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Spread and control of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in Danish pig herds

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Preface

This PhD project was part of a bigger project, the OHLAM (One Health Livestock-Associated MRSA) project, initiated by the Ministry of Environment and Food of Denmark in order to underpin decisions towards a strategy to control LA-MRSA in Denmark and limit its spread to humans.

The background for initiating the OHLAM project was a marked increase in the number of detected human LA-MRSA carriers from 14 in 2007 to 643 in 2013, as well as an increase in the proportion of the total MRSA cases constituted by LA-MRSA from 2.1% to 30.7%. Some of this increase could be explained by revised MRSA guidelines for sampling, but it was still clear that the epidemic was growing, and that there was a need for a strategy to control LA-MRSA.

Generally, there is a lack of knowledge regarding effective interventions against LA-MRSA in pigs as well as in humans. The importance of various transmission routes is not characterized on a large scale and the economic consequences of LA-MRSA at a national scale needs to be further investigated, in order to make informed decisions about handling the epidemic.

The OHLAM project consists of 15 work packages with a high degree of interaction between them. The work presented in this thesis formed part of work package (WP) 1.1 and 1.2. The overall aim of WP 1.1 was to build simulations models for spread of LA-MRSA within and between pig farms and use these models for simulating the effect of possible control strategies**.** It was the intention that these models should be informed by data harvested in other WPs. The aim of WP 1.2 was to investigate the epidemiology of LA-MRSA in the Danish pig population, in particular the influence of vertical transmission, risk factors for introduction and determinants for farm-level LA-MRSA status.

The work that formed the basis for the present thesis was conducted at the Division for Diagnostics and Scientific Advice, National Veterinary Institute, Technical University of Denmark during April 1, 2015 – March 31, 2018, and also included a 2-month stay at Department of Disease Control and Epidemiology, National Veterinary Institute, Uppsala, Sweden (SVA) during September 25, 2017 – November 24, 2017.

The PhD student was enrolled in the VET-BIT PhD School and the PhD project was funded by the Ministry of Environment and Food of Denmark through The Danish Agrifish Agency (J. no. 33010-NIFA-14-612).

Lyngby, March 2018.

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All my co-authors are gratefully acknowledged for their input and contributions.

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Anna Irene

Summary

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is an opportunistic human pathogen with main reservoir in pigs.

Since LA-MRSA first was identified in Denmark in 2007 among isolates collected at two Danish pig farms in 2003, the occurrence have increased dramatically and reached a prevalence of 88% positive farms in 2016. Meanwhile a similar increase in human infections have been observed; most infections are still observed among people with livestock contact, but the development in number of infections among people without livestock contact have followed a similar increasing trend.

Given the high prevalence of LA-MRSA positive farms, total eradication of LA-MRSA in the Danish pig population does not seem feasible, and thus a strong need for exploring options to control the spread of LA-MRSA in Danish pig herds exists. At present it is still not known how LA-MRSA managed to spread so quickly in the Danish pig population and a lot still needs to be understood regarding which factors that determine whether a farm becomes LA-MRSA positive or not.

In the first part of this thesis two studies were conducted with the aim of identifying herd-level risk factors for: 1) herds testing MRSA positive (study 1), and 2) more specifically for herds changing status from negative to positive during 2014-2016 (study 2). The studies were based on data harvested in questionnaire-based phone interviews with farmers and supplemented with data for antimicrobial use, movement of pigs and location of neighbouring farms extracted from three national registers.

Three risk factors already identified in other studies were confirmed. LA-MRSA positive status was associated with large herd size and with number of pig suppliers. In addition, sow herds tested LA-MRSA positive less frequently than herds without sows, and therefore data from sow herds were analysed separately. In univariable analysis, the following factors were associated with sow herds testing LA-MRSA positive: use of wet feed in the sow units; higher weights of piglets at weaning; availability of a delivery room on the farm; cleaning of aisles after pigs were moved; number of pigs per weaner section; number of pigs purchased in the past year, and factors related to rodent control and human traffic in the herd. In herds without sows, the univariable analysis showed that the presence of other animal species on the farm; negative pressure ventilation; full sectioning; frequent visits from the veterinarian; peroral use of tetracyclines for weaners; number of pigs purchased in the past year, and factors related to rodent control and human traffic in the herd were significantly associated with LA-MRSA status. Similar to what have been observed in other studies, many of the factors significantly associated with LA-MRSA status in study 1 was also significantly associated with herd size, and thus it was not possible to identify whether herd size itself or the related factors were the “true” risk factors.

The number of observations in study 2 was small, but three variables (the number of pig suppliers, use of group medication in water vs. administration through feed, and having a company contract for mouse control) were associated with changing LA-MRSA status in the univariable analysis.

Before the implementation of a national control strategy can be decided upon, it is also essential to understand how LA-MRSA spreads and persists within a pig herd, once it has been introduced. In the second part of this thesis a mechanistic model for spread of LA-MRSA within an pig herd was therefore build and subsequently used for studying transmission dynamics and within-farm prevalence after simulating different introductions of LA-MRSA on a farm.

With the current parameterisation of the model, spread of LA-MRSA throughout the farm mainly followed the movement of pigs. The later in the production process LA-MRSA was introduced, the longer it took to spread to the whole farm. After spread of LA-MRSA had reached a steady state, the prevalence of LA-MRSA shedders was predicted to be highest in the farrowing unit, and lowest in the mating unit, independent of where and how LA-MRSA was introduced. Thus the farrowing unit might the area with most potential for intervention against spread of LA-MRSA. Introduction of a low number of intermittently shedding pigs was predicted to frequently result in LA-MRSA not establishing itself in the herd.
Increasing the duration of carriage led to an increased median prevalence, less variance and fewer iterations where LA-MRSA did not become established in the herd. When removing the possibility of pigs becoming persistent shedders, LA-MRSA more frequently faded out and did not become established within the herd.

Not much is known regarding successful interventions against LA-MRSA within pig herds. Consequently the mechanistic model for spread of LA-MRSA within a farm were used for simulating on-farm interventions within four different areas: 1) Reduced antimicrobial consumption, 2) Reduced number of pigs within each section, 3) Reduced mixing of pigs from different litters, batches or pens, and 4) Improved internal biosecurity. It is believed that a reduction in the within-farm LA-MRSA prevalence will result in less spread between farms and reduce the risk of transmission to humans working on the farm.

Reducing the transmission rates after LA-MRSA had become fully established within the herd, resulted in a marked prevalence decrease in the prevalence of LA-MRSA positive pigs within the different stable units, albeit LA-MRSA rarely disappeared completely. This indicates that while reducing antimicrobial consumption might be an important step towards reducing the LA-MRSA occurrence within the herd, other preventive or intervention measures should also be implemented in order to completely clear a herd from LA-MRSA.

Implementation of the other interventions after LA-MRSA had become established within a herd only resulted in marginal changes in the median within-herd prevalence. However, in relation to being able to achieve or maintain a low level of antimicrobial consumption, these factors might still be of importance.

The results of the sensitivity analysis indicated that the assumptions regarding the existence of pigs persistently shedding MRSA have a noticeable influence on the model results.

A secondary of objective of building the simulation model was to identify knowledge gaps regarding spread and control of LA-MRSA. Several knowledge gaps related to infection dynamics exist, including influence of the environment, LA-MRSA load and persistent carriage. Regarding control of LA-MRSA, the main problem is currently a lack of evidence for major effect of any type of intervention other than reducing antimicrobial consumption.

In conclusion, the work presented in this thesis have resulted in:
1) Confirmation of already known risk factors for farms testing LA-MRSA positive (herd type, herd size, number of pig suppliers) and identification of a number of potential new risk factors, albeit many of these were related to herd size, and it therefore was impossible to conclude, whether herd size itself or these factors were the true risk factors.
2) Construction of a mechanistic model for spread of LA-MRSA within a pig herd that can be used for simulating LA-MRSA within herd dynamics following different introductions. The code for the model is publicly available, and the herd part of the model can potentially be re-used together with epidemic models for other pathogens.
3) Simulation of within-herd interventions: Reduced antimicrobial consumption, reduced number of pigs within each section, reduced mixing of pigs, and improved internal biosecurity, of which only reduced antimicrobial consumption had a marked effect on the within-herd prevalence. More intervention scenarios can be simulated, when data becomes available.
4) The observation that once LA-MRSA has become established within a herd, it will spread to all compartments within the farm and be very hard to get rid of.

Sammendrag (Summary in Danish)

Husdyr-associeret methicillin-resistent *Staphylococcus aureus* (husdyr-MRSA) er et opportunistisk patogen med primært reservoir i svinepopulationen.

Siden husdyr-MRSA for første gang blev identificeret i Danmark i 2007 blandt isolater indsamlet i to svinebesætninger i 2003, er forekomsten steget dramatisk og i 2016 blev der fundet husdyr-MRSA i 88% af de undersøgte svinebesætninger. I samme tidsrum er der observeret en tilsvarende stigning i antallet af humane husdyr-MRSA infektioner. De fleste infektioner ses stadigvæk hos personer med kontakt til husdyr, men udviklingen i antallet af infektioner hos personer uden kontakt til husdyr udviser samme stigende tendens.

Givet den høje forekomst af besætninger, der er testet positive for husdyr-MRSA, forekommer det ikke realistisk at udrydde husdyr-MRSA igennem sanering, og derfor er det nødvendigt at undersøge alternative metoder til at få spredningen af husdyr-MRSA i de danske svinebesætninger under kontrol. På nuværende tidspunkt er det stadigvæk uklart, hvordan husdyr-MRSA kunne sprede sig så hurtigt i den danske svinepopulation og, hvilke faktorer, der gør at en svinebesætning bliver husdyr-MRSA positiv.

I den første del af denne afhandling, blev der gennemført to undersøgelser, der havde til formål at identificere risikofaktorer for: 1) at besætninger var husdyr-MRSA positive (undersøgelse 1), eller 2) for at de i løbet af 2014-2016 ændrede status fra at være husdyr-MRSA negative til at være husdyr-MRSA positive (undersøgelse 2). Undersøgelserne var baseret på data indhentet i spørgeskema-baserede telefoninterviews med besætningsejere, suppleret med data fra tre nationale registre for henholdsvis antibiotikaforbrug, flytning af svin, og afstand til andre besætninger.

Tre risikofaktorer, der allerede var identificeret i andre studier blev bekræftet. Status som husdyr-MRSA positiv besætning var relateret til større besætningsstørrelse og antal leverandører af svin. Desuden blev sobesætninger sjældnere testet positive for husdyr-MRSA sammenlignet med besætninger uden søer, og derfor blev data fra besætninger med søer analyseret separat. I univariabel analyse af data fra sobesætninger var følgende faktorer forbundet med at være husdyr-MRSA positiv: brug af vådfoder i so-afsnittene, højere fravænningsvægt, brug af udleveringsrum, rengøring af gangene efter flytning af svin, antal fravænningsgrise pr. sektion, antal svin indkøbt indenfor det seneste år, samt faktorer relateret til gnaverbekæmpelse og menneskelig færdsel i staldene. For besætninger uden søer indikerede de univariable analyser, at positiv husdyr-MRSA status var relateret til tilstedeværelse af andre husdyr på adressen, undertryksventilation, fuldsektionering, hyppige dyrlægebesøg, peroralt forbrug af tetracyklin til fravænningsgrise, antal svin indkøbt indenfor det seneste år, samt faktorer relateret til gnaverbekæmpelse og menneskelig færdsel. Ligesom det også er observeret i en del andre studier, var mange af faktorerne, der var relateret til husdyr-MRSA status også relateret til besætningsstørrelse. Derfor var det i den sidste

ende ikke muligt at konkludere, hvorvidt besætningsstørrelse i sig selv eller de relaterede faktorer er de egentlige risikofaktorer i forhold til husdyr-MRSA.

Antallet af observationer i den anden undersøgelse var lavt, men tre variable var signifikant forbundne med husdyr-MRSA status (antal leverandører af svin, brug af flokmedicinering via vand, samt firmakontrakt for musebekæmpelse).

Før en beslutning kan tages vedrørende en national strategi for bekæmpelse af husdyr-MRSA, er det nødvendigt at forstå, hvordan husdyr-MRSA spreder sig og forbliver i en besætning. Derfor bygger anden del af afhandlingen på udviklingen af en simuleringsmodel for spredning af husdyr-MRSA indenfor en besætning. Denne model blev brugt til at studere spredningsdynamik og forekomst i besætningen efter forskellige introduktioner af husdyr-MRSA.

Med den nuværende parameterisering af modellen, fulgte spredningen af husdyr-MRSA indenfor besætningen primært flytningerne af dyr, og jo senere i produktionscyklus husdyr-MRSA blev introduceret, desto længere tid tog det før bakterien havde spredt sig til hele besætningen. Efter en ligevægtstilstand for spredningen af husdyr-MRSA indenfor besætningen har indfundet sig, vil forekomsten ifølge simuleringerne oftest være højest i farestalden og lavest i løbestalden, uafhængigt af hvor og hvordan husdyr-MRSA blev introduceret. Derfor ser farestalden umiddelbart ud til at være det staldafsnit med størst potentiale for intervention. Ifølge modellen vil introduktion af et lille antal dyr, der kun udskiller husdyr-MRSA fra tid til anden, ofte betyde, at husdyr-MRSA ikke bliver etableret i besætningen, men hurtigt dør ud igen.

I sensitivitetsanalysen blev varigheden af bærertilstanden hos svin, der kun i perioder udskiller husdyr-MRSA forøget, hvilket resulterede i øget medianforekomst af svin, der udskiller husdyr-MRSA, mindre varians og færre iterationer, hvor husdyr-MRSA ikke blev etableret i besætningen. Når muligheden for, at nogle svin blev permanente bærere, blev fjernet, skete det oftere, at husdyr-MRSA ikke blev etableret i besætningen.

På nuværende tidspunkt er der ikke meget viden tilgængelig om succesfulde interventioner overfor husdyr-MRSA i svinebesætninger. Derfor blev simuleringsmodellen brugt til at simulere effekten af besætningsinterventioner med markant forskellige virkningsmekanismer: 1) Reduceret antibiotikaforbrug, 2) Reduceret antal svin pr. sektion, 3) Reduceret sammenblanding af grise fra forskellige kuld, hold eller stier, 4) Forbedret intern smittebeskyttelse. Det forventes, at en reduceret forekomst af husdyr-MRSA inde i besætningerne vil føre til mindre spredning mellem besætninger og minimere risikoen for overførsel af husdyr-MRSA til medarbejderne i besætningen.

Når spredningsraten blev reduceret i simuleringer af spredning i en svinebesætning hvor husdyr-MRSA allerede havde etableret sig, gav det et markant fald i forekomsten i de forskellige staldafsnit, selvom husdyr-MRSA kun sjældent helt forsvandt. Dette indikerer, at selvom en reduktion af antibiotikaforbruget effektivt reducerede forekomsten af svin med husdyr-MRSA i besætningen, og derfor er et vigtigt skridt på vejen til at udrydde husdyr-MRSA, er det kun sjældent nok til helt at udrydde bakterien, og skal derfor stadigvæk suppleres med andre foranstaltninger.

Implementering af de andre interventioner i en svinebesætning hvor husdyr-MRSA allerede var etableret, resulterede kun i marginale ændringer i medianforekomsten i besætningen. Disse interventioner har dog formentligt betydning i forhold til at opnå eller bevare et lavt antibiotikaforbrug.

Sensitivitetsanalyserne indikerede, at antagelsen om tilstedeværelse af svin, der er permanente bærere af husdyr-MRSA, har en betydelig indflydelse på resultaterne af simuleringerne.

Et sekundært formål med at bygge simuleringsmodellen var at identificere områder, hvor der savnes viden i forhold til spredning og kontrol af husdyr-MRSA. Med hensyn til spredning af husdyr-MRSA indenfor besætningen er der adskillige områder relateret til spredningsdynamik, herunder indflydelse af det omgivende miljø, bakterielt load og permanent bærerskab, hvor mere viden er ønskelig. Med hensyn til kontrolforanstaltninger er det primære problem på nuværende tidspunkt mangel på evidens for virkning af andre typer interventioner end sænkning af antibiotikaforbruget.

Denne afhandling har resulteret i:
1) Bekræftelse af allerede kendte risikofaktorer for at besætninger bliver husdyr-MRSA positive (besætningstype, besætningsstørrelse, antal leverandører af svin) og identifikation af et antal nye potentielle risikofaktorer. Dog var mange af disse relateret til besætningsstørrelse, og det var derfor ikke muligt at konkludere, hvorvidt besætningsstørrelsen eller disse faktorer var de egentlige risikofaktorer.
2) En mekanistisk model for spredning af husdyr-MRSA i en svinebesætning. Koden for modellen er offentligt tilgængelig, og besætningsdelen af modellen kan potentielt genbruges sammen med epidemiske modeller for andre sygdomsbakterier.
3) Simulering af interventioner indenfor besætningen: reduceret antibiotikaforbrug, reduceret antal svin pr. sektion, reduceret sammenblanding af svin og forbedret intern smittebeskyttelse, hvoraf kun en sænkning af antibiotikaforbruget havde en markant effekt. Flere typer interventionsscenarier kan simuleres, når data bliver tilgængelige.
4) Iagttagelsen af at når husdyr-MRSA først har etableret sig i besætningen, vil bakterien sprede sig til alle dele af besætningen og være meget svær at komme af med igen.

List of abbreviations

AIC	Akaike Information Criterion
β	Parameter estimate (manuscript I) or transmission rate (manuscript II)
CA-MRSA	Community-acquired methicillin-resistant *Staphylococcus aureus*
CC	Clonal complex
CHR	Central Husbandry Register
DADD	Defined Animal Daily Doses
DVFA	Danish Veterinary and Food Administration
EFSA	European Food Safety Authority
EU	European Union
HA-MRSA	Hospital- or healthcare-associated methicillin-resistant *Staphylococcus aureus*
IS	Intermittent shedder
LA-MRSA	Livestock-associated methicillin-resistant *Staphylococcus aureus*
MLVA	Multiple-Locus Variable-Number Tandem Repeats Analysis
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
OR	Odds ratio
PS	Persistent shedder
QAC	Quaternary ammonium compound
R_0	Basic reproduction ratio (average number of secondary cases from one “infected” case)
VetStat	The Danish Veterinary Medicines Statistics Program

Definitions/terminology

Shedder/carrier

In many papers, the term LA-MRSA carrier is used to describe a pig carrying LA-MRSA in detectable levels. In manuscript II the term LA-MRSA shedder was used for describing a pig that carries LA-MRSA in detectable levels and is assumed to be able to pass on LA-MRSA to other pigs.

Contaminated/Colonised/Infected

There seem to be no clear definitions of the use of these words within this area, but in general 'contaminated' are used when LA-MRSA can be found on the site, but has not necessarily become established. 'Colonised' most often refers to a state, where the organism in question has become established within the host, but not caused clinical disease. The term 'infected' is used, when the human/animal carrying LA-MRSA is exhibiting signs of clinical disease.

Prevalence of LA-MRSA

It can be debated whether it is appropriate to use the term prevalence about LA-MRSA, or if occurrence or presence would be better choices. However, in the present thesis it was decided to use the word prevalence also when it is not referring to disease prevalence.

In general, when citing other papers, the terminology used in the paper in question has been retained, with the exception of ST398 and CC398, where CC398 has been preferred if appropriate.

Table of contents

1. Outline and objectives

This thesis consists of an introduction to livestock-associated methicillin-resistant *Staphylococcus aureus* (LA -MRSA), its spread and occurrence, and an introduction to infectious disease modelling. This is followed by two main chapters; 'Part A - Epidemiology of LA-MRSA in the Danish pig population' and 'Part B - spread and control of LA-MRSA within a pig herd'. Part A includes of one manuscript, while part B includes of two manuscripts. Each part includes a discussion section. The thesis ends with a general discussion of knowledge gaps within the area and a combined discussion of the findings in part A and B, followed by conclusions and perspectives.

'**Part A - Epidemiology of LA-MRSA in the Danish pig population**' is based on an observational study described in manuscript I. The general occurrence of LA-MRSA in the Danish pig population was investigated in two national screenings of pig herds conducted by the Danish Veterinary and Food Administration in 2014 and 2016. The data from these two screenings formed the basis for a risk factor study together with data collected in a questionnaire-based telephone interview survey and data from three national registers. These data included data on movement of pigs (the pig movement database), use of antimicrobials and zinc oxide (the VetStat database), and distance to other farms (the central husbandry register). Some of the information generated in this part of the project was also used in part B as input for parameterisation of the model, or as inspiration for intervention strategies to be tested in the model.

The objectives of part A were to:
1) Investigate herd-level risk factors for farms being classified as LA-MRSA positive (study 1).
2) Investigate herd-level risk factors for farms changing status from LA-MRSA-negative to LA-MRSA-positive during 2014-2016 (study 2).

'Part B - Spread and control of LA-MRSA within a pig herd' is based on two modelling studies described in manuscript II and III. Before the implementation of a national control strategy can be decided upon, it is essential to understand how LA-MRSA spreads and persists within a pig herd, once it is introduced.
For other pathogens, disease spread models have proven to be valuable tools for obtaining a better understanding of disease dynamics, through synthesizing and integrating knowledge from different sources, e.g. literature, experiments and register data. Creating a model for spread of LA-MRSA within a herd could therefore enable us to study the colonization dynamics of LA-MRSA. In addition, such a model would be a useful tool for assessing the short and long term consequences of proposed interventions against LA-MRSA at farm level, and hereby aid decision makers before the implementation of these. The within-herd LA-MRSA prevalence is believed to influence the risk of spread to humans working on the farm, as well as spread to other farms. A better understanding of *within*-herd LA-MRSA dynamics and the effect

of possible interventions is therefore also important in order to study the spread of LA-MRSA *between* herds and assess the effect of possible control strategies in the total Danish pig population.

The objectives of part B of the project were to:

1) Build a stochastic model of spread of LA-MRSA within an integrated pig herd to aid a better understanding of the dynamics of LA-MRSA spread and persistence following different routes of introduction.

2) Use this model for studying the effectiveness of potential strategies for eradication and/or control of LA-MRSA in Danish pig herds.

3) Identify important knowledge gaps in transmission and dispersal of LA-MRSA. This will naturally happen during conceptualisation and parameterisation of the model. The identified knowledge gaps will be discussed in the general discussion section of the thesis.

2. Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA)

2.1 *Staphylococcus aureus*, MRSA and LA-MRSA

Staphylococcus aureus is a human commensal of the skin with main reservoir in the anterior nasal cavity [1,2]. *S. aureus* is also an opportunistic pathogen and is quite resistant to environmental stress, including desiccation, osmotic stress, and limited availability of nutrients, and can therefore survive in a wide range of environments [2,3]. There are many examples of how *S. aureus* has gained resistance against antimicrobial agents to which they are exposed [4]. An example of resistant *S. aureus* is methicillin-resistant *S. aureus* (MRSA), which was first discovered in 1961, only few years after the introduction of methicillin [5].

Today, three main groups of MRSA exist. During the 1970-80s, MRSA was mainly found among hospitalised patients, and therefore this group of MRSA strains have later been referred to as hospital-associated or healthcare-associated MRSA (HA-MRSA) [2,4]. The next wave of MRSA emerged in the mid-1990s, where infections were mainly observed among patients, who had not been hospitalised prior to infection [4]. The strains found among these patients were different from those observed in hospital outbreaks, and this group of strains was named community-acquired MRSA (CA-MRSA) [4]. The third wave of MRSA, which was mainly observed among people with direct or indirect livestock-contact, started in 2005, when the first findings of what is now referred to as livestock-associated MRSA (LA-MRSA) was reported in the Netherlands and France [6,7].

It is believed that LA-MRSA has evolved through a human methicillin-sensitive *S. aureus* clone becoming adapted to pigs, and subsequently acquiring first the *tetM* gene and then the *mecA* gene causing tetracycline- and methicillin-resistance, respectively [8,9].

2.2 LA-MRSA carriage and consequences

2.2.1 LA-MRSA carriage in humans

2.2.1.1 Carrier types and factors influencing carriage in humans

Not all humans exposed to *S. aureus* becomes carriers, but carriage status in those that do become carriers are important, because persistent nasal carriers have an increased risk of *S. aureus* infection, whereas intermittent carriers and non-carriers share the same low risk [10]. The distribution of *S. aureus* carriers in the general human population has been estimated to: persistent carriers (~20%), intermittent carriers (~30%), and non-carriers (~50%) [10–13]. It has however been suggested to no longer distinguish between intermittent carriers and non-carriers, since the *S. aureus* elimination kinetics and antistaphylococcal

antibody profile in these two types of carriers are very similar [10]. Persistent carriers have been known to harbour higher loads of *S. aureus* compared to the two other types of carriers [1,12,14].

Host-related factors are believed to be important determinants for persistent carriage of *S. aureus* [15,16]. However, in a study among twins only a modest effect was found, since the concordance rate for carriage did not differ significantly between pairs of monozygotic twins and same or opposite sex dizygotic twins [17]. A nasal receptor for wall teichoic acid, which is believed to influence colonization with *S. aureus* has been identified [18], but the exact mechanisms involved in colonisation still needs to be described.
It has been suggested that MRSA compete with MSSA strains for colonization of the anterior nares [19], even though, in some studies no association between MSSA and MRSA carriage was found [20,21].

Specifically for LA-MRSA, persistent carriage seem to be strongly related to exposure [22,23], and absence of carriage in periods with no animal contact have led to suggestions of LA-MRSA being a poor persistent colonizer in humans [23]. However, in one study 59% of pig farmers did not clear themselves of LA-MRSA during summer holidays away from the farm [24]. In a study, among 15 farm workers and 45 family members on pig farms in Denmark, Belgium and the Netherlands, the proportion of persistent LA-MRSA carriers was 75-100% [22].

2.2.1.2 Disease

Some of the adhesion and virulence-associated determinants found in other types of MRSA, have not been identified in LA-MRSA and together with low infection rates among carriers, this has led to its pathogenicity in humans being questioned [25–27]. For example, the risk of *S. aureus*-associated toxin syndromes (food poisoning or toxic shock syndrome) is expected to be much lower for LA-MRSA, since the genes involved have rarely been identified in LA-MRSA CC398 [25].

Most LA-MRSA carriers are healthy carriers [28], but in susceptible individuals, LA-MRSA has been implicated in wide range of different conditions (similar to what has been observed for other MRSA types), e.g. skin and soft tissue infections, pneumonia, meningitis, osteomyelitis, endocarditis and bacteraemia [29,30].

In 2016, about 1/3 of all newly registered MRSA carriers in Denmark carried LA-MRSA, but LA-MRSA was only implicated in 16% of all MRSA infections registered this year [28,31]. However, this might be a result of the testing scheme applied when persons with regular contact to pigs or mink come into contact with the hospital system, and can therefore not be taken as indicative of the infection rate among LA-MRSA carriers compared to other types of MRSA carriers. Among 1,981 cases of *S. aureus* bacteremia registered in Denmark in 2016, only seven were caused by LA-MRSA [28]. Most patients with LA-MRSA bloodstream infections had no contact to livestock, albeit most of them lived in rural areas [32]. Hospitalised patients with LA-MRSA infection and contact to livestock are generally younger and healthier than other patients

with MRSA infection, and this difference in demographics might also be part of the explanation for the lower disease burden and less severe complications observed in this patient group [29].

