated meticillin-resistant *Staphylococcus aureus* isolated from humans - emergence of a subclade transmitted without livestock exposure, the Netherlands, 2003 to 2014. Euro Surveillance 21, 30236. https://doi.org/10.2807/1560-7917.es.2016.21.21.30236.

Broens, E.M., Graat, E.A., van de Giessen, A.W., Broekhuizen-Stins, M.J., de Jong, M.C., 2012a. Quantification of transmission of livestock-associated methicillin resistant *Staphylococcus aureus* in pigs. Veterinary Microbiology 155, 381–388. https://doi.org/10.1016/j.vetmic.2011.09.010.

Broens, E.M., Espinosa-Gongora, C., Graat, E.A., Vendrig, N., Van Der Wolf, P.J., Guardabassi, L., Butaye, P., Nielsen, J.P., De Jong, M.C., Van De Giessen, A.W., 2012b. Longitudinal study on transmission of MRSA CC398 within pig herds. BMC Veterinary Research 8, 58. https://doi.org/10.1186/1746-6148-8-58.

Chen, C., Wu, F., 2021. Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) colonisation and infection among livestock workers and veterinarians: a systematic review and meta-analysis. Journal of Occupational and Environmental Medicine 78, 530–540. https://doi.org/10.1136/oemed-2020-106418.

Crombé, F., Argudín, M.A., Vanderhaeghen, W., Hermans, K., Haesebrouck, F., Butaye, P., 2013. Transmission dynamics of methicillin-resistant *Staphylococcus aureus* in pigs. Frontiers in Microbiology 4, 57. https://doi.org/10.3389/fmicb.2013.00057.

Eblé, P., de Koeijer, A., Bouma, A., Stegeman, A., Dekker, A., 2006. Quantification of within- and between-pen transmission of Foot-and-Mouth disease virus in pigs. Veterinary Research 37, 647–654. https://doi.org/10.1051/vetres:2006026.

Elstrøm, P., Grøntvedt, C.A., Gabrielsen, C., Stegger, M., Angen, Ø., Åmdal, S., Enger, H., Urdahl, A.M., Jore, S., Steinbakk, M., Sunde, M., 2019. Livestock-Associated MRSA CC1 in Norway; Introduction to Pig Farms, Zoonotic Transmission, and Eradication. Frontiers in Microbiology 10, 139. https://doi.org/10.3389/fmicb.2019.00139.

European Food Safety Authority, Centre, E., for Disease Prevention and Control, 2022. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2019–2020. EFSA Journal 20, e07209.

Feld, L., Bay, H., Angen, O., Larsen, A.R., Madsen, A.M., 2018. Survival of LA-MRSA in dust from swine farms. Annals of Work Exposures and Health 62, 147–156. https://doi.org/10.1093/annweh/wxx108.

Gillespie, D.T., 1977. Exact stochastic simulation of coupled chemical reactions. The Journal of Physical Chemistry 81, 2340–2361. https://doi.org/10.1021/j100540a008.

Goerge, T., Lorenz, M.B., van Alen, S., Hubner, N.O., Becker, K., Kock, R., 2017. MRSA colonization and infection among persons with occupational livestock exposure in Europe: Prevalence, preventive options and evidence. Veterinary Microbiology 200, 6–12. https://doi.org/10.1016/j.vetmic.2015.10.027.

Kobusch, I., Müller, H., Mellmann, A., Köck, R., Boelhauve, M., 2020. Single Blinded Study on the Feasibility of Decontaminating LA-MRSA in Pig Compartments under Routine Conditions. Antibiotics 9, 141. https://doi.org/10.3390/antibiotics9040141.

Larsen, J., Petersen, A., Sorum, M., Stegger, M., van Alphen, L., Valentiner-Branth, P., Knudsen, L.K., Larsen, L.S., Feingold, B., Price, L.B., Andersen, P.S., Larsen, A.R., Skov, R.L., 2015. Meticillin-resistant *Staphylococcus aureus* CC398 is an increasing cause of disease in people with no livestock contact in Denmark, 1999 to 2011. Euro Surveillance 20, 30021. https://doi.org/10.2807/1560-7917.es.2015.20.37.30021.

Leão, C., Clemente, L., Cara d'Anjo, M., Albuquerque, T., Amaro, A., 2022. Emergence of Cfr-Mediated Linezolid Resistance among Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) from Healthy Pigs in Portugal. Antibiotics (Basel) 11, 1439. https://doi.org/10.3390/antibiotics11101439.