2.2.2 LA-MRSA carriage in pigs

2.2.2.1 Carrier types

Only few longitudinal studies of LA-MRSA carriage in pigs, where carriage types have been reported, exist. In a study of the occurrence of *S. aureus* and LA-MRSA at 20 farms, the 480 tested pigs were classified as non-carriers (23%), intermittent carriers (52%) or persistent carriers (24%) of *S. aureus* based on three samplings per pig at one week intervals [33]. Pigs that persistently carried high loads of *S. aureus* (>10,000 CFU/swab) were identified as "super-carriers", which might be able to play a crucial role in the persistence of the bacteria within a pig herd [33]. The proportion of persistent carriers was highly dependent on farm- and pen-specific factors [33]. This could suggest an influence of the degree of exposure or other environmental factors. As in humans, higher nasal *S. aureus* loads were observed in pigs persistently carrying *S. aureus* (3.6 log CFU; range, 1.9 to 3.9) compared to those only carrying *S. aureus* intermittently (1.4 log CFU; range, 0.3 to 3.3). However, in another study, where a total of 390 pigs on four farms were tested 11 times between farrow and finish, all pigs changed status at least once, i.e. no persistent carriers were identified, which made the authors question the existence of persistent carriers in pigs [34].

2.2.2.2 Host-related factors

In a longitudinal study of colonisation in piglets, it was observed that if a piglet born by an LA-MRSA positive sow remained LA-MRSA negative until weaning, the piglet was at a lower risk of becoming LA-MRSA positive later in life compared to an intermittently colonised piglet from an LA-MRSA negative sow [35]. It has therefore been speculated, that this might be an indication of inherent variation in susceptibility to LA-MRSA [35]. This hypothesis is supported by a study, where a SNP located in a non-coding region was associated with nasal *S. aureus* carriage in pigs and four chemokine genes were identified as candidate genes for *S. aureus* carriage [36]. In addition, when testing nasal samples from pigs from seven different pig lineages, pigs from one specific lineage were less often *S. aureus* positive compared to pigs from other lineages [37].

2.2.2.3 Nasal microbiome

Differential analysis of the nasal microbiome in pigs, identified as carriers or non-carriers of *S. aureus,* has revealed that twenty operational taxonomic units were associated with non-carriers, including species with known probiotic potential and/or antimicrobial effect [38]. Furthermore, an association between nasal carriage of *S. aureus* and other staphylococci in pigs, has been reported in a study, where *S. aureus* rarely was found in the same samples as *S. sciuri*, *S. cohnii*, or *S. saprophyticus* [37].

In two studies, where both the presence of *S. aureus* in general and LA-MRSA in pigs were investigated, co-colonisation with MSSA and LA-MRSA seemed to be possible [33,39].

In another study, it was concluded that farm management can influence the nasal microbiota in pigs, but no association between nasal microbiota and LA-MRSA carriage was found [40].

2.2.2.4 Influence of the pig environment

The environment surrounding pigs shedding LA-MRSA will naturally also become contaminated by LA-MRSA, and it has therefore been suggested that this also plays a role in the spread of LA-MRSA between pigs [41].

In a longitudinal study at four Belgian farms, where pigs were tested 10-11 times between farrow and finish, Verhegghe et al., 2014 [42] found that on the two farms contaminated with low levels only, there were no persistent carriers, and 33% and 17% of the sows were intermittent carriers. On the two highly contaminated farms, 25% of the sows and 47% of the offspring on one farm and 92% of the sows and 37% of the piglets on another farm persistently tested LA-MRSA positive, while the remaining pigs were intermittent carriers. This indicates that the overall contamination level of the farm might influence the fraction of pigs identified as persistent carriers, i.e., it is unknown whether these results reflects "true" persistent carriage or just re-contamination of pigs at the highly contaminated farms.

Results of Multiple-Locus Variable number tandem repeats Analysis (MLVA) revealed that during their life, most pigs were colonized with LA-MRSA belonging to several different MLVA types [42]. On the highly contaminated farms, piglets and sows were often colonized by LA-MRSA belonging to the same cluster; however, all piglets within the same litter did not necessarily carry the same type [42]. This could be an indication of transmission from the environment or from pigs in neighbouring pens. However, on the two less contaminated farms, sows did also not always carry the type most prevalent in their offspring [42].

In a comparison of nasal *S. aureus* carriage in seven different pig lineages, the farm environment seemed to influence the presence of *S. aureus*, since results for the lineages differed between farms [37]. In addition to the possible influence of the overall contamination level of the farm, the risk of LA-MRSA carriage in individuals pigs might also be influenced considerably by differences in management, antimicrobial consumption, stocking density and other local factors [43,44].

2.2.2.5 Other factors

It is unknown, whether dose of exposure influences the duration of LA-MRSA carriage in pigs. However, upon reporting the results of two experimental trials involving inoculation of pigs with LA-MRSA, the authors of one study [45] concluded that the minimum inoculation dose needed for persistent colonization of animals seem to be not less than 10^8 CFU per animal. Similarly, when determining the LA-MRSA dose needed for inoculation of pigs housed in experimental facilities, Szabó et al. [46] found that use of doses ranging from 10^2-10^7 CFU per animal did only result in short-term colonization.

2.2.2.6 Disease in pigs

LA-MRSA has rarely been isolated from diseased pigs, but have been implicated in exudative epidermitis, cutaneous abscesses, septicaemia, mastitis and infections of the urogenital tract and uterus [47,48]. In an investigation of bacteria from 138 lesions at pigs at necropsy, LA-MRSA were isolated as the sole bacteria in 35% of the lesions and therefore the authors suggested that the causative role of LA-MRSA in lesions needs further investigation [49].

2.2.3 Societal impact

2.2.3.1 The MRSA problem in Denmark

The MRSA expert group in Denmark consider LA-MRSA a health- and resource-problem for [50]:

- Individuals, who are immunocompromised or suffer from underlying diseases and need to undergo surgery.
- LA-MRSA carriers including their families, who need to deal with the carriership – especially persons working in pig herds and their families.
- The health system; an increasing amount of resources is needed to prevent spread of LA-MRSA, and to treat patients with LA-MRSA.

The expert group concludes that for the general population LA-MRSA only constitutes a small health threat [50].

2.2.3.2 Stigmatization of MRSA carriers and persons with contact to LA-MRSA positive herds

Stigmatization of MRSA carriers have been reported both in Denmark and other countries [51–54]. Reported problems include: erosion or termination of personal or business relationships; discrimination; bullying; rejections of treatment or access to waiting rooms at health clinics; poor mental health; social withdrawal; feelings of guilt, shame, fear and isolation [51–54].

Specifically regarding LA-MRSA, there has been Danish case stories of farmers' spouses being bullied or avoided at their work place, children of farmers being met with suspicion in day care facilities, and former employees in LA-MRSA positive herds having problems finding a new job [55]. Currently, it is not known to what extent LA-MRSA carriers or farm workers in Denmark experience stigmatization as a consequence of their LA-MRSA status or job.

2.2.3.3 Economic impact in the health care system

Only few assessments of the economic impact of LA-MRSA for the society exist. The yearly cost of LA-MRSA in the Danish health care system was in 2015 estimated to 43 million DKK (~5.77 million €) [31]. The majority of these costs (75%) are used for prevention of spread of LA-MRSA [31]. Since then, additional requirements for isolation of patients and test of persons with regular contact to mink, have added an estimated 5 million DKK to the estimated yearly costs, resulting in a 2017 estimate of 48 million DDK (~6.45 million €) [31]. Similar results were obtained in Norway, relative to the size of the production [31,56].

In a Swedish cost-benefit evaluation of preventing introduction of LA-MRSA in the Swedish pig population, it was estimated that if introduction of LA-MRSA resulted in the same human prevalence in the risk groups as in Denmark or the Netherlands, the costs in the health care system would amount to 0.87-1.23 million € (2011 prices) [57]. In 2016, the number of pigs slaughtered in Sweden amounted to 2.61 million, and the pig production in Sweden is hence markedly smaller than the Danish production (18.22 million pigs slaughtered and 13.51 million live pigs exported in 2016) [58].

Estimates for cost of eradication of LA-MRSA in the pig population are mentioned in the section "3.4 Prevention and interventions".

Based on the costs for the healthcare system we can conclude that LA-MRSA is a costly problem for the society. Therefore spread of LA-MRSA needs to be limited as much as possible, perhaps best in the primary source, which is the pig farm.

3. Spread and occurrence of LA-MRSA

3.1 Occurrence of LA-MRSA

3.1.1 Occurrence of LA-MRSA in animals

3.1.1.1 MRSA in animals worldwide

Since findings of what is now known as LA-MRSA was reported for the first time in the Netherlands and France in 2005 [6,7], LA-MRSA has been detected in many countries in Europe [4] and Asia [59], in addition to Australia [60], Canada [61] and the United states [62].

In Europe, a baseline study was conducted in 2008 with the aim to investigate the MRSA prevalence at breeding and production farms in the EU. In this study, LA-MRSA was found in either breeding or production herds in 19 of the 27 participating countries, and since then, LA-MRSA has been detected in at least seven other countries in Europe [63–70]. Afterwards, the method used in the baseline study, analysis of five pooled dust swab samples of 500 cm^2 each, has been criticised for low sensitivity, limiting the ability to detect MRSA to herds with high within-herd prevalence [71,72]. Thus, the occurrence might have been underestimated. More recent comparisons of the prevalence of positive farms within the different countries are hampered by a lack of data and the use of different methods for sampling. However, high prevalence in the pig population has been reported in both southern and central Europe [73,74], while LA-MRSA has only been sporadically detected in Norway and Sweden [57,75]. The dominant clonal complex (CC) in Europe and Denmark is CC398 [4], albeit other clones also occur, e.g. has CC30 and CC1 been found on Danish pig farms [76].

In Asia, high prevalence of positive farms (>30%) has been reported in Taiwan, China, Hong Kong and Malaysia [59]. The dominant sequence type in Asia is CC9, however findings of CC398 have also been reported in South Korea [59]. In the United States, other *S. aureus* clones of human origin are more commonly found in pigs, and in some studies CC5 has been found to be the dominant livestock-associated type [77]. In Australia, only pigs on one farm have been investigated, and here CC398 and community-adapted CC93 were the dominant clones [60]. Reports from other parts of the world include findings of LA-MRSA in pigs in Peru [78], Brazil [79] and Senegal [80].

Pigs are believed to be the main reservoir for LA-MRSA, even though a high prevalence of positive veal calf farms have also been found in the Netherlands (2010: 88%) [81] and Belgium (2015: 79%) [73]. In addition, findings of LA-MRSA have been reported in a wide range of other animals, including mink, horses, broilers, turkeys, rabbits, pets and rodents [4,82–87].

3.1.1.2 MRSA in the Danish pig population

In 2007, LA-MRSA was retrospectively detected in the Danish pig population, when ten out of hundred isolates collected at three farms in 2005 were re-examined [88,89], due the first reports about findings of

LA-MRSA in the Netherlands and France [6,7]. Since then, the Danish pig population has been screened for MRSA five times. In 2008, 198 production herds and 95 breeding herds were tested as part of the EU baseline survey, and MRSA was detected in 3.5% of the production holdings, but in none of the breeding holdings [74]. As mentioned above, the method used in the baseline study has been criticised for low sensitivity [71,72]. Therefore, the results obtained in this survey are not comparable with results of the later screenings, where pigs were tested using five times five pooled nasal swabs. In 2010 and 2011, MRSA was found in 16% of 99 and 79 tested production herds, respectively [90,91]. In 2014, the prevalence had increased dramatically to 68% and 66% respectively, among the tested 205 production herds and 70 breeding herds. In 2016, 88% of the randomly selected production herds tested positive [28]. Some of the herds tested in 2014 were re-tested in 2016. The 58 re-tested herds, which tested positive in 2014, all remained positive in 2016, whereas 62% of the 53 herds, which had been tested negative in 2014, were now tested MRSA positive [28]. In 2014, results showed some regional variation in the LA-MRSA prevalence (Jutland (70%) , Funen (69%) and Zealand (53%)) [28], and in 2016 the prevalence was 59% among the 27 tested herds in Southeast Zealand, whereas on the island of Bornholm, 62% of the 26 tested herds were found LA-MRSA positive [28].

As observed in other countries [92], the prevalence in the organic pig population in Denmark is considerably lower than in the general pig population. In 2015, LA-MRSA was detected in 6% of 65 randomly selected organic herds [93].

The occurrence of LA-MRSA in Danish pigs at slaughter was investigated in 2009, where 13% of the 789 pigs were found MRSA positive [94], and again in 2011, where LA-MRSA was detected in 44% of swabs from 777 pigs [91], and finally in 2012, where the prevalence was estimated to 77% [95]. However, these results does not necessarily reflect the prevalence among animals within or between farms, as studies from the Netherlands have indicated that LA-MRSA is able to spread during transportation to slaughter and at lairage in the slaughterhouse [96].

3.1.1.3 MRSA in other animals and food in Denmark

The presence of MRSA in the Danish cattle population has been investigated several times. The prevalence in 236 bulk milk samples collected in 2016 was 3%, which is slightly higher than the results of similar investigations conducted in 2014 and 2012 (both 2%) [28].

In 2015, there were two different on farm investigations on MRSA in veal calves. In one investigation, MRSA was detected on 5 of the 50 tested farms (10%), whereas in the other one, MRSA was detected on 2 of 16 farms (12.5%). Interestingly, when the farms in the latter investigation were revisited two weeks later, no animals tested positive [93].

Veal calves have been tested at slaughter in 2010 (192 animals), 2011 (179 animals) and 2015 (93 animals) (using skin swabs in 2010-2011, and an unknown method in 2015), but MRSA was not detected in any of the tested animals [90,91,93].

In an investigation of the diversity of *S. aureus* in small ruminants at slaughter, LA-MRSA CC398 was detected in a sheep, but it was deemed likely that the isolate might originate from cross-contamination at the slaughterhouse, which also slaughtered pigs [97].

In 2015, LA-MRSA was not detected in any of the horses tested at slaughter (N=56) [93], but in an investigation at 74 farms the same year, 17 of 401 horses (4%) from seven of 74 farms (9%) tested MRSA positive [98]. Fourteen of the isolates were CC398, and four of these were closely related to isolates from pigs, while the rest belonged to a horse-adapted sub-lineage [28,98].

Pig offal is used for mink feed, and consequently there is a risk of spread of LA-MRSA from the pig population to mink. In an investigation in 2015, LA-MRSA was detected in healthy animals on 20 of the 50 tested mink farms (40%) and in 20 out of 108 samples from 14 mink feed producers (9%) [87]. During 2015, LA-MRSA was found in 20 of 58 mink submitted for necropsy (34%) [87].

In 2015, LA-MRSA was found in turkeys on one out of 54 investigated poultry farms (2%) (a mixture of conventional and organic chicken farms, as well as turkey farms were tested) [93]. In 2010, MRSA was not detected, when 197 broilers from the same farm were tested at slaughter [90].

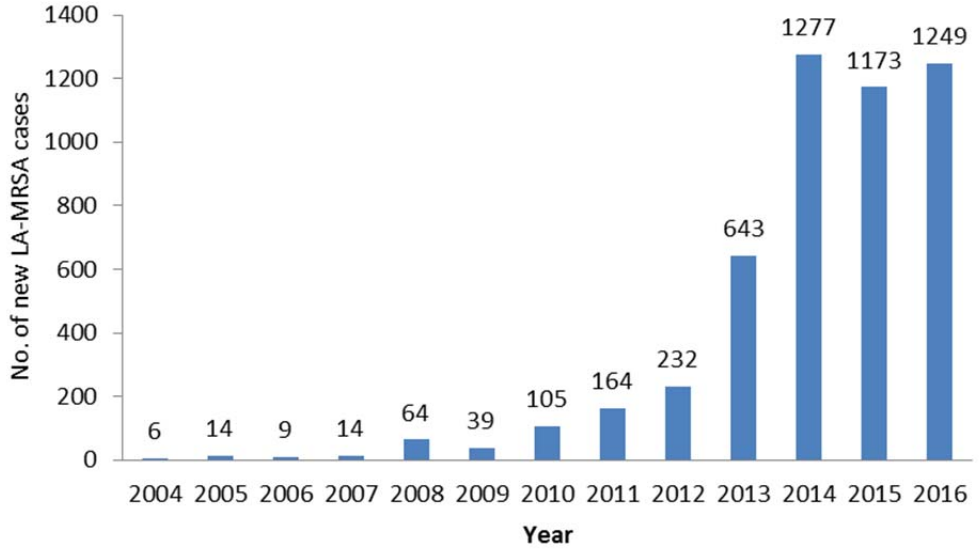
Companion animals most often carry human MRSA strains [99]. In 2015, LA-MRSA was only isolated from one out of 114 dogs tested at veterinary clinics in three different areas of Denmark [93]. The dog was from an area with high density of pig farms. LA-MRSA have also been isolated from flies caught at Danish pig farms [100].

There has been several surveys of the occurrence of MRSA in Danish and imported pork, beef and broiler meat [91]. In 2016, LA-MRSA was found in 48% of 78 samples of Danish pork, compared to 5% in 2009, 6% in 2010 and 10% in 2011 [28]. LA-MRSA clones, that have not been found in the Danish animal reservoir, have been found simultaneously in poultry meat at retail and in humans living in urban areas in Denmark, which indicates that transmission of MRSA from meat to humans might have taken place [101]. However, foodborne transmission or transmission during handling of meat in households are not believed to be major sources of transmission of LA-MRSA to humans, since most LA-MRSA cases live in rural areas, and hence not many cases are observed in areas with high population density, which one would have expected in case of transmission from meat being a major transmission route [102].

3.1.2 Occurrence of LA-MRSA in humans

The voluntary surveillance of MRSA in humans in Denmark started in 1988, and since 2006 MRSA has been notifiable [103]. The first human LA-MRSA cases in Denmark were detected in 2004 [103]. Since then, the number of cases has been increasing considerably with 1,214 new cases in 2017 (preliminary number per 25-01-2018 Statens Serum Institut, 2018) (Fig 1). Registered cases include people with clinical infection as well as healthy carriers. A change in the Danish Health Authorities rules in 2012, made screening of persons with regular contact to pigs mandatory in case of hospitalisation [103], which caused more people of high risk of being carriers to be tested.

Fig. 1. Number of newly registered LA-MRSA cases in Denmark, 2004-2016

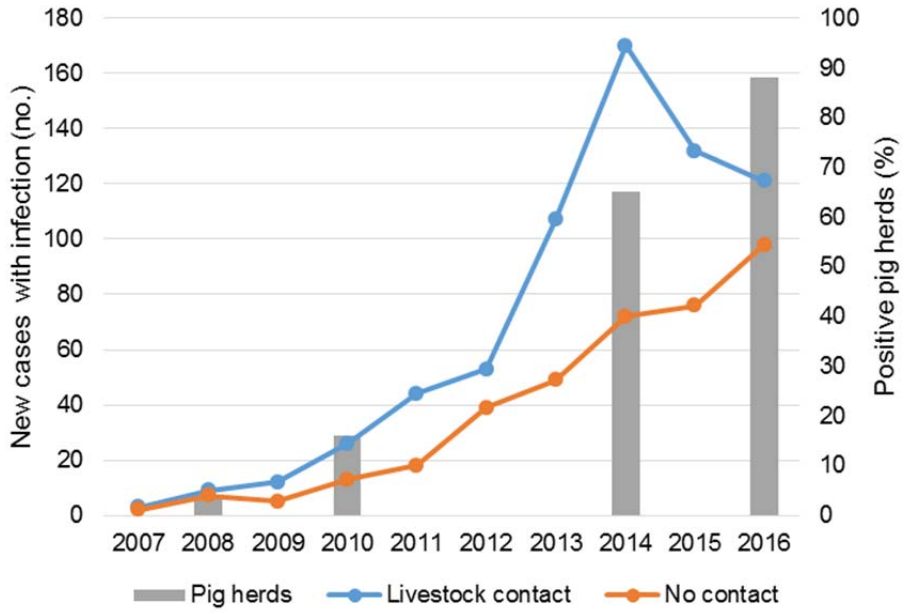


Note: Cases are only registered, when they are tested positive for the first time. New guidelines for sampling were introduced in 2012, leading to more healthy individuals with livestock contact being tested.
Data sources: [28,91,95,103,105–107].

According to the current rules, a person will be swabbed upon admission to a hospital, if the person or a household member have had weekly or more frequent contact with live pigs or worked on mink farms within the past 6 months [108]. Healthcare staff employed in Denmark must be tested every 6 months, if they work in a pig herd on a weekly or more frequent basis, or if a member of his/her household has been tested MRSA-positive [108].

In Fig 2, the development in the number of humans with LA-MRSA infection (i.e. does not include healthy carriers) with and without livestock contact are compared with the development in the prevalence of MRSA positive pig farms over the years. The number of infections in persons without livestock contact followed the increasing prevalence of positive pig herds, whereas the number of newly registered infections in persons with livestock contact saw a decline, most likely because these might already have been registered at an earlier point in time [28].

Fig 2. Human infections among people with and without livestock contact and prevalence of positive pig farms in Denmark, 2007-2016



Source: Figure adopted from Danmap 2016 [28].

In general, the occurrence of human MRSA infections in Denmark is low, compared to most other European countries (<1% of all *S.aureus* bacteraemia cases are caused by MRSA) [109,110]. This has been attributed to the implementation of an efficient search-and-destroy policy in hospitals [110].

3.2 Transmission of LA-MRSA

This section will focus on transmission of LA-MRSA between pigs within farms, in addition to transmission to and between humans. Transmission within farms is relevant in relation to risk of staff becoming LA-MRSA carriers, but also in relation to spread between farms, since results of modelling studies have indicated that increased within-farm prevalence increases the risk of spread to other farms, and thereby influences the prevalence of positive farms [103, J. Shulz personal communication].

3.2.1 Pig-to-pig transmission

In general, the occurrence of LA-MRSA among pigs tends to differ between age groups. In many studies, the highest prevalence of LA-MRSA carriage has been found among piglets or weaners, followed by a decline in prevalence towards slaughter age, albeit the results varies somewhat [35,41,43,61,112–118]. It has been suggested that this age dependency could be related to increased susceptibility to colonization in piglets, because of a poorly developed endogenous microflora [35]. This will then be followed by stress at weaning, in addition to possible mixing of MRSA positive and negative pigs, contamination through human handling during transfer, or transfer to contaminated weaning pens [35]. The prevalence in sows generally seems to be lower than in the other age groups [35,116]. It has however been suggested that this might be influenced by the sampling methodology, as the amount of sample material obtained in a nasal swab might

be influenced by the size of the nasal openings [35]. Furthermore, it might be more difficult to swab sows, compared to younger and more manageable pigs [35].

Several LA-MRSA transmission studies with different aims have been conducted. Some of these took place in animal experimental facilities, where animals were inoculated with a known, often quite high, dose of LA-MRSA, whereas others took place on naturally contaminated farms. The later was especially true for studies of transmission from sows and to offspring. An overview of studies of transmission from sow to offspring is given in Appendix I, table 1, followed by an overview of transmission studies among piglets and weaners in table 2 and table 3 in Appendix I.

It has been demonstrated that perinatal transmission is possible [119], and in a study were sows were classified based on the results of both nasal and vaginal swabs, the odds of piglets being MRSA positive were 12 times higher, when born from a nasally positive sow, compared to a negative sow. The odds were further 3 times higher, if born by a both vaginally and nasally positive sow, compared to one that was only nasally positive.

Significantly higher transmission between both pre-weaning and post-weaning pigs have been observed when tetracyclines and beta-lactam antibiotics are used, compared to when there was no use of these types of antimicrobials [112]. Additionally, in a colonisation study carried out in experimental facilities, higher nasal load was observed, when pigs were fed tetracycline, but in this study it did not seem to influence the transmission between animals housed within the same pen [120].

Currently, the dose of LA-MRSA a pig need to receive in order to become an LA-MRSA shedder is not known, but transmission between pigs have occurred following low dose nasal inoculation ($2*10^4$ CFU/animal) in weaners [45] (Appendix I, table 3).

The reported basic reproduction ratios (R_0) for LA-MRSA in pigs varies greatly, depending on the study and age group of the pigs used, and the reported R_0 values range from <1 to 52.5 [41,112,121].

In two studies, the observed duration of MRSA carriage varied considerably between pigs. In the first study conducted in 6-week old weaners in animal experimental facilities, it varied from 1-39 days, with a median of 7.5 or 18 days depending on the calculation method used [121] (Appendix I, table 3). In the second study, a median duration of 2, 13 or 15 days were observed in three parallel groups of 3-week old piglets [41] (Appendix I, table 2).

3.2.2 Transmission between animals and humans

There are many indications of transmission of LA-MRSA from pigs and to humans, in the form of findings of the same types or clones in pig herds and humans working in these herds, in addition to a higher risk of farmers testing positive for LA-MRSA [4,6,7]. LA-MRSA carriage in farmers have been linked to the

frequency and duration of contact to livestock [23,122,123]. Several studies have shown that most short term visitors on farms only transiently carry LA-MRSA, and that only a minority (12%, 6% and 0% in the three studies listed), are still positive after 24 hours [124–126].

In a systematic review of literature on LA-MRSA carriage among farmers, veterinarians and slaughterhouse workers, the prevalence of LA-MRSA carriers ranged from 0-85% in the 33 reviewed studies [127]. However, the study populations varied from veterinarians, not necessarily working with livestock, to farmers with MRSA positive herds. In a study on 47 MRSA positive pig farms, 86% of the farmworkers, 4% of these farmworkers' family members, 45% of the veterinarians taking care of the herds on these farms, and 9% of these veterinarians' family members tested positive for LA-MRSA [128].

An association between nasal carriage in farmers and LA-MRSA levels in the air on their farms has been demonstrated [123]. Furthermore, among short-term visitors in pig barns who did or did not interact directly with the pigs, LA-MRSA carriage was associated with personal exposure to LA-MRSA in the air [124]. However, those who had interacted with the pigs both had higher personal airborne LA-MRSA exposure and more often carried LA-MRSA [124]. Thus, it was suggested that the increased carriage in those interacting with the pigs might primarily be a result of the increased LA-MRSA concentration in the air, rather than the actual contact to the pigs [124].

There are not many reports of transmission between humans and pigs, but it is assumed that LA-MRSA has been introduced by humans on three sow farms in Norway [75].

3.2.3 Human-to-human transmission

Household members in farmer families are at increased risk of becoming MRSA carriers [23,123,128–130]. For those living on a farm, some of the family members might also enter the stables, while others might not and thus it is not always clear whether these have acquired LA-MRSA through animal contact or through contact to other household members with direct animal contact. Among veterinarians, who carried MRSA, but did not live on farms with livestock, the frequency of LA-MRSA transfer to non-livestock-exposed family members has been estimated to 9% [128].

In Denmark, the number of new LA-MRSA cases with infection and no livestock contact has been increasing since 2007, and reached 121 cases in 2016 [28]. In both Denmark and the Netherlands, it has been observed that the majority of LA-MRSA cases with no livestock contact live in rural areas [131–133]. However, in a study within three Danish municipalities in areas with high pig density, patients with LA-MRSA infection did not live closer to pig farms than the population controls [133]. This could indicate that direct environmental spread from neighbouring farms is maybe less likely, than general community spread in rural areas [133]. Compared to the population with frequent livestock-contact, a greater part of the

population without livestock-contact might be immunocompromised, and thus more susceptible to LA-MRSA infections. Thus spread of LA-MRSA into the general community is a cause of concern [28].

In general, the risk of human-to-human transmission of LA-MRSA is assumed to be lower than for other MRSA types, since the transmissibility of LA-MRSA within hospitals have been estimated to be 4.4 times lower than for non-livestock associated MRSA [134]. However, LA-MRSA has been able to cause outbreaks in two hospitals and a nursing home in the Netherlands [135,136].

3.2.4 Transmission through the environment

The role of the environment in transmission of LA-MRSA is still somewhat unclear. Findings of LA-MRSA in dust have frequently been reported [115,137–139], and the half-life of LA-MRSA in dust has been estimated to 5 days in a study, where it was still possible to cultivate MRSA in the highly contaminated samples 30 days after sampling [137]. LA-MRSA is also able to form robust biofilms, of similar strength to those formed by non-livestock MRSA [140], and therefore it is also expected to be able to survive on surfaces for an extended amounts of time [3]. In addition, faecal shedding of LA-MRSA from pigs has been reported [46], as well as findings in manure, where it is able to survive for at least 16 days at temperatures likely to prevail during normal manure storage in Denmark (15˚C) [141]. Manure has been suggested as a source of human MRSA infections in the United States [142].

In a transmission study on CC5 in pigs, the overall carriage rates in MRSA negative pigs exposed to a naturally MRSA contaminated environment or to inoculated carrier pigs where very similar; 0.11 and 0.09, respectively [143]. However, if only looking at the first five days of exposure, the carriage rate was significantly higher in the group exposed to the contaminated environment [143]. The authors of the study noted that the number of pigs used might be too low to draw a definitive conclusion regarding the influence of the environment [143].

3.3 Risk factors for occurrence of LA-MRSA in pig herds

In theory, LA-MRSA can emerge within herds as a result of horizontal gene transfer of the *SSCmec* cassette and the *mecA* gene from other coagulase-negative staphylococci and to MSSA [144]. However, this is assumed to be a rare event, and therefore in most cases, LA-MRSA is assumed to be introduced in the herd from an external source.

3.3.1 Purchase of pigs

The influence of trade has been investigated in several studies. Findings of identical types or clones on supplier and recipient farms has been demonstrated [75,145,146], and in one study 79% of herds with an LA-MRSA positive supplier of pigs were LA-MRSA positive, whereas only 23% of herds with an LA-MRSA negative supplier tested positive [147]. Buying pigs from more than two suppliers have also been associated with being LA-MRSA positive [148]. However, two network modelling studies on pigs trading in

Denmark with focus on LA-MRSA have been conducted, and in both studies it was concluded that trading alone are not able to explain the rapid spread of LA-MRSA in the Danish pig population [54, personal communication: J. Schulz]. In a Norwegian outbreak investigation, 32 of 51 farms, which had received pigs from LA-MRSA positive farms remained negative, so purchase of pigs from a farm with MRSA positive animals might not always result in the farm becoming contaminated [75]. This was attributed to the fact, that some of the farms had only received few pigs, or had quickly changed supplier after having washed and disinfected the premises. In other cases LA-MRSA had only recently been introduced on the supplier farms, and thus the pigs delivered might not have been LA-MRSA positive [75].

On country level, the number of imported pigs has been identified as a risk factor for having LA-MRSA positive pig farms [149], but this is of limited relevance for the Danish pig population, since the Danish import of pigs is negligible (13 pigs in 2016) [58], and LA-MRSA already is widespread in the country.

3.3.2 Use of antimicrobials and zinc

LA-MRSA isolates harbours *mecA* or *mecC,* which is conferring resistance to methicillin and other beta-lactam antibiotics, e.g. penicillins, cephalosporins and carbapanemes [150]. In addition to this, the vast majority of LA-MRSA has also acquired resistance to tetracyclines [103], which is the most used antimicrobial class in the Danish pig production [28]. However, *Staphylococcus aureus* is known to easily gain resistance towards substances it is exposed to [4], and resistance towards a wide range of other types of antimicrobials has also been identified in LA-MRSA, e.g.: aminoglycosides, fluoroquinolones, lincosamides, macrolides, phenicols and streptogrammins [103,151,152].

Use of antimicrobials LA-MRSA is resistant to, might lead to co-selection and therefore increase the chance of the clone becoming established within the herd [151]. However, the results obtained in on-farm studies are somewhat mixed. Using group-treatment of pigs have been identified as a risk factor for farms testing MRSA positive [44,146,148], but in some studies no significant effect of use of group-medication was detected [147,153]. The influence of antimicrobial usage is supported by the results of transmission studies [112,120] and intervention studies [43,154] (the main results of these studies are summarised in section 3.2.1 and 3.4.2.2).

In Denmark, as in many other European countries, zinc is frequently used for treatment of weaning diarrhoea. Weaners may get prescribed zinc supplementation in levels of up to 2500 mg/kg feed during the first two weeks after weaning [155], and the total consumption of medical zinc amounted to more than 400 tonnes in 2015 [156]. This practise might influence nasal carriage of MRSA, since there seems to be a genetic linkage between *mecA* and *czrC* coding for zinc resistance [157–159]. However, LA-MRSA clones carrying a different *SCCmec* cassette (type IV) does not harbour this gene, and thus not all isolates are resistant to zinc [158].

Still, Moodley et al [120] observed increased nasal carriage after feeding pigs zinc supplemented feed, and in another study a dose-response relationship between nasal MRSA carriage in pigs and zinc supplementation have been demonstrated [160]. In an additional study, in-feed concentrations of zinc were associated with MRSA status of the pigs [161], and in a randomised controlled trial, use of zinc in high concentrations (3000 mg/kg) has increased the prevalence and persistence of MRSA carriage in weaners [162].

Following an EU decision, the use of zinc in therapeutic concentrations will be phased out towards 2022 [163].

3.3.3 Herd size and herd type

In several studies, herd type and herd size have been identified as a farm-level risk factor for being MRSA positive [44,148,153,164]. It has been suggested that the lower risk of farrow-to-finish herds testing positive compared to weaner-to-finisher or grower-to-finisher herds, is caused by herds including sows having less or no purchase of pigs [164]. Organic herds seem to have reduced risk of being MRSA positive [28,92].

Large herd size has been identified as a risk factor in relation to many diseases in pig herds [165]. In a large herd, there will usually be a higher turnover of pigs, more external contacts and more susceptible individuals [165]. Management practices in these herds might differ from those in smaller herds, and antimicrobial use, purchase of gilts and hygiene measures have been found to be associated with herd size [153].

3.3.4 Management factors

Maybe slightly surprising, having all-in/all-out production has been associated with LA-MRSA positive status [148], along with having partially or totally slatted floors [44]. Disinfection of the nursery pens before every new arrival have also been associated with positive LA-MRSA status (OR=14.1) [161]. It was however suggested that this could be caused by some LA-MRSA isolates carrying the *qacG* gene, which makes them resistant to quaternary ammonium compounds [161,166,167]. In relation to the proportion of pigs testing LA-MRSA positive within a farm, several factors related to handling of piglets (tooth clipping and vaccination) seem to be associated with increased carriage rate [43].

3.4 Prevention and interventions

3.4.1 Initiatives against LA-MRSA in Denmark

The first official plan for measures against MRSA in Denmark was initiated in 2014 [168]. Before, several screenings of LA-MRSA in animals and meat had been conducted, and plans for reducing the antimicrobial consumption had been in place on national and EU level for some years [103]. Only the most recent and most LA-MRSA relevant of these will be mentioned here.

In 2010, the “Yellow card” initiative for pigs and cattle was launched [169]. This initiative, which was not specifically driven by MRSA, targeted farmers with high antimicrobial consumption per animal. The idea is that farmers, whose antimicrobial use in an age group exceed a certain threshold (originally set to twice the average consumption and updated yearly), get a so-called “yellow card”. This implies that they will have 9 months to get their consumption below the threshold, or else they will be sanctioned in different ways [170]. In 2016, the yellow card was updated to include weighing of the different antimicrobial classes, with the aim of reducing the use of antimicrobials of human importance, as well as those believed to constitute a high risk in relation to the development of resistance [171]. Since December 31, 2017, 3rd and 4th generation cephalosporins, quinolones and fluoroquinolones have been weighted by a factor 10, and tetracyclines by a factor 1.5, whereas the weighting for all other antimicrobial classes are 1 [171].

In 2010, the pig industry also imposed a voluntary ban on the use of 3rd and 4th generation cephalosporins [103]. Use of this type of antimicrobials has been associated with substantially higher LA-MRSA carriage rate among the pigs [43]. Additionally, a national target of 10% reduction in antimicrobial use for farm animals during 2010-2013 was set and followed by differentiated tax on veterinary antimicrobials in 2013 [103].

In 2014, a five-point plan against LA-MRSA was launched in Denmark [168]. It included the following points:

- Individuals who work with pigs must change their clothes and wash their hands when leaving the pig stables.
- The farmer and his/her veterinarian must draw up an infection protection plan that includes initiatives to reduce infection within the herd and the risk of bacteria being carried out of the herd.
- Routine group medication of pig herds should be discontinued. Such a treatment should only be initiated after a veterinarian has examined the animals and diagnostic samples have been sent for analysis.
- An advisory service for pig workers and health workers should be established.
- It should be investigated, whether the use of antibiotics can be regulated, and whether the incentive for using vaccination as an alternative can be increased.

In 2015, this was followed by a national action plan against LA-MRSA [50]**.** This 4-year plan was based on the recommendations from an expert group set down in 2014. The main points were:

- 15 pct. reduction of the use of antimicrobials for pigs during 2015-2018.
- Hygiene measurements focusing on prevention of spread of LA-MRSA into the community and on the working environment in the stables.
- Reduction of contamination in the herds.
- Surveillance of the development in the prevalence of LA-MRSA over time.
- Investigation of the routes of transmission for LA-MRSA.

- International effort, including a continued pressure to promote a joint EU strategy to reduce antibiotic resistance [168].

Since January 1, 2018 a hygiene course have been made mandatory for everybody, who is professionally handling live pigs [172].

3.4.2 Intervention strategies

In this paragraph, interventions on farm level will be discussed. Technologies which are intended for reducing the within-farm level of MRSA contamination and have only been tested *in vitro*, are also mentioned, since the number of on-farm studies are fairly limited.

3.4.2.1 Eradication of MRSA

In Norway, where LA-MRSA has only been detected sporadically, all pigs from MRSA positive herds are sent for slaughter, followed by thoroughly cleaning and disinfection of the farm [75]. In the Danish pig population, where LA-MRSA is endemic, use of the same strategy would give rise to serious ethical and economic considerations. Preliminary results of a cost of eradication model, indicates that the cost of eradication of LA-MRSA in the Danish pig population would be extremely high (1,837 million € during 15 years) [173]. Thus it is important to explore alternative interventions to limit the spread, which was part of the main purpose of the present PhD project.

3.4.2.2 Changes in the antimicrobial usage pattern

In an 18-month long intervention study on 36 Dutch pig farms, tailor-made interventions were initiated at each farm with focus on further reducing antimicrobial use, improving personnel and farm hygiene, and changing animal contact structures [43]. During the study, the antimicrobial use decreased by 44% and was associated with declining LA-MRSA prevalence in the pigs and in the humans working on the farms. LA-MRSA carriage in pigs was substantially higher at farms using cephalosporins, and the odds for pigs testing LA-MRSA positive were higher if the proportion of group treatments exceeded 0.5 [43].

An association between antimicrobial usage and LA-MRSA carriage in calves has been demonstrated both in a cross-sectional study [132], and later in an intervention study [154].

3.4.2.3 Use of disinfectants

The effect of disinfection procedures on the occurrence of LA-MRSA on pig farms has been investigated in several different studies. In general, it has been proved possible to remove MRSA entirely through disinfection in the absence of animals [75,174]. However, in one study the presence of MRSA was associated with frequent disinfection of nursery pens, and at least one gene associated with resistance against quaternary ammonium compounds (QAC) was detected in all tested isolates [161]. Additionally, seven isolates identified in the same study (17.5% of all isolates) were tolerant to benzalkonium chloride,

and might therefore potentially be able to survive exposure to QAC-based disinfectants in the presence of organic matter [161].

In six Italian pig herds, cleaning and disinfection in the absence of pigs decreased the proportion of positive environmental samples from 50% to 19%, but the effect varied depending on the production phase, with the strongest effect in the farrowing unit [115]. In another investigation, use of disinfectants followed by vacancy periods of 10 days in nursery units did not significantly decrease the prevalence of LA-MRSA after the vacancy period [175].

In a study on use of disinfectant powder in the presence of the pigs, repeated application of the powder under simulated farm condition led to reduced MRSA levels in air and bedding materials. However, the pigs remained positive with variable MRSA loads, which increased, when the application of disinfectant treatments was discontinued [176].

Washing sows in a 3-step procedure on four MRSA-positive Belgian pig farms had no significant effect on MRSA status of the sows' skin or nares, and in 64% of cases the same strain was detected as before washing [177].

3.4.2.4 Other methods

Several other methods have been tested either *in vitro* or in pilot scale, including use of an air washer in combination with a UV-irradiation system on a farm. This gave a relative reduction of 90-99% in the air concentrations of LA-MRSA and airborne bacteria in general, when both parts of the systems were used [178]. However, the authors reported some technical problems related to water consumption and deposition of particles on the UV-irradiators, which needed solving, along with long term testing of the system [178].

Use of competitive exclusion (in this case a mixture of *Bacillus* spp. spores, that should be able to antagonise growth of other bacteria) was not efficient against LA-MRSA, compared to traditional cleaning and disinfection [179]. Another attempt at using biological prevention was the use of bacteriophages against LA-MRSA [180]. A mixture of two phages efficiently killed LA-MRSA *in vitro*, but a reduction was not observed when tested *in vivo* [180]. The authors suggested that this might be caused by different physiological conditions *in vivo*.

A study of the porcine nasal microbiome aimed to determine whether the microbiome of pigs carrying *S. aureus* differs from that of non-carrier pigs. Species with known probiotic potential and antimicrobial effect were identified in the non-carriers, including lactic acid-producing *Leuconostoc spp*. and members of the *Lachnospiraceae* family, which is known for butyrate production [38] and might be of interest in relation to development of new intervention strategies. Already in 2010, it was demonstrated that strains of

Lactobacillus acidophilus and *Lactobacillus casei* are able to strongly inhibit growth of some non-livestock associated MRSA isolates, when tested *in vitro* [181].

4. Infectious disease modelling

4.1 Use of models for decision making

In general, a biological simulation model is an attempt at representing a biological process, by using a computer program for creating a simplified representation of reality. Since models are just approximations of what is going on, predictions will always be subject to uncertainty due to biological variation, the occurrence of unexpected events, and factors related to construction of the model, such as quality of the data available for parameterisation, as well as the assumptions and simplifications made.

Models can be used, when real-life experiments are not ethically, economically or practically feasible. Building models is relatively cheap, and as soon as the model is validated, further changes to examine alternative actions can be incorporated.

Within medical, veterinary and environmental science, mathematical models are used for understanding epidemiological patterns and dynamics, examining alternative control actions, and identifying knowledge gaps to direct further research [182]. During conceptualisation and parameterisation of a model, one will be forced to formulate assumptions and hypotheses, and assess the information already available about the system to be modelled, and thereby get a clear overview of what is already known. Many models have been developed for predicting spread of various exotic diseases [183,184], albeit models for spread of endemic diseases, e.g. within farms, have also been developed [185,186]. Both types of models are valuable tools when assessing short- and long-term consequences of possible intervention strategies, including the costs associated with these. Models have also been used as decision-support tools in real-time during on-going epidemics, e.g. the Foot and Mouth Disease epidemic in the UK in 2001 [187,188] and the Ebola outbreaks in 2014-15 [182], albeit with somewhat varying success [189]. Model-based analysis of such outbreaks, including comparisons of predictions with patterns observed in real-life, can help in improving methodology [182], and thereby aid in preparedness for the next outbreak.

It has frequently been mentioned that models are “Wrong but useful” [190]. This statement can be interpreted as: while uncertainty might heavily influence model predictions, this does not mean that the results of infectious disease modelling cannot be a part of the basis for informing decisions [190]. However, when using models as a decision support tool, there are certain ethics to consider and attempts at building a framework for evaluating whether mathematical models are consistent with ethical good practise and suitable for use as decision support have been described [191]. This framework was based on the principles of independence, transparency (autonomy), beneficence/non-maleficence, and justice, and among many other things the importance of communicating model results, limitations and uncertainties clearly to risk managers was highlighted [191].

Since mathematical models alone rarely full-fill all criteria for being ethical scientific evidence for decision-making, it was suggested that mathematical models may best be utilized in an exploratory context, as one of several sources of scientific evidence to support decisions about disease contingency planning [191]. Development of harmonized international standards for model development has also been called for [191]. Some attempt at this already exist in the form of the ODD (Overview, Design concepts, and Details) protocol [192,193], albeit so far this concept seem to mainly have been applied within ecological modelling [192]. Briefly, the primary objective of the ODD protocol is to standardize the published descriptions of individual-based and agent-based models (ABMs), and thereby ensure transparency and reproducibility [192]. Another standard for describing models has also been suggested in the form of TRACE (TRAnsparent and Comprehensive Ecological modelling documentation) [194], which later was updated and re-defined as "a tool for planning, performing, and documenting good modelling practice" [195].

4.2 Different types of models

Models can be mechanistic or data-driven, albeit hybrids also exist [196]. Mechanistic models are constructed based on an understanding of the mechanisms and biological processes going on within the system of interest, and assumptions about how this system works. This type of model will often be informed by a mixture of data and expert opinions or guestimates. Purely data-driven models are constructed based on available input and output data, and do not necessarily take knowledge about the actual disease dynamics or social dynamics among the affected humans or animals into account [197,198]. Disease models can be either deterministic or stochastic. In deterministic models, point estimates are used for every parameter [199], which results in outcomes given also as point estimates for the average scenario. The same input values will always give the same output and thus the number of re-runs of a deterministic model will not change the result. In stochastic models, variability and uncertainty are taken into account for one or more parameters, based on which one point estimate will be selected for every single step and for each run of the model, depicted from stochastic distributions. Consequently, repeated runs of a stochastic model will give different results, and altogether these results will be presented as a distribution.

Models are also often divided into population-based (compartmental) models, individual-based or agent-based models. In population models, movements of animals between compartments, that represents different infection stages, are modelled collectively [199]. Inclusion of compartments depends on the nature of the disease modelled. Several main types of infectious disease models exist; the simplest type is the SI-model, where individuals can move from being susceptible and to becoming infected, after which they will remain infected [199]. For diseases, where infected individuals can recover and either become infected again or gain immunity that protects against re-infection, SIS or SIR models are used [199].

Another frequently used type of models are SEIR models, which include a pre-infectious stage, called “E” for exposed [199].

In individual-based models, the fate of each individual is modelled rather than proportions of individuals [199]. Although agent-based and individual-based models are challenged by the existence of many parameters in the model and hence the extensive need for data to parametrize the models, they exclusively provide the chance to study dynamics of spread of pathogens and allow for examining control actions mechanistically, which is rather limited when using population models.

In the present PhD project, an individual-based model for spread of LA-MRSA within a pig herd was constructed. In this model an SIS model was used for LA-MRSA, but with two different infectious stages for intermittent shedders (IS) and persistent shedders (PS).

5. Part A: Epidemiology of LA-MRSA in the Danish pig population

5.1 Manuscript I

Risk factors for the occurrence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in Danish pig herds

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Abstract

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is widespread in many European countries including Denmark, where 88% of randomly selected production herds tested positive in 2016.

In the present study, we investigated herd-level risk factors for farms being classified as LA-MRSA positive (study 1), in addition to herd-level risk factors for farms changing status from LA-MRSA negative to LA-MRSA positive during a 2-year period (study 2). Risk factors previously identified in other studies were confirmed in study 1: large herd size, herd type (lower risk in herds with sows) and number of pig suppliers. Due to the effect of herd type, data from sow herds (N=41) and herds without sows (N=166) were analysed separately. A univariable analysis found that the variables significantly associated with LA-MRSA status for sow herds were: use of wet feed in the sow units; higher weights of piglets at weaning; availability of a delivery room on the farm; cleaning of aisles after pigs were moved; number of pigs per weaner section; number of pigs purchased in the past year, and factors related to rodent control and human traffic in the herd. In herds without sows, the univariable analysis showed that the presence of other species of animal on the farm; negative pressure ventilation; full sectioning; frequent visits from the veterinarian; peroral use of tetracyclines for weaners; number of pigs purchased in the past year, and factors related to rodent control and human traffic in the herd were significantly associated with LA-MRSA status. For herds that changed from LA-MRSA negative to positive (study 2), having a company contract for mouse control, having more than one pig supplier and using group medication in the drinking water were the variables associated with LA-MRSA status in the univariable analysis.

We did not succeed in building a biologically meaningful multivariable model based on any of the datasets and, as observed in similar studies, many of the risk factors identified in the univariable analysis were related to herd size. It was therefore not possible to determine whether it was the size of the herd or related factors that were the causal risk factors for being LA-MRSA positive.

Keywords

MRSA; herd size; herd type; questionnaire; pig suppliers; rodent control

Introduction

Since 2005, when it was first reported in the Netherlands and France (Armand-Lefevre, 2005; Voss et al., 2005), livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has become widespread in the pig populations of many countries, including Denmark (Crombé et al., 2013). Its presence is undesirable as it constitutes an occupational health hazard for farm workers, veterinarians and their families, and presents a risk of further dissemination into society (Goerge et al., 2017).

On LA-MRSA-positive pig farms, the bacteria have been isolated from animals, personnel, air, dust, feed and bedding material within the pens, as well as from air and soil samples taken up to 300 m downwind of the farms (Ferguson et al., 2016; Friese et al., 2012; Schulz et al., 2012). Known risk factors for the occurrence of LA-MRSA on pig farms include: large herds; buying weaners or finishers from more than two sources; age of the pigs (increased risk for weaners and nursery pigs); group treatment with antimicrobials; having partially or totally slatted floors; use of zinc for nursery pigs; disinfection; all-in/all-out production, and herd type (Alt et al., 2011; Broens et al., 2011a; Fromm et al., 2014; Slifierz et al., 2015; Tenhagen et al., 2009; van Duijkeren et al., 2008). Farrow-to-finish farms have a lower risk of being LA-MRSA positive compared to weaner-to-finish or grower-to-finish farms (Alt et al., 2011; Fromm et al., 2014; Tenhagen et al., 2009), and in general, lower prevalence has been observed among organic farms compared to conventional farms within the same countries (DANMAP, 2017; van de Vijver et al., 2014). Trade has been identified as a risk factor for the introduction of LA-MRSA in herds (Broens et al., 2011b; Espinosa-Gongora et al., 2012), but there are also indications that introduction might have occurred through human contact (Grøntvedt et al., 2016).

In 2007, LA-MRSA was retrospectively identified among isolates collected from two Danish pig farms in 2005 (Bagcigil et al., 2007; Guardabassi et al., 2007) and it has since spread rapidly in the Danish pig population. In both 2010 and 2011, LA-MRSA was detected in 16% of the tested pig herds (DANMAP, 2012, 2011), whereas in 2014, the prevalence had increased to 66% and 68% among nucleus/multiplier and production herds, respectively. In 2016, 88% of the randomly selected production herds tested positive (DANMAP, 2017).

It is not currently known how LA-MRSA has spread so quickly. In order to initiate efficient interventions to limit the further spread of LA-MRSA in the pig population, it is essential to obtain more knowledge on determinants for its introduction and establishment within a herd.

The main objectives of the present study were to: 1) Investigate herd-level risk factors for farms being classified as LA-MRSA positive (study 1), and 2) Investigate herd-level risk factors for farms changing status from LA-MRSA negative to LA-MRSA positive during 2014-2016 (study 2).

Materials and methods

Screening of herds

Study design

During the period from August to December 2016, the Danish Veterinary and Food Administration (DVFA) tested 221 production herds for LA-MRSA as part of a national screening. These herds were sampled for various reasons: 58 and 53 herds that had tested positive and negative, respectively, in the 2014 screening were re-tested to determine how many had changed LA-MRSA status; 53 herds were randomly selected; 57 herds were selected based on their geographical location in areas not represented in previous screenings. After a herd had been sampled, the owner or person responsible for the herd was invited to participate in a questionnaire-based telephone interview. Data collected during these interviews were supplemented with data extracted from three different national registers: 1) Data on the distance to the nearest pig and mink farms were extracted from the Danish Central Husbandry Register (CHR); 2) Data on pigs received on the farms were extracted from the pig movement database; 3) Data on antimicrobial consumption were extracted from the VetStat database. Study 1 included all tested herds that did not meet the exclusion criteria (see "exclusion criteria, categorisation and handling of missing observations" section for further details), and a subset, consisting of all herds that had tested negative in 2014, were used in study 2.

Sampling

The screening targeted conventional production herds with more than 50 finishers. Sampling staff were asked to sample the pigs that were closest to the stage of leaving the farm, i.e. usually finishers close to slaughter age. Young gilts were sampled in sow herds that had no weaners or finishers other than their own gilt production. At each farm, five pigs from each of five different pens (25 in total) were swabbed in both nares. The five nasal swabs obtained within the same pen were pooled in a tube containing 10 ml Muëller-Hinton broth with 6.5% NaCl. Samples were stored between 1^{o}C and 5^{o}C until lab analysis.

Lab analysis

The analyses were carried out by the DVFA lab. Upon arrival at the lab, the tubes were incubated at 37^{o}C for 18-24 h. The next day, 1 ml of the incubate was transferred to 9 ml tryptone soya broth (TSB) with

3.5 mg/l cefoxitin and 75 mg/l aztreonam, followed by incubation for 18-24 h at 37°C, after which 10 µl of the TSB incubate was streaked on to Brilliance LA-MRSA agar (Oxoid) and incubated for 24 h at 37°C. Finally, two presumptive LA-MRSA colonies were subcultured on blood agar. Verification and typing was carried out by whole-genome sequencing (MiSeq, Illumina) and use of CGE pipelines. When an isolate was confirmed to be LA-MRSA, no subsequent isolates from the herd were sequenced since a herd was declared LA-MRSA positive when at least one sample tested positive.

Questionnaire-based telephone interviews

Interviews

Before sampling, the herd owners received an information letter about the study together with information about the sampling. After sampling, the farmer was contacted by one of three different interviewers, who invited them to participate in the survey and schedule an interview. The interviews were conducted during the period August 30, 2016 – April 12, 2017.

Questionnaire

Each interview included up to 220 questions, depending on which age groups were present on the farm and the answers to the main questions in the questionnaires. The farmers were asked questions related to herd type and size, the surroundings of the farm and contact with other animals, design of the barns used for pigs of different age groups, management (including internal and external biosecurity), feed, ventilation, staff, visitors and health management. The questionnaire was strongly inspired by questionnaires used in other surveys among pig farmers (Dewulf, 2014; Sørensen et al., 2008), but specifically adjusted to LA-MRSA. The full questionnaire is available from the corresponding author upon request.

Register data

Data from the Danish Central Husbandry Register (CHR)

Geographical coordinates (UTM EUREF89, zone 32) for the location of all pig and mink herds in Denmark registered as active during the second half of 2016 were extracted from the Danish Central Husbandry register (CHR). Mink herds were included based on findings of LA-MRSA in healthy animals in 40% of 50 tested farms (Hansen et al., 2017). The distance to the nearest neighbouring pig farm and the nearest larger pig farm (defined as being above the 75% percentile for total number of pigs/year) were calculated and included in the univariable analysis. In addition, the numbers of pig farms within a radius of 1 km, 3 km and 10 km were also calculated and included as explanatory variables. The same parameters were calculated in relation to mink farms.

Data for movement of pigs

In the interviews, farmers were asked about which age groups of pigs (if any) they had purchased within the past year, and how many suppliers they had for each age group. A supplier was considered to be external if the pigs came from a farm with a different CHR registration number, regardless of whether this farm was owned by the same farmer. These data were supplemented with data from the Danish pig movement register, where the number of suppliers and number of pigs received were extracted for the 1-year period prior to sampling, and the data collected during the interviews were used to validate the data extracted from the register.