R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rao, S., Linke, L., Magnuson, R., Jauch, L., Hyatt, D.R., 2022. Antimicrobial resistance and genetic diversity of *Staphylococcus aureus* collected from livestock, poultry and humans. One Health 15,. https://doi.org/10.1016/j.onehlt.2022.100407 100407.

Rosendal, T., Widgren, S., Ståhl, K., Frössling, J., 2020. Modelling spread and surveillance of *Mycobacterium avium* subsp. *paratuberculosis* in the Swedish cattle trade network. Preventive Veterinary Medicine 183,. https://doi.org/10.1016/j.prevetmed.2020.105152 105152.

Schmithausen, R.M., Kellner, S.R., Schulze-Geisthoevel, S.V., Hack, S., Engelhart, S., Bodenstein, I., Al-Sabti, N., Reif, M., Fimmers, R., Korber-Irrgang, B., Harlizius, J., Hoerauf, A., Exner, M., Bierbaum, G., Petersen, B., Bekeredjian-Ding, I., 2015. Eradication of methicillin-resistant *Staphylococcus aureus* and of Enterobacteriaceae expressing extended-spectrum beta-lactamases on a model pig farm. Applied and Environmental Microbiology 81, 7633–7643. https://doi.org/10.1128/aem.01713-15.

Schulz, J., Boklund, A., Toft, N., Halasa, T., 2019. Effects of control measures on the spread of LA-MRSA among Danish pig herds between 2006 and 2015 - a simulation study. Scientific Reports 9, 691. https://doi.org/10.1038/s41598-018-37075-8.

Sieber, R.N., Larsen, A.R., Urth, T.R., Iversen, S., Moller, C.H., Skov, R.L., Larsen, J., Stegger, M., 2019. Genome investigations show host adaptation and transmission of LA-MRSA CC398 from pigs into Danish healthcare institutions. Scientific Reports 9, 18655. https://doi.org/10.1038/s41598-019-55086-x.

Simola, U., Cisewski-Kehe, J., Gutmann, M.U., Corander, J., 2021. Adaptive Approximate Bayesian Computation Tolerance Selection. Bayesian Analysis 16, 397–423. https://doi.org/10.1214/20-BA1211.

Slott Jensen, M.L., Nielsine Skov, M., Pries Kristiansen, H., Toft, A., Lundgaard, H., Gumpert, H., Westh, H., Holm, A., Kolmos, H.J., Kemp, M., 2020. Core genome multi-locus sequence typing as an essential tool in a high-cost livestock-associated meticillin-resistant *Staphylococcus aureus* CC398 hospital outbreak. Journal of Hospital Infection 104, 574–581. https://doi.org/10.1016/j.jhin.2019.12.009.

Smith, T.C., 2015. Livestock-associated *Staphylococcus aureus*: the United States experience. PLoS Pathogens 11, e1004564.

Smith, T.C., Wardyn, S.E., 2015. Human Infections with *Staphylococcus aureus* CC398. Current Environmental Health Reports 2, 41–51. https://doi.org/10.1007/s40572-014-0034-8.

Sørensen, A.I.V., Rosendal, T., Widgren, S., Halasa, T., 2018. Mechanistic modelling of interventions against spread of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) within a Danish farrow-to-finish pig herd. PloS One 13, e0200563.

Toni, T., Welch, D., Strelkowa, N., Ipsen, A., Stumpf, M.P.H., 2009. Approximate Bayesian computation scheme for parameter inference and model selection in dynamical systems. Journal of The Royal Society Interface 6, 187–202. https://doi.org/10.1098/rsif.2008.0172.

Tuominen, K.S., Sternberg Lewerin, S., Jacobson, M., Rosendal, T., 2022. Modelling environmentally mediated spread of livestock-associated methicillin-resistant *Staphylococcus aureus* in a pig herd. Animal 16,. https://doi.org/10.1016/j.animal.2021.100450 100450.

Verkade, E., Kluytmans, J., 2014. Livestock-associated *Staphylococcus aureus* CC398: animal reservoirs and human infections. Infection, Genetics and Evolution 21, 523–530. https://doi.org/10.1016/j.meegid.2013.02.013.

Widgren, S., Engblom, S., Emanuelson, U., Lindberg, A., 2018. Spatio-temporal modelling of verotoxigenic *Escherichia coli* O157 in cattle in Sweden: exploring options for control. Veterinary Research 49, 78. https://doi.org/10.1186/s13567-018-0574-2.

Widgren, S., Bauer, P., Eriksson, R., Engblom, S., 2019. SimInf: A Framework for Data-Driven Stochastic Disease Spread Simulations. Journal of Statistical Software 91, 1–42. https://doi.org/10.18637/jss.v091.i12.