Data for antimicrobial consumption and use of zinc oxide (Vetstat)

All prescriptions of antimicrobials for animals in Denmark (including prescriptions of therapeutic concentrations of zinc oxide as a feed additive) are registered in a central database called Vetstat (Jensen et al., 2004; Stege et al., 2003). For the purpose of the present study, information on age group (for pigs: sows incl. piglets, weaners or finishers), antimicrobial class, amount of medicine, and route of administration was extracted from this database. The extracted data covered a period of one year prior to the sampling date. Furthermore, zinc consumption in kg of active compound and the number of flasks of tetracycline topical spray prescribed within the same one-year period were extracted. All amounts of antimicrobial medicines for parenteral or oral use were converted into defined daily doses per 1 kg of animal (DADDkg) according to the doses published in the DANMAP program (www.danmap.org). The number of Defined Animal Daily Doses (DADD) per 100 animals per day was calculated using biomass estimates as described in Jensen et al., 2014, and these were updated based on more recent production data (Helverskov, 2017). It was assumed that all antimicrobials used perorally for weaners were used for group medication.

Data analysis

Data management

During telephone interviews, printouts of the questionnaires were filled in by the interviewer, including additional comments. These were later entered into a database created in Microsoft Access 2007-2010. The first part of the validation took place during data entry. The full dataset was subsequently validated through cross-tabulation of replies to different questions, and by assessing whether the answers appeared logically and biologically plausible. Answers that seemed very unlikely to be correct were changed to missing observations. Data on the herd size (recorded as the number of sows in the herd, number of weaners sold and number of finishers produced annually) were compared to data from the CHR. If these data differed markedly, the number of animals in the Fertilizer Account Register was used to judge which

entry was most likely to be correct, and the numbers were corrected accordingly. Where discrepancies in the number of suppliers were found between self-reported data and the registered movement data, the register data were considered most reliable.

Exclusion criteria, categorisation and handling of missing observations

Outdoor herds (free-range or housed in barns with outdoor access) and organic herds were excluded from the study, while antimicrobial-free herds remained in the study. Nucleus breeding and/or multiplier herds were also excluded. Continuous variables were either included directly in the analysis, or were categorised if few replies differed from zero or the distribution encouraged dichotomisation or an ordinal scale. A variable for total herd size was created by estimating the total number of pigs present on the farm per day, assuming that the number of pigs produced was evenly distributed throughout the year. In the analyses, a logarithmic transformation of total herd size was used to improve linearity. In the univariable analyses, missing observations were excluded, whereas this would have resulted in the loss of too many observations in the multivariable analysis, so missing observations were recoded as "Not relevant" or "No reply", depending on the reason for the data not being available.

Statistical analysis

Data analysis was conducted in R version 3.2.2 – "Fire Safety" (R Core team, 2015). Data were analysed using logistic regression analysis with farm status (LA-MRSA negative/LA-MRSA positive) as the outcome variable. Odds ratios (OR) for testing LA-MRSA positive, including 95% confidence intervals, were calculated. Overall p-values for categorical variables with more than two levels were calculated using the likelihood ratio test (LRT) with an empty model as reference. Variables were included in the multivariable analysis when: 1) $p<0.20$ in the analysis with recoded missing values, 2) the proportion of observations with recoded missing values did not exceed 10%, 3) cross-tabulation of the variable with LA-MRSA status of the herd did not lead to any cells with zero observations. The analyses were conducted twice: once excluding all *variables* with zero observations in any cells, and once excluding *observations* to get rid of cells with zero *observations,* while allowing the variable to remain in the analysis. The latter was applied only in cases where it was possible to exclude cells with zero observations by deleting one observation only.

Confounding was assessed based on biological knowledge of the factors that might influence each other, and by observing changes in the coefficient (β) following inclusion of the potential confounder. If β changed by more than 20% when including the potential confounder, we deemed that confounding was present. Confounding was controlled for either by splitting the datasets according to levels of the confounder, or by forcing the confounder into the model. If the variable described something already partly or fully covered by another variable or co-linearity was suspected, only one of the variables were selected as a candidate

for inclusion in the multivariable model. The multivariable model was built using manual forward selection, where the next variable to be included was selected based on p-values, and the Akaike Information Criterion (AIC) was calculated using the "add1" function in R. The number of potential variables to include was high compared to the number of observations. No multivariable analysis was done in study 2, because of the relatively small number of observations.

Results and discussion

Inclusion and exclusion

Of the 221 tested herds, 166 were included in study A (75%). Of the 55 excluded herds, 44 herd owners refused to be interviewed or could not be reached during the interview period, and 11 herds were excluded for the following reasons: 1) being a nucleus/multiplier herd (three herds), 2) being organic/having outdoor production/veranda barns (four herds), 3) logistic constraints – the farms did not appear on sampling lists and were only discovered later (three herds), 4) production on the farm had ceased and no relevant personnel were available for interview (one herd). In study B, 40 of 53 herds were included (75%).

Screening outcome

The majority of the randomly selected herds (88%) tested LA-MRSA positive, yet the prevalence was somewhat lower for the area-specific samples (62% for Bornholm and 59% for Southeast Zealand; (DANMAP, 2017). Among the 166 herds included in study A, the overall prevalence of LA-MRSA-positive herds was 76%, and the prevalence in herds with and without sows was 59% and 82%, respectively.

All herds that had tested positive in 2014 also tested positive in 2016. Among the herds that had tested negative in 2014, 62% had changed status to positive in 2016, corresponding to a yearly incidence risk of 38% (DANMAP, 2017). There was a 63% prevalence of LA-MRSA-positive herds among the 40 that had tested negative in 2014 and were included in study B.

Description of the participating herds

The herds could be described as: farrow-to-finish herds (22 herds), sow herds (with no other weaners or finishers present on the farm than those related to gilt production) (7 herds), farrow-to-weaner herds (12 herds), weaner herds (6 herds), weaner-to-finisher herds (20 herds), or finisher herds (99 herds). However, in the dataset, the variable "herd type" only refers to whether or not the herd was a sow herd. In the 41 herds with sows, the numbers of sows ranged from 95 to 2,400 (median=588, mean=633), while the

number of finishers produced annually in all 166 herds ranged from 0 to 45,000 (median=4,000, mean=5,649).

Assessment of confounding

Herd size was found to be related to several other factors, e.g. having a company contract for rat control (Δβ ~ 34%), employees from abroad (Δβ ~ 33%) and number of people working in the herd (Δβ ~ 43%). Therefore, we decided to force herd size (log10 (pigs present per day)) into the multivariable models. The presence of sows on the farm had a significant effect on the LA-MRSA status, but also affected other factors such as the number of employees. Additionally, a considerable number of questions in the interview were only relevant for herds with sows and piglets, and we therefore decided to split the original dataset into two and analyse data for sow herds (N=41) and herds without sows (N=125) separately. Other potential confounders that were tested included age of the premises and numbers of sows on the farm.

Splitting the dataset used in study 1 into two subsets according to the presence of sows also meant that observations relating to weaners were split into two datasets. All univariable analyses on antimicrobial consumption and the use of zinc were therefore repeated on a dataset containing all herds with weaners. However, this did not yield any results of interest (results not presented).

Study 1 - univariable analysis

All herd types

Explanatory variables with $p<0.05$ in the univariable analysis are presented in table 1, except age-group-specific variables. These are instead presented and discussed in the sections on “sow herds” and “herds without sows”. All ORs are presented as the odds of the herd being LA-MRSA positive given that the factor indicated in the explanatory variable was present, relative to the odds of the herd being LA-MRSA positive when it was not present. For numeric variables, this is given as an increase of one unit.

Table 1. Study 1 - univariable analysis: Variables significantly associated with the LA-MRSA status of 166 pig herds

Variable	p-value	OR	95% CI	Yes/no[a]		Median [range]		No. of obs.
				+MRSA	-MRSA	+MRSA	-MRSA	
log10 (pigs present per day)	0.0064	3.00[b]	[1.37; 6.77]	-	-	3.33 [1.84; 4.02]	3.10 [1.76; 3.99]	166
Sows at the farm	0.0035	0.32	[0.15; 0.69]	24/102	17/23	-	-	166
No. of sows (where present)	0.0060	1.28[c]	[1.10; 1.57]	-	-	685 [213; 2,400]	350 [95; 800]	41
Finishers/year	0.0127	1.06[d]	[1.02; 1.11]	-	-	5,000 [0; 45,000]	1,675 [0; 23,000]	166
Farrow-to-finish farm	0.0152	0.32	[0.12; 0.81]	12/114	10/30	-	-	166
Mouse control - company contract	0.0031	3.14	[1.47; 6.75]	99/27	21/18	-	-	165
Rat control - company contract	0.0296	2.33	[1.08; 4.98]	97/29	23/16	-	-	165
Ventilation inlet - negative pressure	0.0094	3.30	[1.32; 8.15]	113/13	29/11	-	-	166
Employees from abroad	0.0385	2.22	[1.05; 4.83]	69/51	14/21	-	-	155
Closed herd	0.0001	0.11	[0.03; 0.32]	5/121	11/29	-	-	166
No. of suppliers in the previous year	0.0006	3.04	[1.71; 6.08]	-	-	1 [1; 11]	1 [1; 2]	163
Pigs received in the previous year	0.0031	1.06[d]	[1.03; 1.12]	-	-	5,399 [0; 56,720]	791 [0; 23,090]	163

a: Yes or no indicates the number of herds of a given LA-MRSA status for which the statement in the variable column is true

b: OR per log10 increase

c: OR per 50 sows

d: OR per 500 pigs

As in other studies, we observed an effect of **herd type** – with a lower risk of the farm testing positive if sows were present (Fromm et al., 2014; Tenhagen et al., 2009). Only those herds with sows and their own gilt production are able to avoid buying pigs from other herds, and in the present study, 37% of the sow herds had not purchased pigs from any other herd (including other herds with the same owner) for one year prior to sampling. The effect of being a **closed herd** (i.e. having no suppliers; OR=0.11), was significant when considering both the full dataset and sow herds only (OR=0.11). The **number of pig suppliers** has also been identified as a risk factor for LA-MRSA in another study (Tenhagen et al., 2009). In the present study, the vast majority of those purchasing pigs (regardless of whether or not the herd included sows) had only one supplier, and only 19.5% of the sow herds and 35.2% of the herds without sows had more than one supplier. The **number of animals purchased** also seemed to have some influence, but the present study did not investigate whether this was linked to the actual number of animals or just the frequency of movements.

In addition to herd type and factors related to the purchase of pigs, the different variables related to herd size and rodent control consistently came out as significant in the univariable analysis of all three datasets in study 1: the full dataset (table 1), the sow dataset (table 2), and the dataset for herds without sows (table 3). **Herd size** has already been identified as a risk factor for herds testing LA-MRSA positive in several other studies (Alt et al., 2011; Broens et al., 2011a; European Food Safety Authority, 2010; Fromm et al., 2014; Tenhagen et al., 2009). Increased risk of introduction of diseases introduced by carrier animals or airborne diseases has been associated with larger herds, and the number of external contacts (trucks and visitors) might increase with increasing herd size (Gardner et al., 2002). Furthermore, many management practices are associated with a larger herd size, some of which might theoretically increase the risk of a herd being LA-MRSA positive, e.g. higher antimicrobial consumption (Broens et al., 2011a), while others should theoretically reduce the risk of disease spread, e.g. sectioning and all-in/all-out production (Gardner et al., 2002).

Questions related **to rodents** were included in the questionnaire, because LA-MRSA has been detected in rats and voles (Pletinckx et al., 2013; van de Giessen et al., 2009), and rats might be able to travel between farms. The presence of rodents and who was responsible for rodent control were significantly related to LA-MRSA status in several of the analyses. However, it was not possible to explore whether LA-MRSA status is directly influenced by the presence of rodents, or whether rodents and rodent control is correlated to other underlying factors. For example, farmers who have specific pathogen free (SPF) production, and farmers producing finishers for the UK market are obliged to have rodent control in place, but a company contract is only specifically required for SPF herds with the highest security level (3 herds in the present

study) (SEGES, 2018, 2016). In contrast, farmers with a voluntary agreement with a rodent control company could have been motivated by relatively severe problems with rodents on their farm or in the area, compared to those without a signed agreement. Additionally, the difference between those relying on the municipality/region for rodent control and those who had a contract with a company was a bit surprising, since a number of municipalities have outsourced the responsibility for rodent control to some of the same private companies.

Sow herds

Explanatory variables that were significantly ($p<0.05$) associated with the LA-MRSA status of the sow herds in the study are listed in table 2. Variables that, after re-coding of missing observations, fulfilled the criteria for potential inclusion in a multivariable model for sow herds are listed in S1 table.

In addition to factors related to the purchase of pigs, herd size, number of suppliers and rodent control, a significant association was found between LA-MRSA status and use of **wet feed** for the sows in the gestation (OR=12.50) and farrowing units (OR=10.11). In a previous study, LA-MRSA was isolated from feed (Friese et al., 2012), but samples were collected directly from the feeder and it was therefore suggested that the findings were a result of secondary contamination from dust, faeces or pigs, rather than primary contamination of the feed itself. The microflora in wet feed is usually dominated by lactic acid bacteria (Brooks et al., 2008), and it is not known whether LA-MRSA would be able to grow in this environment. Since establishing a wet feed system is a fairly large investment (SEGES, 2010), one could speculate that only larger herds might invest in wet-feed equipment, but this could not be confirmed statistically ($p=0.09$). However, the mean number of sows present on farms using wet feed tended to be higher than on farms using dry feed, though the difference was not significant ($p=0.15$).

Higher weight of piglets at weaning was associated with lower risk of the farm being LA-MRSA positive (OR=0.37). Higher weight at weaning is generally assumed to be an indication of higher weaning age. A younger age at weaning has been associated with higher total antimicrobial consumption from birth to slaughter (Postma et al., 2016), but no significant effect of weaning age was found in the present study. Some farmers estimated the average weaning time from the number of days the sows were lactating. As this lactation period may also include the use of sows as nursery sows (foster dams), the lactating period may not be a reliable indicator for how long the piglets have been suckling. However, higher weight at weaning might also be associated with less intensive production, which again may be associated with other management practices that could influence the occurrence of LA-MRSA.

Table 2: Study 1 - univariable analysis: Variables significantly associated with the LA-MRSA status of the sow herds (N=41)

Variable	p-value	OR	95% CI	Yes/no[a]		Median [range]		No. of obs.
				+MRSA	-MRSA	+MRSA	-MRSA	
log10 (pigs present per day)	0.0143	14.83[b]	[2.04; 164.81]	-	-	3.60 [2.76; 3.99]	3.18 [2.42; 3.99]	41
Weaners sold/year	0.0367	1.03[c]	[1.01; 1.07]	-	-	22,000 [0; 80,000]	10,000 [0; 26,500]	41
Weaners produced/year	0.0303	1.04[c]	[1.01; 1.08]	-	-	22,000 [0; 80,000]	10,000 [115; 26,500]	41
No. of sows	0.0180	1.17[d]	[1.05; 1.37]	-	-	685 [213; 2,400]	350 [95; 800]	41
Small occurrence of mice	0.0170	0.19	[0.04; 0.71]	7/17	11/5	-	-	40
Rat control - company contract[e]	0.0200	6.22	[1.44; 33.84]	21/3	9/8	-	-	41
Use of wet feed in the gestation unit[f]	0.0034	12.50	[2.71; 92.02]	15/9	2/15	-	-	41
Weight at weaning (kg)	0.0406	0.37	[0.11; 0.83]	-	-	7 [4; 10]	8 [6;9]	28
Cleaning of aisles after movement	0.0456	6.00	[1.17; 45.83]	22/2	11/6	-	-	41
Washing of aisles after movement[g]	0.0030	9.17	[2.28; 44.40]	20/4	6/11	-	-	41
Delivery room	0.0171	6.67	[1.52; 36.95]	20/3	8/8	-	-	39
Typical no. of weaners/section	0.0446	1.08[h]	[1.01; 1.18]	-	-	43 [15; 92]	27.5 [11; 52]	34
More than three visitors/month	0.0416	4.67	[1.16; 24.20]	12/12	3/14	-	-	41
No. of people working in the herd	0.0030	2.55	[1.47; 5.16]	-	-	5 [2; 15]	3 [2; 6]	41
Employees from abroad	0.0032	9.00	[2.25; 43.52]	18/6	4/12	-	-	40
Closed herd (in the past year)	0.0030	0.11	[0.02; 0.43]	4/20	11/6	-	-	41
No. of suppliers in the past year	0.0356	2.73	[1.19; 7.95]	-	-	1 [0; 4]	0 [0;2]	41
No. of pigs received	0.0132	13.6[c]	[2.10; 140.19]	-	-	289 [0; 20,900]	0 [0; 472]	41

a: Yes or no indicates the number of herds of a given LA-MRSA status for which the statement in the variable column is true
b: OR per log10 increase
c: OR per 500 pigs
d: OR per 50 sows
e: The same results were obtained for mouse control - company contract
f: Use of wet feed in the farrowing unit gave similar results
g: Versus other methods of cleaning or not cleaning (sub-question for 'cleaning of aisles')
h: OR per 10 pigs

Having a **delivery room** was associated with higher risk of being LA-MRSA positive. However, having a delivery room was also associated with a large herd size (OR=8.70). Furthermore, some of the farmers who did not have a delivery room had alternative procedures in place, such as a delivery paddock or a delivery truck for transporting pigs to the road, which might have a similar (or even stronger) protective effect. Unfortunately, we only have information on this for a limited number of the farms.

Surprisingly, **cleaning the aisles** after moving pigs (OR=6.00) and, more specifically **washing the aisles** (OR=9.17), were associated with farms testing positive for LA-MRSA. A biological explanation could be that cleaning might disturb dust containing LA-MRSA, and washing might create aerosols.

Several factors related to **human traffic** in the herd (number of visitors or employees, employees from abroad) were significantly related to the LA-MRSA status of the sow herds. More people entering the herd will increase the risk of human introduction, but this could also be a proxy for herd size. Having employees from abroad was included in the study due to previous reports about introduction by employees from abroad (Grøntvedt et al., 2016). However, given the high prevalence in Danish pig herds, this may be of less relevance and could also be an effect of herd size, since the relationship between having employees from abroad and herd size was close to the threshold for significance (p=0.0530; OR=7.38).

Having **bigger epidemiological units**, measured by the typical number of weaners per section (OR = 1.08 per 10 pigs), was also associated with herds testing positive. Other factors related to having many pigs together in one unit (i.e. air space stocking density, floor space stocking density and herd size) have previously been identified as risk factors for the spread of other swine diseases (Gardner et al., 2002).

Herds without sows

Explanatory variables where $p<0.05$ for the LA-MRSA status of herds without sows are listed in table 3. Variables that, after re-coding of missing observations, fulfilled the criteria for potential inclusion in the multivariable model for herds without sows are listed in S3 table.

Table 3: Study 1 - univariable analysis: Variables significantly associated with the LA-MRSA status of the herds without sows (N=125)

Variable	p-value	OR	95% CI	Yes/no[a]		Median [range]		No. of obs.
				+MRSA	-MRSA	+MRSA	-MRSA	
log10 (pigs present per day)	0.0072	3.76[b]	[1.45; 10.24]	-	-	3.27 [1.84; 4.02]	2.97 [1.76; 3.58]	125
Other animal species on the farm	0.0351	0.32	[0.11; 0.96]	13/88	7/15	-	-	123
Mouse control - company contract[c]	0.0410	2.71	[1.03; 7.08]	78/24	10/12	-	-	124
Rat control - municipality/region[c]	0.0155	0.28	[0.10; 0.80]	14/88	8/14	-	-	124
Full sectioning - finisher unit	0.0104	4.05	[1.36; 11.86]	79/12	13/8	-	-	112
Ventilation inlet - negative pressure	0.0203	3.62	[1.18; 10.68]	91/11	16/7	-	-	125
Days between visits from vet ≤ 35	0.0317	2.93	[1.08; 7.79]	82/18	14/9	-	-	123
Three or more visitors/month	0.0325	0.28	[0.09; 0.94]	9/90	6/17	-	-	122
Only one person working in the herd	0.0169	0.32	[0.12; 0.82]	22/76	11/12	-	-	121
No. of suppliers in the previous year	0.0365	2.75	[1.27; 8.73]	-	-	1 [1; 11]	1 [1; 2]	125
No. of pigs received	0.0489	1.04[d]	[1.01; 1.10]	-	-	6901 [229; 56,720]	5041 [229; 2,390]	122
Peroral use of tetracyclines (yes/no)[e]	0.0420	13.50	[1.35; 317.77]	18/4	1/3	-	-	26

a: Yes or no indicates the number of herds of a given LA-MRSA status for which the statement in the variable column is true

b: OR per log10 increase

c: Many herds, but not all gave the same replies for mouse and rat control

d: OR per 500 pigs received

e: Set to NA for herds that had no weaners (also significant if these were set to zero), only four herds with weaners were negative

A protective effect of having **other animal** species on the farm was observed in herds without sows (OR=0.32). Among the 20 farms (16%) with other animal species present, eleven had cattle, nine had horses, and one had sheep or goats. A similar protective effect of having other animals on the farm was obtained in a meta-analysis using pooled data from several LA-MRSA risk factor studies (Fromm et al., 2014), in which this effect was also associated with floor type and having outdoor access. It was therefore suggested that these features could be characteristic of traditional family farms. None of the pigs in the present study had outdoor access, though the presence of other animals was also associated with herd size (OR=0.30, p=0.0175), supporting the theory that presence of other animal species might be indicative of less intensive farming/ hobby type herds.

Having a **ventilation system** with negative pressure was associated with positive LA-MRSA status (OR=3.62), while natural ventilation was associated with lower risk of positive LA-MRSA status (OR=0.03). However, only six farms had natural ventilation, while the vast majority had negative pressure ventilation systems (107 farms), and a small number had other types of systems. It has been suggested that depending on the type of ventilation system and the construction of the barns, internal spread of LA-MRSA throughout the whole building via dust might be able to occur (Friese et al., 2012). However, the role of ventilation in the introduction and persistence of LA-MRSA within the herd still needs to be elucidated. Herds with curtain ventilation or barns with open sides were excluded from the study, but the exact type of ventilation in place on the remaining six farms with natural ventilation remains unknown. Furthermore, having natural ventilation was also associated with a smaller herd size (p=0.0016).

In the analysis of data from herds without sows, having **full sectioning** in the finisher unit (OR=4.05) and **frequent visits from the veterinarian** (on average ≤35 days between visits; OR=2.93) were both associated with testing LA-MRSA positive, but both factors were also related to a larger herd size (full sectioning: OR=8.35, p=0.0003; ≤35 days between visits: OR=13.78, p<0.001). The observed association between herd size and frequency of visits from a veterinarian was expected, since it is mandatory for all large[1] Danish pig herds to have a health advisory agreement with a veterinarian – most often including at least nine mandatory visits per year (Ministry of Environment and Food of Denmark, 2016). One could also hypothesise that visits from the veterinarian might be a potential source of introduction, but our dataset showed no significant association with the veterinarian or clinic used, or how many other pig herds the veterinarian or clinic served (results not presented). However, due to the limited number of pig herds in the dataset relative to the number of pig veterinarians in Denmark, many veterinarians only featured once in the dataset. Visits from veterinarians are of course also just part of the **human traffic** in the herd in general

[1] >300 sows, gilts or boars, >3,000 finishers or >6,000 weaners

(see discussion in the “sow herds” section above). Herds with no sows and only one person working in the herd (OR=0.32) and with more than three visitors per month on average (OR=0.28) were negatively associated with LA-MRSA status. This latter effect was contrary to expectations, and opposite to that observed for the sow herds.

As mentioned in the introduction, the use of zinc for nursery pigs and the use of group treatment with antibiotics have previously been identified as risk factors for being LA-MRSA positive (Fromm et al., 2014; Slifierz et al., 2015). In the present study, the **peroral use of tetracyclines for weaners,** which was assumed to be equivalent to group treatment, was associated with LA-MRSA status (OR=13.50). None of the other factors related to the use of zinc or antimicrobial consumption were found to be significant. The vast majority of herds with weaners used zinc (85%), and the amount used was not significantly associated with LA-MRSA status when scaled to the number of weaners produced annually. In the questionnaire, farmers were asked whether they routinely initiated group treatment, but there was no significant difference in relation to LA-MRSA status.

Study 2 - univariable analysis

The dataset in study 2 was not segregated based on the presence of sows due to the relatively small number of observations and only seven of the participating herds being sow herds. Only three explanatory variables were significantly associated with LA-MRSA status: the **number of pig suppliers** within the past year (OR=15.17 [2.46; 296.40], p=0.0143), **use of group medication in water** (vs. administration through feed; OR=12.00 [1.44; 261.37], p=0.0406), and having a **company contract for mouse control** (OR=6.00 [1.51; 27.06], p=0.0136). The effect of herd size (log10 (pigs present per day)) was close to the threshold for significance (OR= 3.76; p=0.0594).

Administration of group medication through water has previously been associated with increased antimicrobial consumption (Fertner et al., 2016). Fertner et al. speculated that this might be related to the potentially large number of animals served by each waterline, which makes it more difficult to treat smaller groups of pigs.

General discussion

In study 1, many of the explanatory variables that were associated with LA-MRSA status in the univariable analysis were also associated with herd size. Several of the factors associated with herds without sows being LA-MRSA negative (no full sectioning; long intervals between visits from the veterinarian; having other animal species on the farm; no contract with a rodent control company and having only one person working with the pigs) might also be associated with less intensive production, in addition to smaller herds

in general. Similarly, a higher weight at weaning; having no delivery room; a lower number of visitors and no employees from abroad were associated with sow herds being LA-MRSA negative, and these are also factors one could speculate might be linked to less intensive production.

In our first attempt to build a multivariable model for the sow herds (exclusion of variables to avoid cells with zero observations in cross-tabulations), the LA-MRSA status was significantly related to herd size (log10(pigs present per day)), use of wet feed in the gestation unit and use of tetracyclines and colistin for sows and piglets. However, both tetracycline and colistin use in sows and piglets were strongly confounded with herd size and were also related to each other. Adding them to the model led to a 106% increase in the regression parameter for wet feed and a 112% increase in the regression parameter for herd size. In addition, only one of the negative herds used colistin, so the basis for estimation was also very limited. These two factors were therefore not included in the model. Adding the use of wet feed only caused a 4% change in the regression parameter for herd size, but these two parameters still seemed to modify each other, leading to a very large confidence interval for both ORs (S3 table).

When excluding observations to avoid zero cells in cross-tabulation of variables, only herd size and cleaning of aisles after moving pigs remained in the model. However, these also strongly modified each other (S3 table). For herds without sows, the LA-MRSA status of the farm was associated with the average number of visitors per month, having a company contract for rat control and the herd size, regardless of which model-building approach was taken. However, these models do not seem to be biologically meaningful. In the present investigation, the number of observations was low relative to the number of factors investigated, so the possibility of some being significant just by chance cannot be excluded.

In questionnaire surveys, there is always a risk of misunderstandings, recall bias or an inclination to give "politically correct" answers. To minimise the effect of this bias, several variables were cross-checked with register data where possible. For example, 21/41 sow farmers considered their herd to be closed, whereas according to data from the movement database, only 15 of those sow farms had no entries of pigs from other herds within the past year.

Conclusions

Sow herds tested LA-MRSA positive less frequently than herds without sows. Many of the factors significantly associated with LA-MRSA status in study 1 also seemed to be associated with herd size, and it was therefore not possible to determine whether herd size itself or the related factors were the "true" risk

factors for an LA-MRSA-positive status. Similar problems caused by associations with herd size have been observed in other studies (Broens et al., 2011a).

We did not succeed in building any biologically meaningful multivariable models, though the results obtained in study 1 suggest that herds remaining LA-MRSA negative might be smaller herds with less intensive production. The dataset available for study 2 was small, and only three variables (the number of suppliers, use of group medication in water vs. administration through feed, and having a company contract for mouse control) were associated with LA-MRSA status in the univariable analysis. The reasons for some herds being able to maintain negative status are believed to be multifactorial, and this study was impeded by a relatively low number of observations and possibly by potential factors of relevance not being recorded.

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References

Alt, K., Fetsch, A., Schroeter, A., Guerra, B., Hammerl, J.A., Hertwig, S., Senkov, N., Geinets, A., Mueller-Graf, C., Braeunig, J., Kaesbohrer, A., Appel, B., Hensel, A., Tenhagen, B.-A., 2011. Factors associated with the occurrence of MRSA CC398 in herds of fattening pigs in Germany. BMC Vet. Res. 7, 69. doi:10.1186/1746-6148-7-69

Armand-Lefevre, L., 2005. Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. Emerg. Infect. Dis. 11, 711–714.

Bagcigil, F.A., Moodley, A., Baptiste, K.E., Jensen, V.F., Guardabassi, L., 2007. Occurrence, species distribution, antimicrobial resistance and clonality of methicillin- and erythromycin-resistant staphylococci in the nasal cavity of domestic animals. Vet. Microbiol. 121, 307–315. doi:10.1016/j.vetmic.2006.12.007

Broens, E.M., Graat, E.A.M., Van, P.J., Wolf, D., Van De Giessen, A.W., De Jong, M.C.M., 2011a. Prevalence and risk factor analysis of livestock associated MRSA-positive pig herds in The Netherlands. Prev. Vet. Med. 102, 41–49. doi:10.1016/j.prevetmed.2011.06.005

Broens, E.M., Graat, E. A. M., Van der Wolf, P.J., Van de Giessen, A. W., Van Duijkeren, E., Wagenaar, J. A., Van Nes, A., Mevius, D.J., de Jong, M.C.M., 2011b. MRSA CC398 in the pig production chain. Prev. Vet. Med. 98, 182–189. doi:10.1016/j.prevetmed.2010.10.010

Brooks, P.H., 2008. Fermented liquid feed for pigs. CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour. 3, 1–17. doi:10.1079/PAVSNNR20083073

Crombé, F., Argudín, M.A., Vanderhaeghen, W., Hermans, K., Haesebrouck, F., Butaye, P., 2013. Transmission Dynamics of Methicillin-Resistant *Staphylococcus aureus* in Pigs. Front. Microbiol. 4, 57. doi:10.3389/fmicb.2013.00057

DANMAP, 2017. DANMAP 2016 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. https://www.danmap.org

DANMAP, 2012. DANMAP 2011 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. https://www.danmap.org

DANMAP, 2011. DANMAP 2010 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. https://www.danmap.org

Dewulf, J., 2014. An online risk-based biosecurity scoring system for pig farms. Vet. J. Irel. 4, 426–429.

Espinosa-Gongora, C., Broens, E.M., Moodley, A., Nielsen, J.P., Guardabassi, L., 2012. Transmission of MRSA CC398 strains between pig farms related by trade of animals. Vet. Rec. 170, 564–564. doi:10.1136/vr.100704

European Food Safety Authority, 2010. Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holding with breeding pigs, in the EU, 2008. Part B: factors associated with MRSA contamination of holdings.

Ferguson, D.D., Smith, T.C., Hanson, B.M., Wardyn, S.E., Donham, K.J., Ferguson, D.D., Smith, T.C., Hanson, B.M., Wardyn, S.E., 2016. Detection of Airborne Methicillin-Resistant *Staphylococcus aureus* Inside and Downwind of a Swine Building, and in Animal Feed: Potential Occupational, Animal Health, and Environmental Implications. J. Agromedicine 21, 149–153. doi:10.1080/1059924X.2016.1142917

Fertner, M., Boklund, A., Dupont, N., Toft, N., 2016. Changes in group treatment procedures of Danish finishers and its influence on the amount of administered antimicrobials. Prev. Vet. Med. 126, 89–93. doi:10.1016/j.prevetmed.2016.01.034

Friese, A., Schulz, J., Hoehle, L., Fetsch, A., Tenhagen, B.-A., Hartung, J., Roesler, U., 2012. Occurrence of MRSA in air and housing environment of pig barns. Vet. Microbiol. 158, 129–135. doi:10.1016/j.vetmic.2012.01.019

Fromm, S., Beißwanger, E., Käsbohrer, A., Tenhagen, B.-A., 2014. Risk factors for MRSA in fattening pig herds – A meta-analysis using pooled data. Prev. Vet. Med. 117, 180–188. doi:10.1016/j.prevetmed.2014.08.014

Gardner, I.A., Willeberg, P., Mousing, J., 2002. Empirical and theoretical evidence for herd size as a risk factor for swine diseases. Anim. Heal. Res. Rev. 3. doi:10.1079/AHRR200239

Goerge, T., Barbara, M., Alen, S. Van, Hübner, N., Becker, K., Köck, R., 2017. MRSA colonization and infection among persons with occupational livestock exposure in Europe: Prevalence , preventive options and evidence. Vet. Microbiol. 200, 6–12. doi:10.1016/j.vetmic.2015.10.027

Grøntvedt, C.A., Elstrøm, P., Stegger, M., Skov, R.L., Skytt Andersen, P., Larssen, K.W., Urdahl, A.M., Angen, Ø., Larsen, J., Åmdal, S., Løtvedt, S.M., Sunde, M., Bjørnholt, J.V., 2016. Methicillin-Resistant *Staphylococcus aureus* CC398 in Humans and Pigs in Norway: A “One Health” Perspective on Introduction and Transmission. Clin. Infect. Dis. 63, 1431–1438. doi:10.1093/cid/ciw552

Guardabassi, L., Stegger, M., Skov, R., 2007. Retrospective detection of methicillin resistant and susceptible *Staphylococcus aureus* ST398 in Danish slaughter pigs. Vet. Microbiol. 122, 384–6. doi:10.1016/j.vetmic.2007.03.021

Hansen, J.E., Larsen, A.R., Skov, R.L., Chriél, M., Larsen, G., Angen, Ø., Larsen, J., Lassen, D.C., Pedersen, K., 2017. Livestock-associated methicillin-resistant *Staphylococcus aureus* is widespread in farmed mink (Neovison vison). Vet. Microbiol. 207, 44–49. doi:10.1016/j.vetmic.2017.05.027

Helverskov, O., 2017. Landsgennemsnit for produktivitet i svineproduktionen (in Danish). http://svineproduktion.dk/publikationer/kilder/notater/2017/1716

Jensen, V.F., de Knegt, L. V., Andersen, V.D., Wingstrand, A., 2014. Temporal relationship between decrease in antimicrobial prescription for Danish pigs and the "Yellow Card" legal intervention directed at reduction of antimicrobial use. Prev. Vet. Med. 117, 554–564. doi:10.1016/j.prevetmed.2014.08.006

Jensen, V.F., Jacobsen, E., Bager, F., 2004. Veterinary antimicrobial-usage statistics based on standardized measures of dosage 64, 201–215. doi:10.1016/j.prevetmed.2004.04.001

Ministry of Environment and Food of Denmark, 2016. Bekendtgørelse om sundhedsrådgivningsaftaler for svinebesætninger (in Danish).

Pletinckx, L.J., Verhegghe, M., Crombé, F., Dewulf, J., De Bleecker, Y., Rasschaert, G., Butaye, P., Goddeeris, B.M., De Man, I., 2013. Evidence of possible methicillin-resistant *Staphylococcus aureus* ST398 spread between pigs and other animals and people residing on the same farm. Prev. Vet. Med. 109, 293–303. doi:10.1016/j.prevetmed.2012.10.019

Postma, M., Backhans, A., Collineau, L., Loesken, S., Sjölund, M., Belloc, C., Emanuelson, U., Beilage, E., Nielsen, E.O., Stärk, K.D.C., Dewulf, J., 2016. Evaluation of the relationship between the biosecurity status, production parameters , herd characteristics and antimicrobial usage in farrow-to-finish pig production in four EU countries. Porc. Heal. Manag. 2, 1–11. doi:10.1186/s40813-016-0028-z

R Core team, 2015. R Core Team. R - A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http//www.R-project.org/.

Schulz, J., Friese, A., Klees, S., Tenhagen, B.A., Fetsch, A., Rösler, U., Hartung, J., 2012. Longitudinal Study of the Contamination of Air and of Soil Surfaces in the Vicinity of Pig Barns by Livestock-Associated Methicillin-Resistant *Staphylococcus aureus*. Appl. Environ. Microbiol. 78, 5666–5671. doi:10.1128/AEM.00550-12

SEGES, 2018. SPF-Sundhedsregler for SPF-besætninger (in Danish). http://spfsus.dk/~/media/system/0/1/f/e/01fedce06e8f5f6d85d3be0b05a93228/spf-sundhedsregler020118.ashx

SEGES, 2016. Produktstandard for Englandsgrise, Januar 2016 (in Danish). https://www.danishcrown.dk/ejer/svineleverandoer/danish-crown-afregning/danish-crown-kontrakt/specialgrise/

SEGES, 2010. Vådfoder kontra tørfoder (in Danish). http://svineproduktion.dk/Viden/I-stalden/Foder/Foderstrategi/Vaadfoder_kontra_toerfoder

Slifierz, M.J., Friendship, R.M., Scott Weese, J., 2015. Methicillin-resistant *Staphylococcus aureus* in commercial swine herds is associated with disinfectant and zinc usage. Appl. Environ. Microbiol. 81, 2690–2695. doi:10.1128/AEM.00036-15

Stege, H., Bager, F., Jacobsen, E., Thougaard, A., 2003. VETSTAT - the Danish system for surveillance of the veterinary use of drugs for production animals. Prev. Vet. Med. 57, 105–115. doi:10.1016/S0167-5877(02)00233-7

Sørensen, A.I.V., Lundsby, K., Larsen, L.S., Wingstrand, A., 2008. Karakteristik af danske slagtesvinebesætninger 2007-2008 (in Danish). http://orbit.dtu.dk/fedora/objects/orbit:89478/datastreams/file_6339741/content

Tenhagen, B., Fetsch, A., Alt, K., Käsbohrer, A., Bräunig, J., Appel, B., 2009. MRSA in herds of fattening pigs in Germany - Associated risk factors, in: Safe Pork 2009 - Québec City, Québec, Canada. pp. 124–127.

van de Giessen, A.W., van Santen-Verheuvel, M.G., Hengeveld, P.D., Bosch, T., Broens, E.M., Reusken, C.B.E.M., 2009. Occurrence of methicillin-resistant *Staphylococcus aureus* in rats living on pig farms. Prev. Vet. Med. 91, 270–273. doi:10.1016/j.prevetmed.2009.05.016

van de Vijver, L.P.L., Tulinski, P., Bondt, N., Mevius, D., Verwer, C., 2014. Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) in organic pig herds in the Netherlands. Zoonoses Public Health 61, 338–345. doi:10.1111/zph.12076

van Duijkeren, E., Ikawaty, R., Broekhuizen-Stins, M., Spalburg, E., de Neeling, A., Allaart, J., van Nes, A., Wagenaar, J., Fluit, A., 2008. Transmission of methicillin-resistant *Staphylococcus aureus* strains between different kinds of pig farms. Vet. Microbiol. 126, 383–389. doi:10.1016/j.vetmic.2007.07.021

Voss, A., Loeffen, F., Bakker, J., Klaassen, C., Wulf, M., 2005. Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg. Infect. Dis. 11, 1965–1966. doi:10.3201/eid1112.050428

Supplementary Materials

S1 Table: Study 1 - Variables included in the multivariable analysis – sow herds

S2 Table: Study 1 - Variables included in the multivariable analysis – herds without sows

S3 Table: Results of multivariable analysis in study 1

S1 Table: Study 1 - variables included in the multivariable analysis – sow herds

Variable	p-value[a]	OR	95% CI	Non-missings[b]	Min. Group[c]
log10(pigs present per day)	0.0048	14.83[b]	[2.04; 164.81]	41	numeric
Small vs. larger occurrence of mice	0.0178	0.19	[0.05; 0.72]	40	6
Rat control - company control	0.0238	5.93	[1.37; 32.30]	41	1
Solid floor (100%/combination) - gest. unit	0.0497	0.24	[0.05; 0.95]	41	4
Deep litter - gest. unit	0.1721	0.20	[0.01; 1.76]	38	1
Wet feed vs. dry feed - gest. unit	0.0045	11.67	[2.51; 86.19]	41	16
Straw bedding (deep/limited) - farr. unit	0.1940	0.23	[0.01; 1.59]	41	1
Wet feed vs. dry feed - farr. unit	0.0100	10.11	[2.14; 75.27]	38	1
Cleaning of aisles after moving pigs[d]	0.0456	6.00	[1.17; 45.83]	40	2
Shower mandatory when leaving farm	0.0695	3.95	[0.97; 20.47]	41	3
Delivery room present on CHR	0.0395	6.67[e]	[1.52; 36.95]	39	1
Negative pressure ventilation – inlet[d]	0.1030	0.15	[0.01; 1.13]	40	1
More than three visitors per month	0.0416	4.67	[1.16; 24.20]	41	3
No. of personnel taking care of the pigs	0.0025	2.69	[1.53; 5.62]	41	numeric
Employees working with pigs at other CHRs	0.1867	0.13	[0.00; 1.32]	41	1
Employees from abroad[d]	0.0032	9.00	[2.25; 43.52]	40	4
Fixed routine/work order (youngest first)[d]	0.1657	0.27	[0.03; 1.61]	40	2
Closed herd	0.0030	0.11	[0.02; 0.43]	41	4
No. of pigs received (per 100 pigs)	0.1710	1.27	[0.43; 6.63]	41	numeric
Use of probiotics/ alternative medicine	0.1970	2.50	[0.65; 11.06]	41	4
Used for sows (y/n) - Tetracycline	0.0507	0.19	[0.03; 0.87]	41	2
Used for sows (y/n) - Simple penicillins	0.1890	2.92	[0.61; 16.35]	41	3
Used for sows (y/n) - Colistin	0.0936	6.59	[1.01; 130.44]	41	1
Used for sows (y/n) - Combined penicillins	0.1474	0.38	[0.10; 1.39]	41	6

a: For categorical variables with more than two levels, the p-value originates from the LRT against an empty model. For the remainder, the p-value originates from a univariable logistic regression

b: No. of observations that are neither "no reply" nor "not relevant". The min. number required to be considered for inclusion in the multivariable analysis was set to 37 (41 obs. minus 10%)

c: Smallest number of obs. in a group when cross-tabulated with LA-MRSA status

d: Only included in the second approach in the multivariable analysis, where single observations were deleted to eliminate cells in cross-tabulations with no observations

e: OR presented for delivery room present vs. not present (OR for "No reply" vs. the other categories were not significant)

S2 Table: Study 1 - Variables included in the multivariable analysis – herds without sows

Variable	p-value[a]	OR	95% CI	Non-missings[b]	Min. group[c]
log10 (pigs present per day)	0.0072	3.76[d]	[1.45; 10.24]	125	numeric
Area (North; South; East; SE; Bornholm)	0.1208	See footnote[e]		125	3
Other animal species in CHR	0.1081	0.32	[0.11; 0.97]	125	1
Dist. to nearest horses	0.0707	1.11	[1.01; 1.27]	114	numeric
Rat control - region/municipality	0.0155	0.28	[0.10; 0.80]	124	8
Typical no. of pigs per pen - finisher unit	0.1530	0.95	[0.88; 1.02]	116	numeric
Negative pressure ventilation – inlet[f]	0.0060	4.98	[1.55; 15.86]	122	7
Mean no. of visitors per month	0.0318	0.73	[0.52; 0.95]	122	numeric
Days between visits from the vet ≤35[f]	0.0317	2.93	[1.08; 7.79]	123	9
More than one person taking care of the pigs[f]	0.0169	3.17	[1.22; 8.23]	121	11
No. of suppliers in the previous year	0.0365	2.75	[1.27; 8.73]	122	numeric
No. of pigs received	0.0489	1.04[g]	[1.01; 1.10]	122	numeric
No. of pig farms within 3 km	0.1046	0.91	[0.82; 1.02]	125	numeric
Dist. to nearest pig farm in CHR (per 100 m)	0.1990	1.06[h]	[0.97; 1.17]	125	numeric
Linamid - yes/no, finishers	0.1270	2.11	[0.81; 5.66]	119	9
Pleuromutilin - ADDs per 100 finishers	0.0923	0.72	[0.46; 1.05]	119	numeric

a: For categorical variables with more than two levels, the p-value originates from the LRT against an empty model. For the remainder, the p-value originates from a univariable logistic regression

b: No. of observations that are neither "no reply" nor "not relevant". The min. number required to be considered for inclusion in the multivariable analysis was set to 113 (125 obs. minus 10%)

c: Smallest number of obs. in a group when cross-tabulated with LA-MRSA status

d: OR per log10 increase

e: Mainly driven by the difference between North and Bornholm: OR=0.24 [0.06; 0.97], p=0.0403. (No other contrasts resulted in p<0.05)

f: Only included in the second approach in the multivariable analysis, where single observations were deleted to eliminate cells in cross-tabulations with no observations

g: OR per 500 pigs

h: OR per 100 m distance

S3 Table: Results of multivariable analysis in study 1

Variable	P-value	OR	[95% CI]
Sow herds - model 1			*AIC: 40.86*
Use of wet feed in the gestation unit	0.00893	12.6	[2.3; 11.7]
Herd size (log10(pigs present per day))	0.01585	81.8	[3.9; 640]
Sow herds - model 2			*AIC: 40.31*
No cleaning of aisles after moving pigs	0.02131	0.04	[0.00; 0.42]
Herd size (log10(pigs present per day))	0.00354	152.9	[8.10; 8,183.12]
Herds without sows			*AIC: 101.36*
Average no. of visitors per month	0.00838	0.56	[0.34; 0.80]
Rat control – municipality/region (y/n)	0.00688	0.19	[0.06; 0.64]
Herd size (log10(pigs present per day))	0.01817	3.78	[1.26; 11.86]

5.2. Discussion (Part A)

Many of the potential risk factors identified in univariable analysis in study 1 in manuscript I were also associated with herd size, and therefore it was not possible to conclude, whether herd size itself or factors related to herd size were the true risk factors for farms having status as LA-MRSA positive. This is a commonly reported problem in risk factor studies [153,165]. Specifically for Danish pig herds, a relation between herd size, stocking density and pig density in the surrounding area have been reported [165], but these factors were not associated with LA-MRSA status in the present study. In manuscript I, it was decided to report all factors associated with both LA-MRSA status and herd size, rather than just concluding that LA-MRSA status was associated with herd size, despite the risk of misinterpretation. For economic reasons, most farmers will probably not be willing to markedly change the size of their herd, whereas the associated management procedures might be easier to adjust, and therefore it is still very important to clarify what characterize these herds [165].

Many of the questions included in the questionnaire used for the study (the full questionnaire is available in Danish in Appendix II), were not directly related to introduction of LA-MRSA, and thus one could argue, that it might have been unlikely to observe an effect of these. However, many of these factors, e.g. use of zinc, cleaning and sorting of pigs, were hypothesized to influence establishment or spread of LA-MRSA within the herd, and thus still might influence the overall LA-MRSA status of the farm.

Factors not included in the investigation, which might have been of relevance in relation to the risk of introduction of LA-MRSA, include number of trucks visiting the premises per month, frequency of introduction of new pigs (currently only number of suppliers and number of animals received per year were included), as well as the use of temporary workers. Also, in the few open questions included in the questionnaires, the farmers themselves had lots of different comments and theories about LA-MRSA, of which it might be of interest to include some in another study, e.g. use of disposable gloves.

The questionnaire also included questions about use of probiotics and dust reducing initiatives, which both sometimes have been mentioned as potential interventions against LA-MRSA, albeit any probiotics currently used most likely would be aimed at improving the pigs' gut flora. However, these were only rarely used in the herds included in the study and therefore it could not be investigated whether this had any impact on the herd-level LA-MRSA status.

It might be of interest to further investigate several of the factors identified in univariable analysis in a set-up, where herd size is controlled for. This includes the use of wet feed, which had some of the highest ORs in the study, and also seems relevant seen from a biological perspective. Also the results for some of the factors, that only occurred infrequently and therefore could not form the basis for any firm conclusions, could warrant further investigation. This is for example the case for natural ventilation, which was

associated with negative LA-MRSA status, but did only occur in six herds. Differences in ventilation and air flow are interesting in relation to LA-MRSA, seen in the light of that LA-MRSA positive pigs have been able to lose LA-MRSA when inserted in free-range production [28]. Furthermore, it could also have been interesting to explore, whether there was any effect of which veterinarian or clinic the herds used (both in relation to antimicrobial use and risk of potentially transmitting LA-MRSA between herds). However, most veterinarians were only used by one of the herds in the present dataset, so to further investigate this, a much bigger dataset is required.

6. Part B: Spread and control of LA-MRSA within a pig herd

6.1 Manuscript II

A mechanistic model for spread of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) within a pig herd

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PLOS ONE

RESEARCH ARTICLE

A mechanistic model for spread of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) within a pig herd

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Abstract

Before an efficient control strategy for livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) in pigs can be decided upon, it is necessary to obtain a better understanding of how LA-MRSA spreads and persists within a pig herd, once it is introduced. We here present a mechanistic stochastic discrete-event simulation model for spread of LA-MRSA within a farrow-to-finish sow herd to aid in this. The model was individual-based and included three different disease compartments: susceptible, intermittent or persistent shedder of MRSA. The model was used for studying transmission dynamics and within-farm prevalence after different introductions of LA-MRSA into a farm. The spread of LA-MRSA throughout the farm mainly followed the movement of pigs. After spread of LA-MRSA had reached equilibrium, the prevalence of LA-MRSA shedders was predicted to be highest in the farrowing unit, independent of how LA-MRSA was introduced. LA-MRSA took longer to spread to the whole herd if introduced in the finisher stable, rather than by gilts in the mating stable. The more LA-MRSA positive animals introduced, the shorter time before the prevalence in the herd stabilised. Introduction of a low number of intermittently shedding pigs was predicted to frequently result in LA-MRSA fading out. The model is a potential decision support tool for assessments of short and long term consequences of proposed intervention strategies or surveillance options for LA-MRSA within pig herds.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) are a group of *S. aureus* that have acquired the *MecA* or *MecC* gene, which make them resistant to most β-lactam antibiotics [1]. Three main groups of MRSA exist. Hospital-acquired MRSA (HA-MRSA) was identified in the late 1980s and was the dominant source of MRSA infections until community-acquired MRSA (CA-MRSA) emerged in the mid-1990s [2]. Livestock-associated MRSA (LA-MRSA) in humans was identified for the first time in the Netherlands in 2005 [3,4].

The pig population is the main reservoir for LA-MRSA, but LA-MRSA are also found in a wide range of other animals, including cattle, horses, chickens, turkeys, rats, dogs and cats

and analysis, decision to publish, or preparation of the manuscript.

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[2,5±9]. The majority of LA-MRSA strains harbor *tetM* and sometimes also *tetK* [10,11], which causes resistance to tetracyclines, the most used antimicrobial group in the Danish pig production [5]. Other resistance genes are often present in LA-MRSA as well, in addition to the zinc resistance determinant *czrC* [12±14].

Like other *S. aureus*, LA-MRSA is an opportunistic pathogen in humans, where it colonizes the anterior nares. Only a minority of humans exposed to LA-MRSA become carriers and of these most will be asymptomatic carriers. However in those susceptible, LA-MRSA is capable of causing a large variety of conditions, ranging from mild skin and soft tissue infections to more severe conditions, e.g. pneumonia, meningitis and septicemia [15].

The majority of humans identified as LA-MRSA carriers have either been farm workers, veterinarians or members of households including farm workers/veterinarians. Thus, the main routes of transmission are assumed to be direct animal contact or direct exposure to air within the barns or indirect animal contact through close contact with individuals having direct animal contact [4,16].

In recent years, LA-MRSA has received considerable attention in Denmark due to an increased number of individuals being identified as carriers of this pathogen, albeit this partly could be explained by a revision of the national sampling guidelines causing more people at high risk of being carriers to be tested. In 2015, LA-MRSA CC398 accounted for 18% (208/1,147) of all reported MRSA infections in Denmark [5]. Nevertheless, compared to other European countries, the overall MRSA prevalence in Denmark remains low [17]. However, with 30.9 million pigs slaughtered or exported in 2015 [5], the national pig population constitute a potential LA-MRSA reservoir of a considerable size. In the last screening conducted by the Danish Veterinary and Food Administration in 2016, LA-MRSA was detected in 88% of randomly selected production herds [18].

Before the implementation of a national control strategy can be decided upon, it is essential to understand how LA-MRSA spreads and persists within a pig herd, once it is introduced. For that purpose, we built a mechanistic Monte Carlo simulation model for spread of LA-MRSA within an integrated pig herd. This model can be used for studying the colonization dynamics of LA-MRSA and for assessing the short and long term consequences of proposed interventions against LA-MRSA at farm level in terms of efficiency and cost-effectiveness, and thus be used as decision support before the implementation of these. It can also be used for investigating how a cost-effective surveillance system for early detection of LA-MRSA on a farm and subsequent decontamination could be designed. To the best of our knowledge, this is the first individual-based simulation model for spread of LA-MRSA within a pig herd to be described.

The objective of this study is to develop a model to aid a better understanding of the dynamics of LA-MRSA spread within an integrated pig farm following different routes of introduction.

Materials and methods

A dynamic mechanistic Monte Carlo simulation model for the spread and persistence of LA-MRSA within a pig herd was built in R version 3.2.2± ªFire Safetyº [19]. The model is individual-based and uses discrete time-steps set to one day each.

Herd model

Herd type and size. The herd model represents an integrated sow herd with all age groups from farrow-to-finish at one site. We aimed at modelling a typical Danish medium-sized production farm, comprising 500 sows and with an annual production of 15,400 slaughter pigs

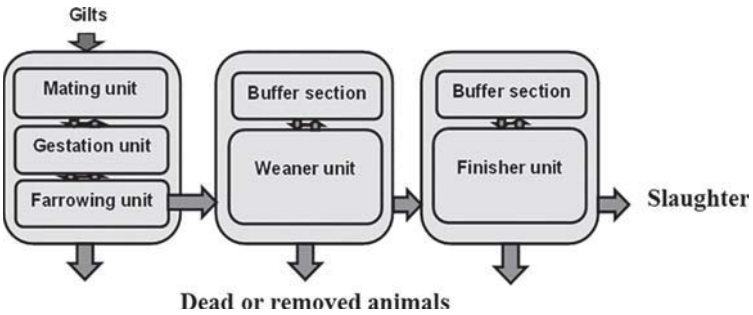

Fig 1. Flow between stable units in a simulated Danish integrated herd.

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[20,21]. Since the majority of Danish integrated herds purchase gilts from other herds [22], we included purchase of these in the model. It was assumed that the herd relied solely on artificial insemination and thus there was no influx of boars.

Farm design. In the model, pigs were housed in five different units; a sow barn containing three units: 1) a mating and control unit, 2) a gestation unit, 3) a farrowing unit; and two separate barns containing 4) a weaner unit and 5) a finisher unit, respectively (Fig 1). The weaner unit and the finisher unit both included a buffer section, where pigs were housed if they were not ready to be moved to the finisher unit or to be sent for slaughter together with the rest of their batch. With the exception of the gestation unit, each stable unit was divided into several different sections (rooms), where each section housed a varying number of pens depending on the age group (Table 1). Pigs were moved between the units according to age (Table 1). Gilts awaiting first insemination were housed in a separate section in the mating unit, whereas sows awaiting return to oestrus before re-insemination were housed together with other sows in the mating unit awaiting service. In the farrowing unit, it was assumed that sows selected as nursery sows (foster dams) were moved to the section where the piglets to be nursed were born.

Production cycle. We simulated a farm with weekly batch production in 21 sow batches and all-in/all-out production on section level. One full sow production cycle (mating, gestation, farrowing and nursing) was assumed to take 147 days (S1 Fig). At start of simulation each sow batch consisted of 23±26 sows of different ages and parities, which were at the same stage in the sow cycle (S1 Table).

Re-insemination of sows. It was assumed that sows were ready to be inseminated five days after weaning. The probability of insemination failure was 0.12, where sows to be re-inseminated were selected through a binomial process [23]. For simplification, it was assumed

Table 1. Housing in different stable units in a hypothetical farrow-to-finish pig herd with 500 sows.

	Mating unit	Gestation unit	Farrowing unit	Weaner unit	Finisher unit
Time spent in the unit	Day 1–33 in each sow cycle	Day 34–113 in each sow cycle	Sows: Day 114–147 Piglets: Day 1–28	Day 29–77	Day 78-slaughterage
Pigs in the unit	Sows, gilts	Gestating sows	Sows + piglets	Weaners	Finishers
Sectioning in the unit	Full	None	Full	Full	Full
System within the unit	Individual housing of sows	Loose-housing	Individual housing with piglets	Max. 30 pigs per pen	Max. 15 pigs per pen
	Max. 5 gilts per pen	One pen per batch			
No. of sections	5 + 1 for gilts	1	5	8 + 1 buffer	14 + 1 buffer
No. of pens per section	40 (12 for gilts)	12	35	14 (3 in buffer)	24 (10 in buffer)
Snout contact btw. neighboring pens	Yes	Not relevant	Yes	Yes	Yes

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that both return of oestrus and lack of pregnancy without return to oestrus would be discovered three weeks after insemination. Consequently, re-insemination of the majority of the open sows would be attempted, while the remaining ones would be selected for strategic culling based on parity. The probabilities of re-insemination being attempted are given in S1 Table. Re-inseminated sows would be permanently moved to the sow batch, where the other sows in the batch had been inseminated in the same week as them.

Use of nursery sows (foster dams). Litter size (live-born piglets only) was drawn from a normal distribution and rounded into integers (S2 Table). For litters consisting of more than 14 piglets, the surplus piglets were foster bred by a nursery sow. A two-step nursery sow system was used; surplus piglets from several sows were given to a sow that until then had been nursing her own 8-day old piglets. The 8-day old piglets from this sow were moved to a second sow, whose own piglets were ready to be weaned. After nursery piglets had been weaned, nursery sows would remain part of the sow batch to which they were moved upon selection as nursery sows.

Removal of sows. Four different processes for culling/deaths of sows were incorporated in the model. Strategic culling took place either immediately after weaning or after insemination had failed to result in pregnancy. Deaths or emergency culling could occur anytime with a probability depending on parity and current stage in the sow cycle (S3 Table). In all processes, the probability of a sow getting removed increased with the number of parities. At the very latest the sows were culled after their eighth litter had been weaned.

Replacement of sows. Gilts were included in the herd at least seven weeks prior to their first insemination, which was assumed to take place, when they were at least 243 days old, which is within the age range generally recommended in Denmark (230±260 days) [24]. The size of the gilt stock on the farm was evaluated on a weekly basis, and new animals were added when needed. Three days prior to insemination, the size of the sow batch ready for insemination was evaluated, and if it consisted of less than 23 pigs, new gilts were added to reach this number, in order to maintain a constant supply of piglets.

Weaning and placement into pens. Piglets were weaned after four weeks. Since it is common practise on many Danish farms to sort the pigs according to size, piglets from different litters were randomly mixed during movement to the weaner unit, and again upon entering the finisher unit. It was assumed that pigs were selected for slaughter twice a week, and that 20% of the pigs in the batch would be ready for slaughter earlier or later than the rest of the batch (S4 Table). In the event of a stable section running full, for simplicity and in order to ensure stability of the model, it was assumed that the surplus pigs was sold or slaughtered.

Use of buffer sections in the weaner and finisher unit. It was assumed that for a certain proportion of the weaners and finishers, the pigs would not be big enough to follow the rest of the batch when they were moved from the weaner to the finisher unit or sent for slaughter. These pigs were assumed to be moved to a buffer section within either the weaner or finisher unit, where they might be mixed with pigs from other batches. In the weaning unit, 20% of weaners scheduled to leave the unit would remain in the buffer unit for another week. These pigs were sampled randomly and each pig could be repeatedly selected for an additional one week stay in the buffer stable again in up to three consecutive samplings. In the finisher unit, the remaining pigs in a section would be moved to the buffer stable, if the number of animals left within a section decreased to below a certain threshold (calibrated to 150 animals) and the animals were at least 158 days old.

Removal of piglets, weaners and finishers. The probability of death or removal of piglets was age-dependent with a higher probability of removal during the first days of their lives (S5 Table). For weaners and finishers, we assumed a constant daily probability of death or removal (S5 Table).

Epidemic model

Definitions. In the present model, we did not take into consideration, whether the pigs are truly colonized by LA-MRSA or only contaminated. Instead we used the terms intermittent shedder (IS) and persistent shedder (PS) to define a pig, which either temporarily or permanently harbours LA-MRSA in the nasal cavity in levels detectable by the method used by Broens et al. 2012 [25], and is able to spread LA-MRSA to another pig. For simplification, it was assumed that all pigs harbouring LA-MRSA in the nasal cavity were equally likely to spread it to other pigs. It was also assumed that `recovery' implies that the animal is no longer shedding LA-MRSA, but that no immunity towards re-acquisition was acquired. All parameters have been based on data for LA-MRSA CC398, when available in the literature. Where no published data for CC398 were available, parameters have been based on LA-MRSA belonging to other clonal complexes or general estimates for *S. aureus*. In the rest of this text, MRSA will refer to LA-MRSA unless stated otherwise.

Structure. The infection model was structured as an SIS compartmental model with one susceptible stage and two separate infectious stages for IS and PS (Fig 2) and one overall transmission rate (β) for going to one of the infectious stages. The probability of pigs becoming PS was assumed to depend on the infectious pressure in their environment as well as host-related factors. A proportion (mean: 24%) of randomly selected pigs (equal to maximum q on Fig 2) was assigned the potential to become PS (assumption based on [26]). The probability of these pigs actually becoming PS after exposure (q) was dependent on the prevalence of pigs shedding MRSA in the section, where they were housed. For simplicity, we introduced a prevalence threshold (most likely value: 70%), where the threshold level was estimated from [26]. Two different probabilities were applied for below (most likely value: 10%) or above the threshold (most likely value: 75%) (S6 Table). Both probabilities and the prevalence threshold were drawn from pert distributions. The proportion of pigs with the potential to become persistent shedders was sampled from a normal distribution (S6 Table). It was assumed that pigs stopped shedding after a given number of days (D_{IS} or D_{PS}) and went back to being susceptible. However for the vast majority of the PS, this does not happen.

Transmission parameters. The transmission rates for MRSA used in the model were based on the results of a transmission study conducted on four Dutch farms, where pigs were followed from farrow to finish [25] (S7 Table). In this study, the transmission rates were determined separately for pre-weaning and post-weaning pigs. In our model, the transmission rates estimated in the Dutch study [25] for post-weaning pigs were used both for weaners and finishers, as well as for transmission between gilts and sows in the mating unit or gestation unit,

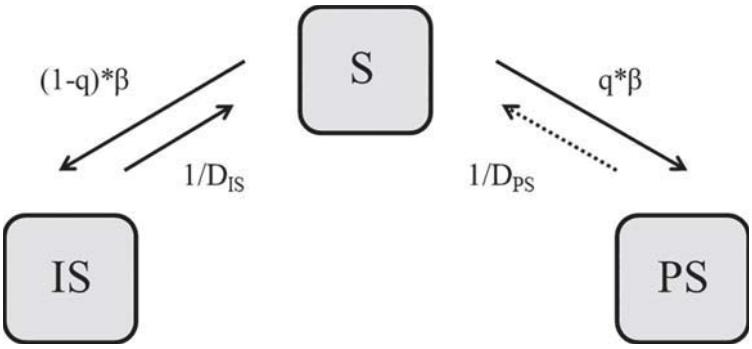

Fig 2. Infection model for MRSA. S = Susceptible, IS = Intermittent shedder, PS = Persistent shedder, β = Overall transmission rate, q = fraction of shedders becoming persistent shedders, D_{IS} = Duration of shedding for intermittent shedders, D_{PS} = Duration of shedding for persistent shedders, $D_{PS} >> D_{IS}$.

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whereas the pre-weaning rates were used for transmission between pre-weaning pigs and for transmission from sow to offspring after day 1.

Due to the uncertainty related to the transmission parameters, we decided to simulate all scenarios three times using one of three different sets of transmission rates each time (S7 Table) in an attempt to model both worst and best case scenarios for every situation plus a scenario in between. Since the transmission rates among pre- and post-weaning pigs were also determined both with and without use of risk antimicrobials in the Dutch study [25], the highest and the lowest set of rates used in our model were based on their results. Use of risk antimicrobials was defined on pen level as at least one pig within the pen receiving tetracyclines or β-lactam antibiotics within a time interval between samplings [25]. The set of medium rates were created based on the average values of the two other sets, to represent a farm with a moderate use of antibiotics, relative to the two other levels (S7 Table). The transmission rates used for the individual iterations were sampled from pert distributions.

Transmission of MRSA from sows to new-born piglets on the day of farrowing was modelled as a simple probability of the offspring being MRSA positive given it had been born by an MRSA positive dam, where the probability was sampled from a pert distribution with a probability interval based on the results of a study of the effect of sow status on piglet colonisation age [27] (S7 Table). The probability of piglets born by an MRSA-negative sow becoming MRSA shedders during their first day of life changed depending on the presence or absence of MRSA shedders within the section. If no shedders were present, the probability was set to zero. Otherwise, a probability drawn from a pert distribution (based on [27]) was used (S7 Table). After the first day in the piglets' life, the pre-weaning transmission rates estimated by [25] were used both for spread of MRSA between piglets being nursed by the same sow and for spread between the sow and its piglets.

Four different transmission routes for spread of MRSA between pigs were modelled: 1) Transmission within the same pen; 2) Transmission between pens within the same section; 3) Transmission between sections; 4) Transmission between stables. The transmission rates for within pen and between pen transmission were based on data from [25] (S7 Table; calculations described in S1 Appendix). No data for transmission between sections or stables were available, and these rates will naturally depend on local conditions, e.g. the design of the stables, ventilation system and biosecurity measures in place. In our model, the spread between sections and stable units were assumed to be a fraction of the between-pen transmission rate used in the scenario in question. It was assumed that more handling of animals would take place in the farrowing and mating units compared to in the other units. Therefore, the fraction of spread between pens applied for spread between sections within these units was assumed to be 0.20, while 0.15 in the other units (S7 Table). We did not differentiate between spread from different sources e.g. pigs, humans, equipment, dust.

Transmission between pigs within a given unit was assumed to be density-dependent, i.e. the contact rate between pigs is assumed to be dependent on the number of pigs within the entity (pen, section or unit). The probability of a given pig (pig_j) becoming an MRSA shedder as a result of contamination from pigs within the entity, where it was housed, was given by:

$$\mathrm{Prob}_{E(j)} = 1 - e^{-\beta_{Ej} * \Delta T * \frac{I_{Ej}}{N_{Ej}}}, \quad (1)$$

where β_{Ej} is the within entity transmission rate for transmission of MRSA during a time step; I_{Ej} is the number of infectious pigs within the entity, where pig_j is housed, during that time step; ΔT is the difference in days between the current and previous time step (which was always equal to 1); and N_{Ej} is the total number of pigs within the entity, where pig_j is housed during that time step. Entity can be equal to: pen (WP = spread within pen); section (BP = spread

between pens within the same section); unit (BSe = spread between sections within the same unit) or farm (BSt = spread between stable units within the same farm).

For each susceptible pig (pig_j) the total daily probability of becoming an MRSA shedder ($Tot_{ProbInf(j)}$) during a time step was calculated as:

$$Tot_{ProbInf(j)} = 1 - ((1 - Prob_{WP(j)}) * (1 - Prob_{BP(j)}) * (1 - Prob_{BSe(j)}) * (1 - Prob_{Bst(j)})), \quad (2)$$

where $Prob_{WP}$, $Prob_{BP}$, $Prob_{BSe}$, and $Prob_{BSt}$ are the probabilities of becoming shedder as a result of within-pen, between-pen, between-section and between-stable spread, respectively.

Based on human studies we assumed that IS and PS also constituted two distinct groups in pigs with distinctly different durations of shedding [28]. Duration of shedding for IS was sampled from a pert distribution based on a transmission study carried out under experimental conditions [29] (S2 Table). It was assumed that PS had no probability of recovery during the first 100 days of shedding and thereafter a 0.01 probability of recovery (selected for simplification, based on periods of 84 and 154 days used for carriage classification in two human studies [28,30]). The assumption of no recovery was based on the relatively short lifespan of slaughter pigs and reports of humans carrying the same *S. aureus* strain for up to eight years [31].

Model output and validation

Model run. The model was run for six years following a burn-in period of four years before MRSA was introduced. The length of the burn-in period was based on the time needed for the number of pigs to stabilise, after simulation had been initiated.

The minimum number of iterations needed was determined, based on when convergence had been reached, assessed as the number of iterations needed for the variance of the total prevalence of MRSA-positive pigs in the herd at the end of run-time to stabilize (S2 Fig). Based on this 200 iterations were assessed to be enough to reach convergence. Nevertheless, the model was run in 500 iterations per scenario, in order to ensure higher stability of the outcomes, because the model was run with different sets of transmission rates and we expected the stability to vary.

Introduction of MRSA. In order to investigate spread and persistence of MRSA following different scenarios of MRSA introduction in an MRSA-free herd, various introductions were simulated: 1) Single or multiple introductions (fortnightly repeated for three months); 2) Introductions in different age groups (gilts, weaners or finishers); 3) Introductions of various numbers of shedders (1, 3, 10, 50 or 100) and; 4) Introduction of IS or PS. Not all combinations of these four parameters were modelled and only the most interesting results are presented in this paper.

Output parameters. The following model output parameters were used for comparison and visualisation of the scenarios modelled: 1) Development of the prevalence of MRSA shedders over time; 2) Proportion of iterations where MRSA fades out following introduction and time before fade-out; 3) MRSA prevalence in the different stable units.

Validation. Before the epidemic model was added, the herd model was validated using the rationalism method (assessing whether the output changed as expected following changes in the input values) and the tracing method (following individual animals over time) [32]. Production outputs simulated in the herd model were compared to production data from a sample of Danish herds [23]. The majority of the code for the model was also verified by an expert/another programmer (face validity).

Sensitivity and robustness analysis. The sensitivity analysis mainly focused on assessing the effect of duration of shedding, and how the status as IS or PS was assigned.

The pert distribution used for duration of shedding for IS was altered from a most likely value of 7.5 days (min = 1 day, max = 26 days) to 18 days (min = 6 days, max = 29 days) based

on data from the same study as the original value [29], where a different definition of when pigs were to be considered MRSA positive was applied (S2 Table). The ranges of values obtained based on either definition were both consistent with data from another study, where the duration of carriage ranged from 1±39 days [33].

Spread following introduction of one IS gilt were modelled with two different modifications of the concept of how to select pigs to become IS or PS: 1) All pigs will become IS upon exposure (no PS); 2) Whether pigs become IS or PS is solely determined by host-factors (no influence of the prevalence of MRSA shedders in the room).

Since all scenarios had already been modelled using three different sets of transmission rates, only one additional set of transmission rates was introduced during sensitivity analysis. In this set, the same transmission rates were used for both pre- and post-weaning pigs. The transmission rate used for within-pen transmission was sampled from a pert distribution based on values calculated from the results of an inoculation study [33], where mean values (for three groups of pigs) of the reported transmission rates and the lower and higher 95% confidence interval limits were used as the most likely value, minimum and maximum, respectively (S2 Table). Since only within-pen transmission rates were reported, it was assumed that the ratio of between-pen and within-pen transmission was the same as for the transmission rates used in the standard scenario. As a result, the between-pen transmission rate was calculated by multiplying the within-pen rate with the average ratio of between-pen and within-pen transmission rates in the lowest and highest sets of transmission rates (S2 Table).

The robustness of the model was assessed by changing more than one parameter at a time (S8 Table).

Results

Validation

Any unexpected output discovered using the tracing and rationalism method or during expert validation were further investigated and followed by corrections of the code, when needed.

Various production parameters were included in the herd model output and compared to real-life production data from swine Danish herds, in order to externally validate the model and check if the parameters were appropriately calibrated (S9 Table). The model output and real-life data generally had good agreement.

Spread of MRSA

When low or medium transmission was assumed, introduction of MRSA by one IS gilt was in most cases predicted to result in MRSA fading out (Fig 3A). When high transmission were assumed, then based on the median values, spread from the mating unit to other units was not observed, before enough time had elapsed for some of the gilts to be pregnant and be moved to the gestation unit (Fig 3B and 3C). After introduction in the farrowing stable the number of shedders saw a marked increase, followed by spread into the weaner unit and later into the farrowing unit (Fig 3). MRSA mainly seemed to be following the routes of the animals. However, if MRSA was introduced in the weaner unit or finisher unit, the simulations indicated that spread to the sow units was still likely to occur, despite animals not being moved backwards (S3 and S4 Figs). The later in the production process MRSA was introduced (gilts → weaners → finishers), the slower spread and thereby longer time before the prevalence in the stables units stabilized (Fig 3 and S3 and S4 Figs).

Following introduction of a PS instead of an IS into either stable unit, similar developments in median prevalence of MRSA shedders over time were predicted, except that in most cases MRSA was not predicted to fade out, when low or medium transmission rates were used (Fig

Legend added after publication:

Mat Gest Far Wean Fini

Mat = Mating unit
Gest = Gestation unit
Farr = Farrowing unit
Wean = Weaner unit
Fini = Finisher unit

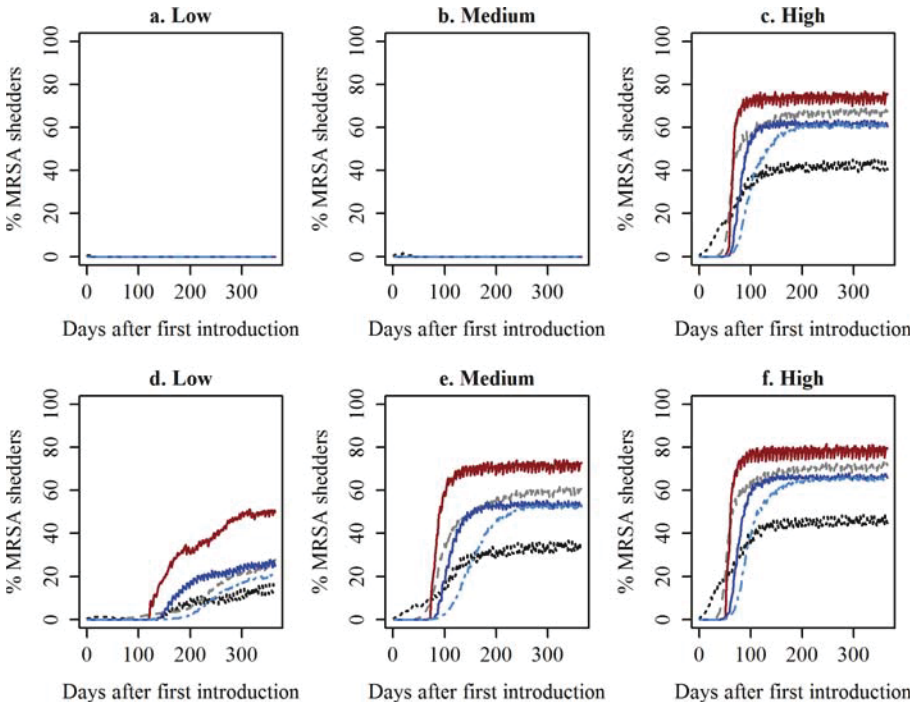

Fig 3. Development in the median prevalence of MRSA shedders following introduction of one MRSA shedding gilt. Predicted median prevalence over time following introduction of one intermittently (a-c) or persistently shedding gilt (d-f), when using low (a+d), medium (b+e) or high (c+f) transmission rates.
Mat = Mating unit, Gest = Gestation unit, Farr = Farrowing unit, Wean = Weaner unit, Fini = Finisher unit.

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3D). The proportion of MRSA shedders in the five different stable units, six years after introduction of an IS or a PS in the mating unit is illustrated in a violin plot in Fig 4. As seen from the distribution of the prevalences of MRSA shedders obtained in the 500 iterations (the width of the `violins'), MRSA seems to either fade out when introduced by an IS, or show a pattern similar to when introduced by a PS, where the observed prevalences clustered around the median.

The model predicts that when spread of MRSA kicks off, the predicted prevalence of MRSA shedders within each stable unit reaches an equilibrium (Fig 4). As expected, the age group in which MRSA was introduced had no marked influence on the equilibrium prevalence (S5 and S6 Figs).

Table 2 shows the total median proportion of MRSA shedders in the herd six years after various introductions, as well as the proportion of iterations, where MRSA faded out, including the number of days elapsed between introduction and fade-out. In general the higher transmission rate used the higher prevalence after stabilisation and the lower proportion of iterations, where MRSA fades out (Table 2). When the lower sets of transmission rates was applied, MRSA was predicted to be able to remain in the herd for years, and still eventually fade out. In theory, MRSA fade-out following introduction by a PS is in most cases only possible after the initial PS has been removed from the farm, and therefore MRSA could remain in the herd for a long time despite the infection not becoming established, if it was introduced by an animal with a long lifespan, e.g. a gilt (Table 2).

The introduction of more animals increased the probability of MRSA becoming established on the farm (S7 Fig and S10 Table), and shorter time passed before an equilibrium was reached

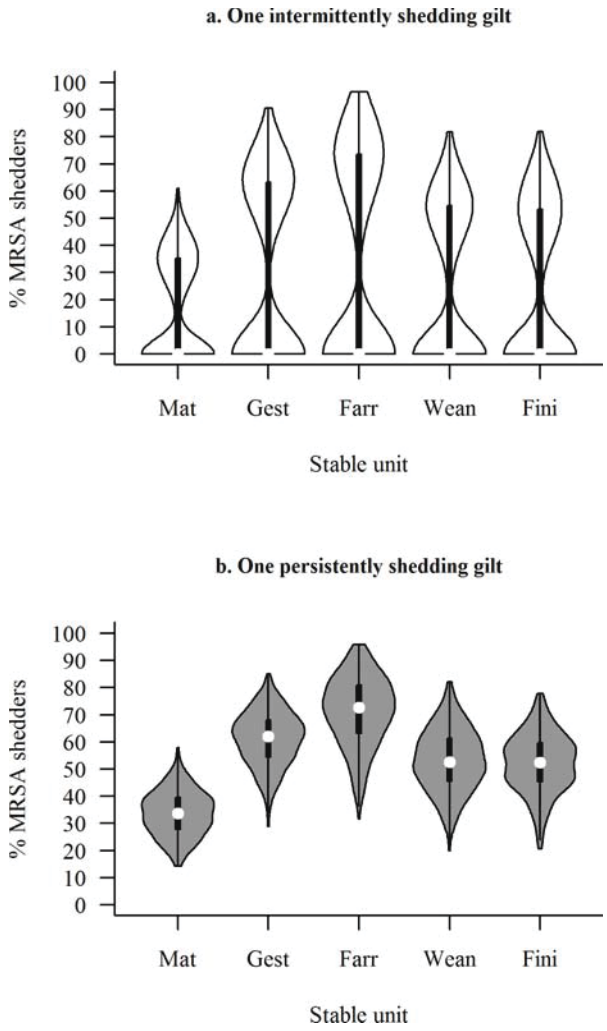

Fig 4. Violin plot of the prevalence following introduction of one gilt shedding MRSA intermittently or persistently. Predicted prevalence of MRSA shedders six years after introduction, when medium transmission rates were used (distribution of 500 iterations). The median prevalences are indicated by white dots. Mat = Mating unit, Gest = Gestation unit, Farr = Farrowing unit, Wean = Weaner unit, Fini = Finisher unit.

https://doi.org/10.1371/journal.pone.0188429.g004

(S8 Fig). However, already with the introduction of thirty instead of ten finishers, the time needed for the MRSA prevalence to stabilize was very similar (S8 Fig). When comparing single or multiple introductions, exemplified by the introduction of one, three or ten IS gilts either once or once every fortnight for three months, the patterns predicted were very similar, since the only major difference was an increased probability of fade-out following single introductions, in particular for one shedder only (S9 and S10 Figs and S10 Table).

Sensitivity and robustness analysis

When modelling introduction of one intermittently shedding gilt with different alternative parameterisations, increasing the duration of shedding led to an increased median prevalence, less variance and fewer iterations, where MRSA faded out (Fig 5 and S8 Table). Removing the possibility of any pigs becoming PS led to MRSA more frequently fading out. Modelling persistent carriage as only being dependent on host-related factors did lead to less cases, where

Table 2. Predicted prevalence and fade-out of MRSA in a simulated pig herd following single introductions.

Transmission rates	Introduction scenario	Shedder prevalence		Fade out	Duration	
		Median	5th-95th percentile	(% iterations)	Median	Range
Low	1 IS gilt	0.0	0–38.0	87.0	13.0	1–142
	1 PS gilt	0.0	0–0.01	88.4	507.0	469–557
	1 IS weaner	0.0	0–0	99.2	14.5	1–257
	1 PS weaner	0.0	0–0	95.4	150.0	11–658
	1 IS finisher	0.0	0–0	99.6	14.0	2–312
	1 PS finisher	0.0	0–0	98.4	94.0	1–425
Medium	1 IS gilt	0.0	0–68.6	51.0	13.0	2–100
	1 PS gilt	56.4	39.4–70.2	0.0	-	-
	1 IS weaner	43.9	0–69.0	46.0	11.0	2–347
	1 PS weaner	56.1	0–69.7	7.0	150.0	3–346
	1 IS finisher	0.0	0–68.5	58.4	15.0	1–314
	1 PS finisher	54.1	0.68.9	27.4	100.0	80–444
High	1 IS gilt	64.7	0–79.6	26.4	9.0	2–80
	1 PS gilt	67.0	48.2–79.4	0.0	-	-
	1 IS weaner	64.6	0–82.3	20.6	7.0	1–153
	1 PS weaner	68.0	54.5–80.3	0.4	100.5	9–192
	1 IS finisher	64.7	0–80.6	28.4	8.0	1–128
	1 PS finisher	67.8	51.1–78.8	0.0	-	-

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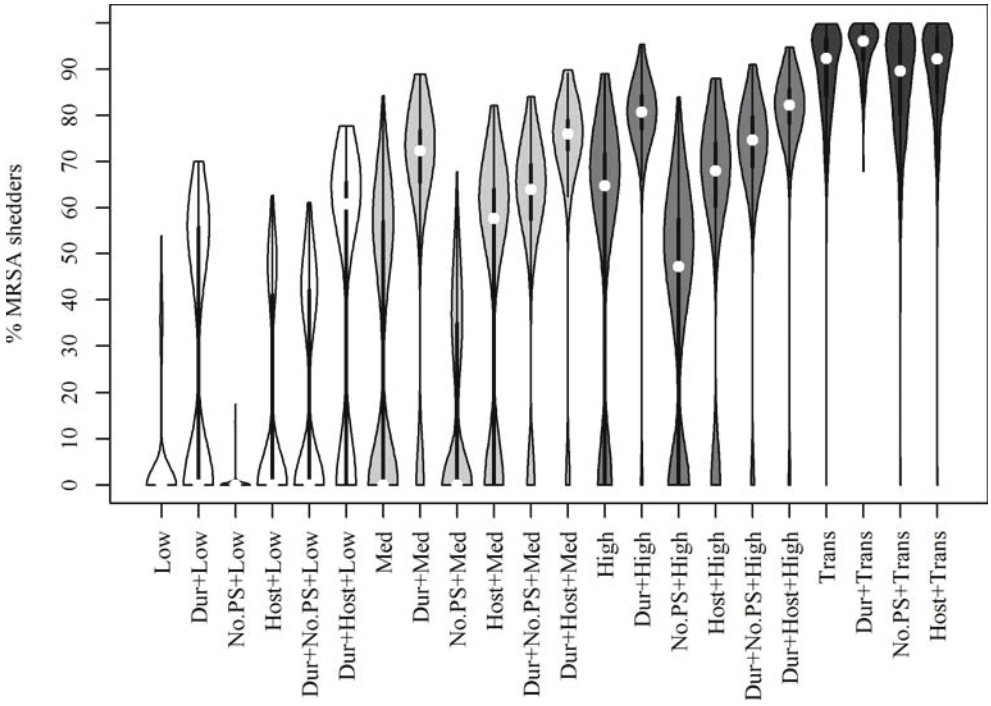

Fig 5. Results of sensitivity- and robustness analysis. Predicted prevalence six years after introduction of one intermittently shedding gilt (distribution of 500 iterations). Last part of each label indicates the transmission rate used. Dur = duration of shedding for IS altered, No.PS = no persistent shedders, Host = shedder type solely determined by host factors (no influence of prevalence in the room), Trans = transmission rates altered.

https://doi.org/10.1371/journal.pone.0188429.g005

MRSA faded out, compared to using the original distribution. The alternative set of higher transmission rates introduced in the robustness analysis for all age groups predicted higher median prevalence and less variation between the results of the different iterations, except when there was no persistent shedders.

Discussion

In the present study, we modelled the spread of MRSA between animals within a pig farm mechanistically. The observed effects of different simulated introductions were in line with what one would expect a priori. Our results show that once MRSA has become established in a herd, it will maintain a prevalence that varied depending on factors such as the pig unit and transmission rate, e.g. the median prevalence reaching up to 76% following introduction of one IS gilt when high transmission is assumed (Fig 3C). The variation in the within-herd prevalence in different age groups has been reported before ([2] and S11 Table). Many studies have reported an increase in MRSA prevalence after weaning, followed by a decline in the prevalence before slaughter age (S11 Table), but as expected there was variation and others did not observe any significant difference [34]. In a Swiss study, where individual pigs were followed over time, the highest proportion of pigs changing status from negative to positive were observed when piglets were from 1±14 days old, where the highest proportion of pigs changing status from positive to negative were observed in the last part of the finisher period (between 15±19 weeks and 21±25 weeks of age) [35]. Due to the parameterisation of our model, the prevalence was generally predicted to be highest in piglets in the farrowing unit, before decreasing in the weaner and finisher units, where it persisted at similar levels, albeit slightly lower in the finisher unit. This can be changed though and the model can relatively easily be calibrated to other prevalence levels within the different units by for instance adjusting the transmission rates, which the model is already prepared for. For the purpose of the current study, calibration for specific situations is not necessary. Nevertheless, this may be important when studying the impact of interventions to control MRSA within the herd and the success of these interventions given different MRSA within-herd prevalences.

Introduction of more MRSA shedders and multiple introductions led to faster spread. Despite the assumption of no use of risk antimicrobials (tetracyclines and β-lactam antibiotics) in the herd (and therefore use of the low transmission rates associated with this), introduction of MRSA shedders in a few cases (0.6±13.0%) still led MRSA to spread throughout the herd and become established. Thus a low antimicrobial usage within a herd may not always be sufficient to prevent MRSA from spreading and becoming established, once it has been introduced.

The observation of MRSA being able to fade out following introduction of a few IS, does not seem unrealistic given that during an investigation on Norwegian pig farms, 32 of 51 farms did not become MRSA positive, despite having positive suppliers [36]. For twelve farms, this was explained by the farms only being sporadically supplied from the infected farms, which is therefore comparable to the scenarios modelled.

In our model predictions, MRSA spreads relatively easy between the different units of the farm. MRSA is mainly spread forward in the production chain through movement of pigs, but spread to all units was also predicted when MRSA was introduced in the weaner or finisher section. This was a consequence of our assumptions, since to our best knowledge no between-compartment transmission rates for MRSA on pig farms have been published. Therefore we assumed the between-section and between-unit transmission rates to be a smaller fraction of the between-pen transmission rate, 0.15±0.20 and 0.02 respectively. The true risk of transmission will dependent on multiple local factors i.e. internal biosecurity and design and location

of stable units in relation to each other. However, given detection of MRSA in substantial levels in the air inside and outside pig barns [16,37] and the risk of carry-over with workers and equipment, we find it justified to assume that this spread could occur.

The transmission rates used were based on data from a study carried out at four Dutch farms [25]. As for all other studies based on a limited amount of animals, prudence is needed, when interpreting the results. Differences between farms regarding management, antimicrobial use and stable design will potentially influence the transmission rates. This can be reflected in the model by for instance adjusting the transmission rates to reflect different prevelance situations as discussed above.

During 2007±2012 the overall use of antimicrobials for farm animals in the Netherlands was reduced with 56% [38]. However in 2010, the year after the study the transmission rates originates from was conducted, the total antimicrobial use for pigs in the Netherlands was 35% higher than in Denmark [39]. With regard to the groups of drugs considered risk antimicrobials [25], especially the use of tetracyclines for pigs was markedly higher in the Netherlands, whereas some narrow spectrum penicillins were used more in Denmark [39]. On the other hand, in Denmark weaners may get prescribed zinc supplementation in the feed, whereas this is not allowed in the Netherlands [40]. This practise might also influence nasal carriage of MRSA, since there seems to be a genetic linkage between *mecA* and *czrC*, which is coding for zinc resistance [14,41,42]. Thus using transmission rates based on no use of zinc, as is the case in our model, might lead to an underestimation of the transmission frequency in the weaning units [43], whereas using transmission rates based on higher antimicrobial consumption might lead to overestimation.

In our model we used transmission rates based on naturally contaminated pigs housed in ordinary farms. Another approach would have been to rely on data from a transmission study, where pigs housed in animal experimental facilities had been inoculated with MRSA [33]. These transmission rates are considerably higher than the rates used in our model. However, despite the risk of underestimating the true rate of transmission, we believe that for our purpose, it will be more appropriate to use data from naturally contaminated pigs housed in an ordinary farm environment, since management practices, animal density and environmental spread play an important role in transmission [44].

The association between the MRSA status of the sow and the probability of piglets testing MRSA positive have been confirmed in several studies [45±47]. In our model, the probability of transmission from sow to new-born piglets was based on predictions from a study, where piglets had been sampled within one hour after birth and again after one day [47]. It has been suggested that piglets might get transiently rather than persistently colonized from their dam [45]. However this has not been taken into account in the model, meaning that the proportion of piglets becoming PS might be overestimated.

After considering different model structures, we chose to assume that IS and PS constituted two distinct groups in pigs, based on evidence in humans [28] and potential evidence in pigs [26].

The proportion of pigs assumed to have the potential to become PS was based on a study at 20 Danish pig farms, where the proportion of pigs persistently testing positive for *S. aureus* was 24% [26]. In a study conducted at four Belgian farms, no PS was found at two mildly contaminated farms (17±33% IS), while 25±92% of sows at two highly contaminated farms did persistently test MRSA positive (all the remaining sows at these two farms were IS) [48]. Therefore it seems reasonable that we introduce a prevalence dependency in the model, and thereby take the effect of the contamination level into account.

Results of the sensitivity analysis demonstrated that increasing the duration of carriage led to equilibrium occurring at a markedly higher prevalence compared to the default values (Fig 5 and S8 Table). This was also the case in the robustness analysis, when increased duration was

combined with parameter changes that otherwise were expected to decrease the equilibrium prevalence. Altogether, these results indicate that duration of carriage has a considerable influence on the results obtained. This duration may potentially be influenced by many different factors, such as dose of exposure, genetics and the nasal microbiome of the pig [26,49±52]. Removing persistent shedders in the sensitivity analysis interestingly also had a markedly effect, which indicates that there might be some potential in control options targeted at this particular subgroup of animals. As expected, increasing the transmission rate had a pronounced effect and resulted in higher equilibrium prevalences and in some cases, the predictions reached 100% (Fig 5 and S8 Table).

As for all simulation models, the precision, uncertainty and validity of the model predictions will depend on the availability and quality of data for parameterisation of the model and the assumptions and simplifications made. Therefore prudence is called for when interpreting model predictions, which only should be taken as indicative of how MRSA might spread. Despite these limitations, our simulation model can assist in: highlighting knowledge gaps for future research; providing insights in the dynamics of spread of MRSA; the study of possible hypothetical scenarios; and investigation of possible intervention strategies or surveillance options.

Supporting information

S1 Appendix. Estimation of transmission rates.
(PDF)

S1 Table. Model input: Probabilities of sow parities at simulation start and re-insemination attempts.
(PDF)

S2 Table. Model input: Litter size, duration of shedding and transmission rates used for sensitivity analysis.
(PDF)

S3 Table. Model input: Probability of removal of sows.
(PDF)

S4 Table. Model input: Assumed slaughter age distribution.
(PDF)

S5 Table. Model input: Removal of piglets, weaners and finishers.
(PDF)

S6 Table. Model input: Probability of pigs becoming persistent shedders.
(PDF)

S7 Table. Model input: Transmission rates and probabilities.
(PDF)

S8 Table. Model output: Results of sensitivity- and robustness analysis.
(PDF)

S9 Table. Model output: Simulated production parameters compared to Danish production data.
(PDF)

S10 Table. Model output: Predicted fade out of MRSA in a simulated pig herd and time elapsed between introduction and fade out following single or multiple introductions.
(PDF)

S11 Table. Summary of MRSA prevalence in different age groups in observational studies.
(PDF)

S1 Fig. The sow cycle modelled in a hypothetical farrow-to-finish herd.
(PDF)

S2 Fig. Model output: Convergence after introduction of one intermittently shedding gilt.
(PDF)

S3 Fig. Model output: Development in the median prevalence of MRSA shedders following introduction of one MRSA shedding weaner.
(PDF)

S4 Fig. Model output: Development in the median prevalence of MRSA shedders following introduction of one MRSA shedding finisher.
(PDF)

S5 Fig. Model output: Violin plot of the prevalence following introduction of one weaner shedding MRSA intermittently or persistently.
(PDF)

S6 Fig. Model output: Violin plot of the prevalence following introduction of one finisher shedding MRSA intermittently or persistently.
(PDF)

S7 Fig. Model output: Violin plot of the prevalence following introduction of one, ten or thirty finishers shedding MRSA intermittently.
(PDF)

S8 Fig. Model output: Development in the median prevalence of MRSA shedders following introduction of one, ten or thirty IS finishers.
(PDF)

S9 Fig. Model output: Violin plot of the prevalence following introduction of one, three or ten gilt shedding MRSA intermittently every fortnight for three months.
(PDF)

S10 Fig. Model output: Development in the median prevalence of MRSA shedders following single or multiple introductions.
(PDF)

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References

1. Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn C, et al. Structural Comparison of Three Types of Staphylococcal Cassette Chromosome mec Integrated in the Chromosome in Methicillin-Resistant *Staphylococcus aureus* Structural Comparison of Three Types of Staphylococcal Cassette Chromosome mec Integrated in the Chr. Antimicrob Agents Chemother. 2001; 45: 1323–1336. https://doi.org/10.1128/AAC.45.5.1323-1336.2001 PMID: 11302791
2. Crombé F, Argudín MA, Vanderhaeghen W, Hermans K, Haesebrouck F, Butaye P. Transmission Dynamics of Methicillin-Resistant *Staphylococcus aureus* in Pigs. Front Microbiol. 2013; 4: 57. https://doi.org/10.3389/fmicb.2013.00057 PMID: 23518663
3. Armand-Lefevre L. Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. Emerg Infect Dis. 2005; 11: 711–714. https://doi.org/10.3201/eid1105.040866 PMID: 15890125
4. Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin—resistant *Staphylococcus aureus* in pig farming. Emerg Infect Dis. 2005; 11: 1965–1966. https://doi.org/10.3201/eid1112.050428 PMID: 16485492
5. DANMAP. DANMAP 2015—Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark [Internet]. 2016. Available: http://www.danmap.org/~/media/Projektsites/Danmap/DANMAPreports/DANMAP2015/DANMAP2015.ashx
6. Friese A, Schulz J, Zimmermann K, Tenhagen B-A, Fetsch A, Hartung J, et al. Occurrence of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* in Turkey and Broiler Barns and Contamination of Air and Soil Surfaces in Their Vicinity. Appl Environ Microbiol. 2013; 79: 2759–2766. https://doi.org/10.1128/AEM.03939-12 PMID: 23417001
7. Leonard FC, Markey BK. Meticillin-resistant *Staphylococcus aureus* in animals: A review. Veterinary Journal. 2008. pp. 27–36. https://doi.org/10.1016/j.tvjl.2006.11.008
8. Nemeghaire S, Roelandt S, Argudín MA, Haesebrouck F, Butaye P. Characterization of methicillin-resistant *Staphylococcus aureus* from healthy carrier chickens. Avian Pathol. 2013; 42: 342–6. https://doi.org/10.1080/03079457.2013.805183 PMID: 23777220
9. van de Giessen AW, van Santen-Verheuvel MG, Hengeveld PD, Bosch T, Broens EM, Reusken CBEM. Occurrence of methicillin-resistant *Staphylococcus aureus* in rats living on pig farms. Prev Vet Med. 2009; 91: 270–273. https://doi.org/10.1016/j.prevetmed.2009.05.016 PMID: 19523703
10. Larsen J, Clasen J, Hansen JE, Paulander W, Petersen A, Larsen AR, et al. Co-presence of *tet (K)* and *tet (M)* in livestock-associated methicillin-resistant *Staphylococcus aureus* CC398 is associated with increased fitness during exposure to sub-lethal concentrations of tetracycline. Antimicrob Agents Chemother. 2016; 60: AAC.00426–16. https://doi.org/10.1128/AAC.00426-16 PMID: 27161637
11. Guardabassi L, Larsen J, Weese JS, Butaye P, Battisti A, Kluytmans J, et al. Public health impact and antimicrobial selection of meticillin-resistant staphylococci in animals. Integr Med Res. 2013; 1: 55–62. https://doi.org/10.1016/j.jgar.2013.03.011 PMID: 27873579
12. de Neeling AJ, van den Broek MJM, Spalburg EC, van Santen-Verheuvel MG, Dam-Deisz WDC, Boshuizen HC, et al. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. Vet Microbiol. 2007; 122: 366–72. https://doi.org/10.1016/j.vetmic.2007.01.027 PMID: 17367960
13. Guardabassi L, Stegger M, Skov R. Retrospective detection of methicillin resistant and susceptible *Staphylococcus aureus* ST398 in Danish slaughter pigs. Vet Microbiol. 2007; 122: 384–6. https://doi.org/10.1016/j.vetmic.2007.03.021 PMID: 17467199
14. Cavaco LM, Hasman H, Aarestrup FM. Zinc resistance of *Staphylococcus aureus* of animal origin is strongly associated with methicillin resistance. Vet Microbiol. 2011; 150: 344–348. https://doi.org/10.1016/j.vetmic.2011.02.014 PMID: 21411247

15. Cuny C, Wieler L, Witte W. Livestock-Associated MRSA: The Impact on Humans. Antibiotics. 2015; 4: 521–543. https://doi.org/10.3390/antibiotics4040521 PMID: 27025639

16. Bos MEH, Verstappen KM, van Cleef B a GL, Dohmen W, Dorado-García A, Graveland H, et al. Transmission through air as a possible route of exposure for MRSA. J Expo Sci Environ Epidemiol. 2016; 26: 263–269. https://doi.org/10.1038/jes.2014.85 PMID: 25515375

17. ECDC. Annual Epidemiological Report 2014. Antimicrobial resistance and healthcare-associated infections. European Centre for Disease Control and Prevention, Stockholm; 2015. Available: http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-annual-epidemiological-report.pdf

18. Danish Veterinary and Food Administration. Resultaterne af screening for husdyr-MRSA i svin i 2016 (in Danish). 2017. pp. 1–2. Available: https://www.foedevarestyrelsen.dk/Nyheder/Aktuelt/Documents/MRSAekspertgruppe—resultateneforekomstafhusdyr-MRSAisvin2016.pdf

19. R Core team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. 2015. pp. 275–286. Available: http://www.R-project.org/.

20. Danish Agriculture & Food Council. Statistics 2015 pigmeat. 2016. Available: http://www.agricultureandfood.dk/prices-statistics/annual-statistics

21. Danish Pig Research Centre. Program: Holddrift (in Danish). Danish Pig Research Centre; 2010. Available: http://vsp.lf.dk/Services/Beregninger/Planlaegningogstyringafholddrift.aspx

22. Hybschmann GK, Ersbøll AK, Vigre H, Baadsgaard NP, Houe H. Herd-level risk factors for antimicrobial demanding gastrointestinal diseases in Danish herds with finisher pigs: A register-based study. Prev Vet Med. 2010; 98: 190–197. https://doi.org/10.1016/j.prevetmed.2010.10.005 PMID: 21071103

23. Jessen O. Landsgennemsnit for produktivitet i svineproduktionen 2015 (in Danish). 2016. Available: vsp.lf.dk/~/media/Files/PDF—Publikationer/. . ./Notat_1611.pdf

24. SEGES. Løbning af søer (in Danish). 2017. Available: http://svineproduktion.dk/Viden/I-stalden/Management/Soeer/Loebning-af-soeer.

25. Broens EM, Espinosa-Gongora C, Graat EAM, Vendrig N, Van Der Wolf PJ, Guardabassi L, et al. Longitudinal study on transmission of MRSA CC398 within pig herds. BMC Vet Res. 2012; 8: 58. https://doi.org/10.1186/1746-6148-8-58 PMID: 22607475

26. Espinosa-Gongora C, Dahl J, Elvstrøm A, van Wamel WJ, Guardabassi L. Individual predisposition to *Staphylococcus aureus* colonization in pigs on the basis of quantification, carriage dynamics, and serological profiles. Appl Environ Microbiol. 2015; 81: 1251–6. https://doi.org/10.1128/AEM.03392-14 PMID: 25501475

27. Verhegghe M, Pletinckx LJ, Crombé F, Weyenberg S Van, Haesebrouck F, Butaye P, et al. Cohort study for the presence of livestock-associated MRSA in piglets: Effect of sow status at farrowing and determination of the piglet colonization age. Vet Microbiol. 2013; 162: 679–686. https://doi.org/10.1016/j.vetmic.2012.09.014 PMID: 23067724

28. van Belkum A, Verkaik NJ, de Vogel CP, Boelens HA, Verveer J, Nouwen JL, et al. Reclassification of *Staphylococcus aureus* nasal carriage types. J Infect Dis. 2009; 199: 1820–6. https://doi.org/10.1086/599119 PMID: 19419332

29. Broens EM, Graat E a M, van de Giessen AW, Broekhuizen-Stins MJ, de Jong MCM. Quantification of transmission of livestock-associated methicillin resistant *Staphylococcus aureus* in pigs. Vet Microbiol. Elsevier B.V.; 2012; 155: 381–388. https://doi.org/10.1016/j.vetmic.2011.09.010 PMID: 21963419

30. Nouwen JL, Ott A, Kluytmans-Vandenbergh MF, Boelens HA, Hofman A, van Belkum A, et al. Predicting the *Staphylococcus aureus* nasal carrier state: derivation and validation of a "culture rule." Clin Infect Dis. 2004; 39: 806–811. https://doi.org/10.1086/423376 PMID: 15472812

31. Vandenbergh MFQ, Yzerman EPF, Van Belkum A, Boelens HAM, Sijmons M, Verbrugh HA. Follow-Up of *Staphylococcus aureus* Nasal Carriage after 8 Years: Redefining the Persistent Carrier State. J Clin Microbiol. 1999; 37: 3133–3140. PMID: 10488166

32. Halasa T, Nielen M, Huirne RBM, Hogeveen H. Stochastic bio-economic model of bovine intramammary infection. Livest Sci. Elsevier B.V.; 2009; 124: 295–305. https://doi.org/10.1016/j.livsci.2009.02.019

33. Crombé F, Vanderhaeghen W, Dewulf J, Hermans K, Haesebrouck F, Butaye P. Colonization and transmission of methicillin-resistant *Staphylococcus aureus* ST398 in nursery piglets. Appl Environ Microbiol. 2012; 78: 1631–1634. https://doi.org/10.1128/AEM.07356-11 PMID: 22194292

34. Khanna T, Friendship R, Dewey C, Weese JS. Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. Vet Microbiol. 2008; 128: 298–303. https://doi.org/10.1016/j.vetmic.2007.10.006 PMID: 18023542

35. Bangerter PD, Sidler X, Perreten V, Overesch G. Longitudinal study on the colonisation and transmission of methicillin-resistant *Staphylococcus aureus* in pig farms. Vet Microbiol. Elsevier B.V.; 2016; 183: 125–134. https://doi.org/10.1016/j.vetmic.2015.12.007 PMID: 26790945

36. Grøntvedt CA, Elstrøm P, Stegger M, Skov RL, Skytt Andersen P, Larssen KW, et al. Methicillin-Resistant *Staphylococcus aureus* CC398 in Humans and Pigs in Norway: A "One Health" Perspective on Introduction and Transmission. Clin Infect Dis. 2016; 63: 1431–1438. https://doi.org/10.1093/cid/ciw552 PMID: 27516381

37. Schulz J, Friese A, Klees S, Tenhagen BA, Fetsch A, Rösler U, et al. Longitudinal Study of the Contamination of Air and of Soil Surfaces in the Vicinity of Pig Barns by Livestock-Associated Methicillin- Resistant *Staphylococcus aureus*. Appl Environ Microbiol. 2012; 78: 5666–5671. https://doi.org/10.1128/AEM.00550-12 PMID: 22685139

38. Speksnijder DC, Mevius DJ, Bruschke CJM, Wagenaar JA. Reduction of veterinary antimicrobial use in the Netherlands. The dutch success model. Zoonoses Public Health. 2015; 62: 79–87. https://doi.org/10.1111/zph.12167 PMID: 25421382

39. Bondt N, Jensen VF, Puister-Jansen LF, van Geijlswijk IM. Comparing antimicrobial exposure based on sales data. Prev Vet Med. Elsevier B.V.; 2013; 108: 10–20. https://doi.org/10.1016/j.prevetmed.2012.07.009 PMID: 22897857

40. Federation of Veterinarians of Europe. FVE position paper on the use of Zinc Oxide. 2014. Available: http://www.fsvf.fr/fs/FVE_mai_2014/c8oea-FVE_AG_Biarritz_mai2014_pt_9b_Draft_FVE_position_on_ZnO.pdf

41. Aarestrup FM, Cavaco L, Hasman H. Decreased susceptibility to zinc chloride is associated with methicillin resistant *Staphylococcus aureus* CC398 in Danish swine. Vet Microbiol. 2010; 142: 455–7. https://doi.org/10.1016/j.vetmic.2009.10.021 PMID: 19939591

42. Cavaco LM, Hasman H, Stegger M, Andersen PS, Skov R, Fluit AC, et al. Cloning and occurrence of *czrC*, a gene conferring cadmium and zinc resistance in methicillin-resistant *Staphylococcus aureus* CC398 isolates. Antimicrob Agents Chemother. 2010; 54: 3605–3608. https://doi.org/10.1128/AAC.00058-10 PMID: 20585119

43. Moodley A, Nielsen SS, Guardabassi L. Effects of tetracycline and zinc on selection of methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type 398 in pigs. Vet Microbiol. 2011; 152: 420–423. https://doi.org/10.1016/j.vetmic.2011.05.025 PMID: 21664077

44. Gibbons JF, Markey BK, Jahns H, Boland F, Abbott Y, Burns A, et al. Investigation of the persistence and transmission of MRSA CC 5 in pigs following intra-nasal inoculation. Vet Microbiol. 2013; 162: 771–778. https://doi.org/10.1016/j.vetmic.2012.10.001 PMID: 23116587

45. Burns A, Shore AC, Brennan GI, Coleman DC, Egan J, Fanning S, et al. A longitudinal study of *Staphylococcus aureus* colonization in pigs in Ireland. Vet Microbiol. 2014; 174: 504–513. https://doi.org/10.1016/j.vetmic.2014.10.009 PMID: 25465665

46. Weese JS, Zwambag A, Rosendal T, Reid-Smith R, Friendship R. Longitudinal Investigation of Methicillin-Resistant *Staphylococcus aureus* in Piglets. Zoonoses Public Health. 2011; 58: 238–243. https://doi.org/10.1111/j.1863-2378.2010.01340.x PMID: 20586995

47. Verhegghe M, Pletinckx LJ, Crombé F, Vandersmissen T, Haesebrouck F, Butaye P, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 in pig farms and multispecies farms. Zoonoses Public Health. 2013; 60: 366–74. https://doi.org/10.1111/zph.12007 PMID: 22925210

48. Verhegghe M, Crombé F, Pletinckx LJ, Haesebrouck F, Butaye P, Herman L, et al. Genetic diversity of livestock-associated MRSA isolates obtained from piglets from farrowing until slaughter age on four farrow-to-finish farms. 2014; 1–13. https://doi.org/10.1186/s13567-014-0089-4 PMID: 25217275

49. Graveland H, Wagenaar JA, Bergs K, Heesterbeek H, Heederik D. Persistence of livestock associated MRSA CC398 in humans is dependent on intensity of animal contact. PLoS One. 2011; 6. https://doi.org/10.1371/journal.pone.0016830 PMID: 21347386

50. Garcia-Graells C, van Cleef BAGL, Larsen J, Denis O, Skov R, Voss A. Dynamic of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* CC398 in Pig Farm Households: A Pilot Study. PLoS One. 2013; 8. https://doi.org/10.1371/journal.pone.0065512 PMID: 23741497

51. Ruimy R, Angebault CC, Lix Djossou F, Dupont C, Epelboin L, Jarraud S, et al. Are Host Genetics the Predominant Determinant of Persistent Nasal *Staphylococcus aureus* Carriage in Humans? J Infect Dis. 2010; 202: 924–934. https://doi.org/10.1086/655901 PMID: 20677941

52. Skallerup P, Espinosa-Gongora C, Jørgensen CB, Guardabassi L, Fredholm M. Genome-wide association study reveals a locus for nasal carriage of *Staphylococcus aureus* in Danish crossbred pigs. BMC Vet Res. 2015; https://doi.org/10.1186/s12917-015-0599-y PMID: 26612358

S1 Appendix: Estimation of transmission rates

The transmission rates (β) used in our simulation model was drawn from a pert distribution.

The values used as most likely, min. and max. value in the distribution was calculated from the reproduction ratio (R_0) and corresponding the 95% CI reported by Broens et al., 2012a, combined with the duration of carriage (D) used in the same study (estimated by Broens et al., 2012b) as:

$$\beta = R_0/D, \quad \text{(A1)}$$

where D was 17.4 days.

Broens et al., 2012a introduces the term total infection pressure (IP) defined as:

IP = IP within the pen + IP other pens + IP environment, (A2)

where IP within the pen = proportion of infectious pigs within the pen, IP other pens = proportion of infectious pen within the compartment, but not in the same pen, and IP environment = proportion of positive environmental wipes.

Based on, this Broens et al., 2012a also introduced a variable (pIP) to describe the relative effect of transmission through direct contact with pen mates:

pIP = IP within the pen /IP (A3)

R_0 reported by Broens et al., 2012a, when pIP=1 was used for estimation of within-pen transmission rates, whereas between- pen transmission rates were estimated based on R_0 when pIP=0.

References

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

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

Table S1: Model input: Sow parities at simulation start and re-insemination attempts

Parity	1	2	3	4	5	6	7	8
Probability matrix for sow parities at start of simulation	0.22	0.20	0.17	0.14	0.11	0.09	0.05	0.02
Probability of re-insemination being attempted*	1.0	0.7	0.6	0.5	0.4	0.3	0.2	0.0

*: Sørensen G, Christiansen MG. Udsætningsstrategi (in Danish). Danish Research Centre for pigs; 2013. pp. 1–8. Available: http://vsp.lf.dk/Viden/Reproduktion/Udsaetningsstrategi.aspx

Table S2. Model input: Litter size, duration of shedding and transmission rates used for sensitivity analysis

Parameter	Distribution	Mean	SD	Most likely	Min	Max
Litter size[1]	Normal	15.9	1.5	-	-	-
Shedding duration (days)[2]	Pert	-	-	7.5	1	26
Alt. Shedding duration (days	Pert	-	-	18	6	29
Alt. BetaWP[3]	Pert	-	-	1.57	0.86	2.92
Alt. BetaBP[4]	Pert	-	-	0.31	0.17	0.57

1: Jessen, 2016 [1]. The SD is an assumption. The numbers drawn were rounded to integers.

2: Broens, 2012a [2].

3: Within pen transmision rates (Alt. BetaWP) : Crombé et al., 2012 [3].

4: Calculated from Crombé et al, 2012 and BetaWP/BetaBP ratio in Broens et al, 2012b [3,4].

References

1. Jessen O. Landsgennemsnit for produktivitet i svineproduktionen 2015 (in Danish) [Internet]. 2016. Available: vsp.lf.dk/~/media/Files/PDF - Publikationer/.../Notat_1611.pdf

2. Broens EM, Graat EAM, van de Giessen AW, Broekhuizen-Stins MJ, de Jong MCM. Quantification of transmission of livestock-associated methicillin resistant *Staphylococcus aureus* in pigs. Vet Microbiol. Elsevier B.V.; 2012a;155: 381–388. doi:10.1016/j.vetmic.2011.09.010

3. Crombé F, Vanderhaeghen W, Dewulf J, Hermans K, Haesebrouck F, Butaye P. Colonization and transmission of methicillin-resistant *Staphylococcus aureus* ST398 in nursery piglets. Appl Environ Microbiol. 2012;78: 1631–1634. doi:10.1128/AEM.07356-11

4. Broens EM, Espinosa-Gongora C, Graat EAM, Vendrig N, Van Der Wolf PJ, Guardabassi L, et al. Longitudinal study on transmission of MRSA CC398 within pig herds. BMC Vet Res. 2012b;8: 58. doi:10.1186/1746-6148-8-58

Table S3. Model input: Probability of removal of sows

Parity	1	2	3	4	5	6	7	8
Daily probability of removal between insemination and farrowing[1]	0.00018	0.00014	0.00015	0.00024	0.00016	0.0002	0.00018	0.0003
Daily probability of removal after farrowing and before weaning[2]	0.00085	0.00079	0.00094	0.00103	0.00094	0.00076	0.00127	0.00085
Probability of removal immediately after weaning[3]	0.040	0.024	0.026	0.050	0.108	0.256	0.354	1.000

All probabilities were calculated based on data from Sørensen & Christiansen, 2013 and assumptions regarding duration of the different stages in the sow cycle.

1: The total probability for the whole period ranged from 0.016 to 0.034

2: The total probability for the whole period ranged from 0.025 to 0.034

3: The probability of removal immediately after weaning was adjusted for removal of sows experiencing insemination failure, where re-insemination was not attempted (according to the probabilities in table S1)

References

Sørensen G, Christiansen MG. Udsætningsstrategi (in Danish). Danish Research Centre for pigs; 2013. pp. 1–8. Available: http://vsp.lf.dk/Viden/Reproduktion/Udsaetningsstrategi.aspx

Table S4. Model input: Assumed slaughter age distribution

Age (days)	% pigs per batch
151	2
158	8
165	80
172	8
179	2

Table S5. Model input: Removal of piglets, weaners and finishers

	Total probability	Daily probability	
Piglets[1]	0.13	Day 1	0.03752
		Day 2	0.02010
		Day 3	0.01474
		Day 4-7	0.00670
		Day 8-14	0.00322
		Day 15-28	0.00094
Weaners[2]	0.03		0.00060
Finishers[2]	0.06		0.00007

1: Assumptions for daily probabilities of removal are based on [1].
2: Total probability based on [2]. Finisher mortality was calculated as total mortality in finisher herds minus mortality in weaner herds.

References

1. Pedersen LJ, Berg P, Jørgensen E, Bonde MK, Herskin MS, Knage-rasmussen KM, et al. Pattegrisdødelighed i DK (in Danish). 2010. Available: http://web.agrsci.dk/djfpublikation/djfpdf/Rapport_86_husdyrbrug_53458_samlet.pdf
2. Jessen O. Landsgennemsnit for produktivitet i svineproduktionen 2015 (in Danish). 2016. Available: vsp.lf.dk/~/media/Files/PDF - Publikationer/.../Notat_1611.pdf

Table S6. Model input: Probability of pigs becoming persistent shedders

Parameter	Distribution	Mean/ most likely value	Min	Max/SD
Probability of having the potential to become a persistent shedder	Normal	0.24	-	0.01
Prevalence threshold	Pert	0.70	0.50	1.00
Probability of persistent shedding below threshold	Pert	0.1	0.01	0.40
Probability of persistent shedding above threshold	Pert	0.75	0.50	1.00

Note: All parameters were based on assumptions inspired by Espinosa-Gongora et al., 2015.

Probability of having the potential to become a persistent shedder: The probability of an individual pig being assigned the potential to become a persistent carrier, provided it will be exposed to sufficiently high levels of MRSA.

Prevalence threshold: The prevalence cut-off value for when higher or lower probability for potential persistent shedders becoming persistent shedders should be applied.

Probability of persistent shedding below threshold: The probability of potential persistent shedders becoming persistent carriers, if the prevalence within the room, where they are housed is below or equal to the PrevCutOff.

Probability of persistent shedding above threshold = Probability of potential persistent shedders becoming persistent carriers, if the prevalence within the room, where they are housed is above PrevCutOff.

References

Espinosa-Gongora C, Dahl J, Elvstrøm A, van Wamel WJ, Guardabassi L. Individual predisposion to *Staphylococcus aureus* colonization in pigs on the basis of quantification, carriage dynamics, and serological profiles. Appl Environ Microbiol. 2015;81: 1251–6. doi:10.1128/AEM.03392-14

Table S7. Model input: Transmission rates and probabilities

Route	Agegroup	Most likely value[1]	Min.[2]	Max.[2]	Source/calculated from
Transmission rates					
Within pen (Low)	Weaner	0.0701	0.0345	0.1425	Broens et al., 2012
	Finisher	0.0701	0.0345	0.1425	Broens et al., 2012
	Others	0.0701	0.0345	0.1425	Broens et al., 2012
	Sow→offspring	0.1816	0.0655	0.5069	Broens et al., 2016
	Piglets	0.1816	0.0655	0.5069	Broens et al., 2016
Between pens (Low)	Weaner	0.0138	0.0103	0.0178	Broens et al., 2012
	Finisher	0.0138	0.0103	0.0178	Broens et al., 2012
	Others	0.0138	0.0103	0.0178	Broens et al., 2012
	Mixed	0.0351	0.0195	0.0632	Broens et al., 2012
Within pen (Med)	Weaner	0.1247	0.0503	0.3144	Average of low and high
	Finisher	0.1247	0.0503	0.3144	Average of low and high
	Others	0.1247	0.0503	0.3144	Average of low and high
	Sow→offspring	0.3230	0.0951	1.1190	Average of low and high
	Piglets	0.3230	0.0951	1.1190	Average of low and high
Between pens (Med)	Weaner	0.0241	0.0149	0.0394	Average of low and high
	Finisher	0.0241	0.0149	0.0394	Average of low and high
	Others	0.0241	0.0149	0.0394	Average of low and high
	Mixed	0.0624	0.0284	0.1399	Average of low and high
Within pen (High)	Weaner	0.1793	0.0661	0.4862	Broens et al., 2012
	Finisher	0.1793	0.0661	0.4862	Broens et al., 2012
	Others	0.1793	0.0661	0.4862	Broens et al., 2012
	Sow→offspring	0.4644	0.1247	1.7310	Broens et al., 2016
	Piglets	0.4644	0.1247	1.7310	Broens et al., 2016
Between pens (High)	Weaner	0.0345	0.0195	0.0609	Broens et al., 2012
	Finisher	0.0345	0.0195	0.0609	Broens et al., 2012
	Others	0.0345	0.0195	0.0609	Broens et al., 2012
	Mixed	0.0897	0.0374	0.2167	Broens et al., 2012
Between sections	Farrowing sec.	0.20*BetaBPO			Assumption
	All other sec.	0.15*BetaBPO			Assumption
Between stables	All	0.02*BetaBPO			Assumption
Probabilities					
Sow to piglets	Newborns (pos. dam)	0.75	0.56	0.91	Verhegghe et al., 2013
(day 1)	Newborns (neg. dam)[3]	0.35	0.26	0.46	Verhegghe et al., 2013

1: All values for transmission rates are calculated from R0 values, whereas the the probabilities are mean predictions read from a figure.

2: The lower and upper limits of 95% confidence intervals for the most likely value were used as min. and max. values in the pert distribution.

3: Probability of infection as newborn (during the first day of life) given that the piglet is born by an uninfected sow, but in a unit with infected animals. If there are no infected animals within the section, this value will be zero

References

Broens EM, Espinosa-Gongora C, Graat EAM, Vendrig N, Van Der Wolf PJ, Guardabassi L, et al. Longitudinal study on transmission of MRSA CC398 within pig herds. BMC Vet Res. 2012b;8: 58. doi:10.1186/1746-6148-8-58

Table S8: Results of sensitivity- and robustness analysis

Parameterisation (Transmission rate + modification)	Shedder prevalence		Fade out	Days before fade-out	
	Median	5th-95th percentile	(% iterations)	Median	Range
Low	0.0	0-38.0	87.0	13.0	1-142
Med	0.0	0-68.6	51.0	13.0	2-100
High	64.7	0-79.6	26.4	9.0	2-80
Low+Dur	0.0	0-63.5	51.8	27	7-268
Med+Dur	72.3	0-83.4	17.0	24	8-81
High+Dur	80.7	69.6-89.2	3.8	21	7-45
Low+No.PS	0.0	0-0	98.4	14	2-1902
Med+No.PS	0.0	0-51.5	58.2	16	1-108
High+No.PS	47.2	0-70.6	28.8	12	2-199
Low+Host	0.0	0-52.8	72.4	11	2-248
Med+Host	57.7	0-72.3	34.4	9	1-34
High+Host	67.9	0-81.4	19.0	8	1-38
Low+Dur+No.PS	0.0	0-51.9	57.6	23	7-590
Med+Dur+No.PS	63.9	0-77.3	15.0	21	10-78
High+Dur+No.PS	74.6	0-85.6	6.4	18.5	10-52
Low+Dur+Host	60.8	0-71.4	37.6	21	7-139
Med+Dur+Host	76.0	0-83.7	9.6	16.5	7-60
High+Dur+Host	82.2	68.2-90.1	4.6	18	6-43
Trans	92.4	71.5-99.5	0.2	10	-
Trans+Dur	96.0	84.8-99.7	0.0	-	-
Trans+No.PS	89.6	65.5-99.4	0.4	4.5	4-5
Trans+Host	92.3	71.5-99.4	0.2	4	-

Dur = duration of shedding for IS altered,
No.PS = no persistent shedders,
Host = shedder type solely determined by host factors (no influence of prevalence in the room),
Trans = low, medium or high transmission rates based on Broens et al., 2012 replaced by rates based on Crombé et al., 2012.

References

Broens EM, Espinosa-Gongora C, Graat EAM, Vendrig N, Van Der Wolf PJ, Guardabassi L, et al. Longitudinal study on transmission of MRSA CC398 within pig herds. BMC Vet Res. 2012;8: 58. doi:10.1186/1746-6148-8-58

Crombé F, Vanderhaeghen W, Dewulf J, Hermans K, Haesebrouck F, Butaye P. Colonization and transmission of methicillin-resistant *Staphylococcus aureus* ST398 in nursery piglets. Appl Environ Microbiol. 2012;78: 1631–1634. doi:10.1128/AEM.07356-11

Table S9: Model output: Simulated parameters compared to Danish production data.

Parameter	Model performance[1]	DK production 2015[2]
No. of liveborn piglets per litter	15.3	15.9
No. of weaned piglets per litter	13.5	13.8
Lactation time, days	28*,D	30
Dead before weaning, %	13.2	13.4
Litters from first parity sows,%	20.0	23.5
From weaning to insemination, days	6.0D	5.7
Farrowing rate, %	87.5	88.1
Re-inseminated, %	6.2	5.3
Slaughterpigs, dead or rejected at the abattoir, %	3.7D	3.7

* Nursery sows not included

D: Defined as this value

1: Medians of medians per iteration based on 6 years run with 500 iterations (after 3 years pre-run)

2: Jessen, O. / VSP (2016): Notat 1611: Landsgennemsnit for produktivitet i svineproduktionen, 2015

Table S10. Model output: Predicted fade out of MRSA in a simulated pig herd and time elapsed between introduction and fade out following single or multiple introductions.

Transmission rates	Introduction scenario	Shedder prevalence		Fade out (% iterations)	Duration (days)	
		Median	5th-95th percentile		Median	Range
Low	3 IS gilts	0.0	0-39.1	79.6	21	2-304
	3 PS gilts	31.2	16.1-45.9	0.2	1248	-
	10 IS gilts	17.7	0-44.3	48.2	31	3-322
	10 PS gilts	32.0	17.1-45.7	0.0	-	-
	10 IS weaner	0.0	0-31.5	92.8	142	2-669
	10 PS weaner	0.0	0-44.2	75.8	192	142-584
	10 IS finisher	0.0	0-0	97.8	86	2-528
	10 PS finisher	0.0	0-40.1	87.2	129	94-758
	30 IS finisher	0.0	0-35.3	92.8	96	7-604
	50 IS finishers	0.0	0-32.9	93.8	100	15-744
	3 IS gilts/14 days	27.5	0-45.8	29.8	94	54-537
	10 IS gilts/14 days	31.0	0-46.0	9.4	106	77-448
	50 IS weaners/14 days	0.0	0-44.6	51.2	268	170-1040
	100 IS weaners/14 days	27.1	0-48.3	39.0	276	156-758
	50 IS finishers/14 days	0.0	0-45.6	65.2	206	100-528
	100 IS finishers/14 days	0.0	0-44.5	54.0	226	130-640
Medium	3 IS gilts	53.2	0-67.8	23.6	23	1-178
	3 PS gilts	56.5	39.9-72.0	0	-	-
	10 IS gilts	55.3	0-69.2	8.0	33	5-106
	10 PS gilts	56.3	40.4-68.4	0	-	-
	10 IS weaner	56.8	36.0-69.8	4.2	133	9-394
	10 PS weaner	56.7	41.4-70.6	0.0	-	-
	10 IS finisher	55.1	0-70.4	18.2	94	7-408
	10 PS finisher	56.2	0-70.3	5.4	156	100-410
	30 IS finisher	56.2	0-71.1	10.8	111	21-268
	50 IS finishers	56.9	34.8-69.1	4.2	124	36-318
	3 IS gilts/14 days	56.0	39.5-69.7	2	104.5	59-131
	10 IS gilts/14 days	56.2	39.9-70.6	0	-	-
	50 IS weaners/14 days	56.4	42.2-71.9	0.2	436	-
	100 IS weaners/14 days	57.1	41.7-70.1	0.0	-	-
	50 IS finishers/14 days	56.1	39.2-71.2	0.6	254	151-260
	100 IS finishers/14 days	57.0	40.0-69.9	0.0	-	-

The table continues on the next page.

Table S10, continued from page 1.

Transmission rates	Introduction scenario	Shedder prevalence		Fade out (% iterations)	Duration (days)	
		Median	5th-95th percentile		Median	Range
High	3 IS gilts	67.5	0-80.4	6.0	19.5	2-54
	3 PS gilts	68.3	54.8-59.9	0	-	-
	10 IS gilts	67.5	52.7-59.5	0.6	39	20-49
	10 PS gilts	67.7	53.2-80.3	0	-	-
	10 IS weaner	68.6	53.1-81.5	0	-	-
	10 PS weaner	68.0	52.4-79.9	0	-	-
	10 IS finisher	67.4	51.6-81.6	0.6	134	94-142
	10 PS finisher	67.8	53.7-81.5	0	-	-
	30 IS finisher	67.9	53.3-80.7	0	-	-
	50 IS finishers	67.5	52.1-80.2	0	-	-
	3 IS gilts/14 days	67.0	52.9-80.4	0.2	120	-
	10 IS gilts/14 days	66.4	47.4-80.6	0	-	-
	50 IS weaners/14 days	67.8	53.3-80.8	0	-	-
	100 IS weaners/14 days	68.3	53.2-81.3	0	-	-
	50 IS finishers/14 days	67.3	52.6-81.4	0	-	-
	100 IS finishers/14 days	67.6	53.4-81.9	0	-	-

Table S11: Summary of MRSA prevalence in different age groups in observational studies

MRSA prevalence in group[1]	Age in days	Broens et al., 2012[2]	Crombé et al., 2012	Dewaele et al., 2011[3]	Khanna et al., 2008	Merialdi et al., 2012	Merialdi et al., 2013[4]	Nathaus et al., 2010	Pletinckx et al., 2013[5]	Verhegghe et al., 2013[6]	Weese et al., 2011
Sows	Unspec.	33[7]/77[8]	26	96/59	-	-	53.3	10.3	1.4-90.7	17-33/50-100	-
Piglets	See notes	>60	41	100/50	20	1.6	53.3	15.5[9] 48.3[11]	4.8-100	0-36/>90	1[10] 20[11]
Weaners	See notes	-	-	100/100[12] 100/100[16]	28	100	56.7 60.0[17]	25.9[13] 51.7[18]	73.3-100	69-91/>95[14]	34[15] 65[13] 50[17] 42[19]
Finishers	See notes		26	100/-[20] 78/-[23] 87/-[24]	26[21]	18.3	31.7		51.1-85.7	60-75/75-80[22]	

Notes:
1: Prevalence of positive nasal swabs, if no other sample type is indicated
2: Based on nasal or rectal swabs
3: Results for two farms presented as farm A/farm B
4: Based on environmental swabs
5: Significant differences were reported between pre- and post-weaned piglets, between post-weaned piglets and fattening pigs, between 11-17 weeks old and 18-20 weeks old fattening pigs and between sows and fattening pigs
6: Reported as results for two low- / to high-contaminated farms. Some of the values are only approximate values, since they have been read from a graph (day 165 for the low-contaminated farms + all values from the highly contaminated farms, except the prevalence range for the sows)
7: Before farrowing
8: At the end of weaning
9: Day 1-3
10: Day 1
11: Day 21
12: Day 28-56
13: Day 42

14: Day 52-58
15: Day 28
16: Day 56-84
17: Day 56
18: Day 63
19: Day 72
20: Day 84-112
21: Agegroup defined as ‘grower-finisher’
22: Day 165
23: Day 112-140
24: Day 140-162

References

1. Broens EM, Espinosa-Gongora C, Graat EAM, Vendrig N, Van Der Wolf PJ, Guardabassi L, et al. Longitudinal study on transmission of MRSA CC398 within pig herds. BMC Vet Res. 2012;8: 58. doi:10.1186/1746-6148-8-58

2. Crombé F, Vanderhaeghen W, Dewulf J, Hermans K, Haesebrouck F, Butaye P. Colonization and transmission of methicillin-resistant *Staphylococcus aureus* ST398 in nursery piglets. Appl Environ Microbiol. 2012;78: 1631–1634. doi:10.1128/AEM.07356-11

3. Dewaele I, Messens W, Man I De, Delputte P, Herman L, Butaye P, et al. Sampling, prevalence and characterization of methicillin-resistant *Staphylococcus aureus* on two Belgian pig farms. Vet Sci Dev. 2011;1: 4–8. doi:10.4081/vsd.2011.e1

4. Khanna T, Friendship R, Dewey C, Weese JS. Methicillin resistant Staphylococcus aureus colonization in pigs and pig farmers. Vet Microbiol. 2008;128: 298–303. doi:10.1016/j.vetmic.2007.10.006

5. Merialdi G, Galletti E, Rugna G, Granito G, Franco A, Battisti A, et al. Longitudinal study on MRSA nasal colonization in a farrow to finish pig herd. 4th European Symposium of Porcine Health Management. 2012. p. 1.

6. Merialdi G, Galletti E, Guazzetti S, Rosignoli C, Alborali G, Battisti A, et al. Environmental methicillin-resistant *Staphylococcus aureus* contamination in pig herds in relation to the productive phase and application of cleaning and disinfection. Res Vet Sci. 2013;94: 425–427. doi:10.1016/j.rvsc.2012.10.020

7. Nathaus R, Blaha T, Tegeler R, Meemken D. *Staphylococcus aureus* in zwei Schweine- zuchtbestanden (Intra-herd prevalence and

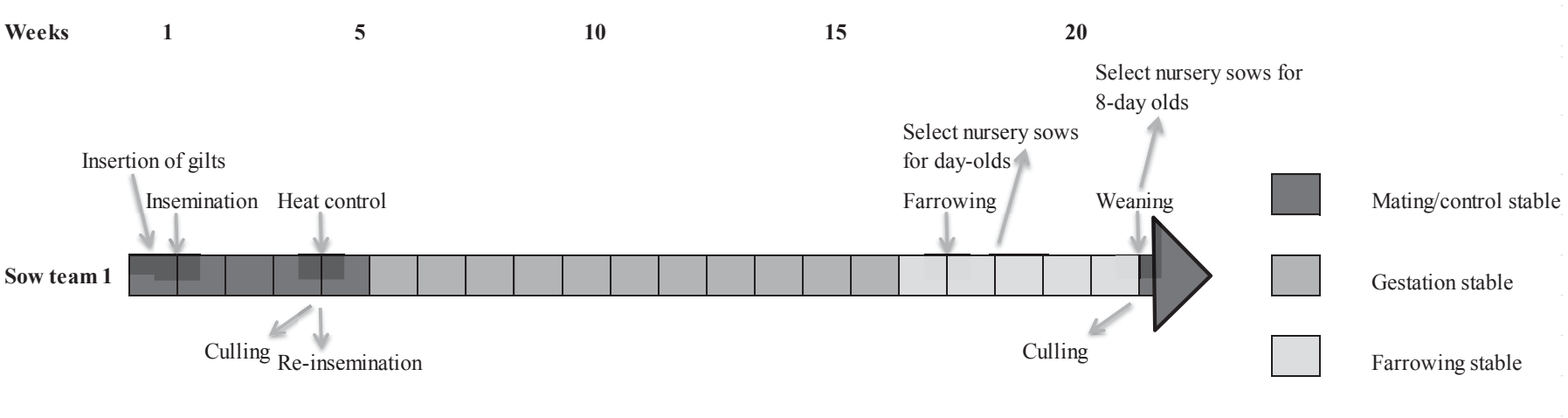
colonisation dynamics of Methicillin-resistant Staphylococcus au reus (MRSA) in two pig breeding herds) (In German with abstract in English). Berl Munch Tierarztl Wochenschr. 2010;1236: 221–228. doi:10.2376/0005-9366-123-221

8. Pletinckx LJ, Verhegghe M, Crombé F, Dewulf J, De Bleecker Y, Rasschaert G, et al. Evidence of possible methicillin-resistant *Staphylococcus aureus* ST398 spread between pigs and other animals and people residing on the same farm. Prev Vet Med. 2013;109: 293–303. doi:10.1016/j.prevetmed.2012.10.019

9. Verhegghe M, Pletinckx LJ, Crombé F, Weyenberg S Van, Haesebrouck F, Butaye P, et al. Cohort study for the presence of livestock-associated MRSA in piglets: Effect of sow status at farrowing and determination of the piglet colonization age. Vet Microbiol. 2013;162: 679–686. doi:10.1016/j.vetmic.2012.09.014

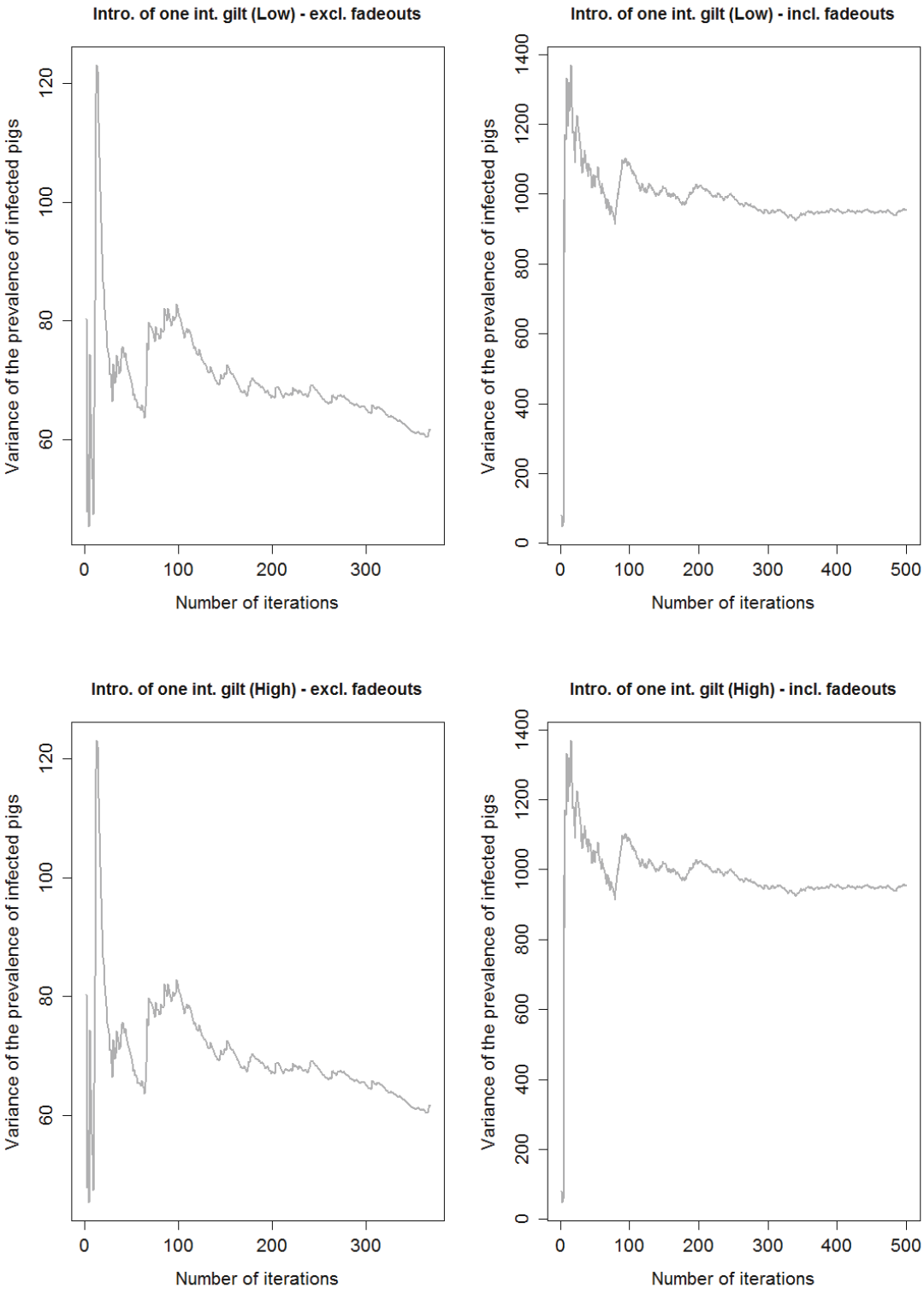
10. Weese JS, Zwambag A, Rosendal T, Reid-Smith R, Friendship R. Longitudinal Investigation of Methicillin-Resistant *Staphylococcus aureus* in Piglets. Zoonoses Public Health. 2011;58: 238–243. doi:10.1111/j.1863-2378.2010.01340.x

Figure S1: The sow cycle modelled in a hypothetical farrow-to-finish herd.



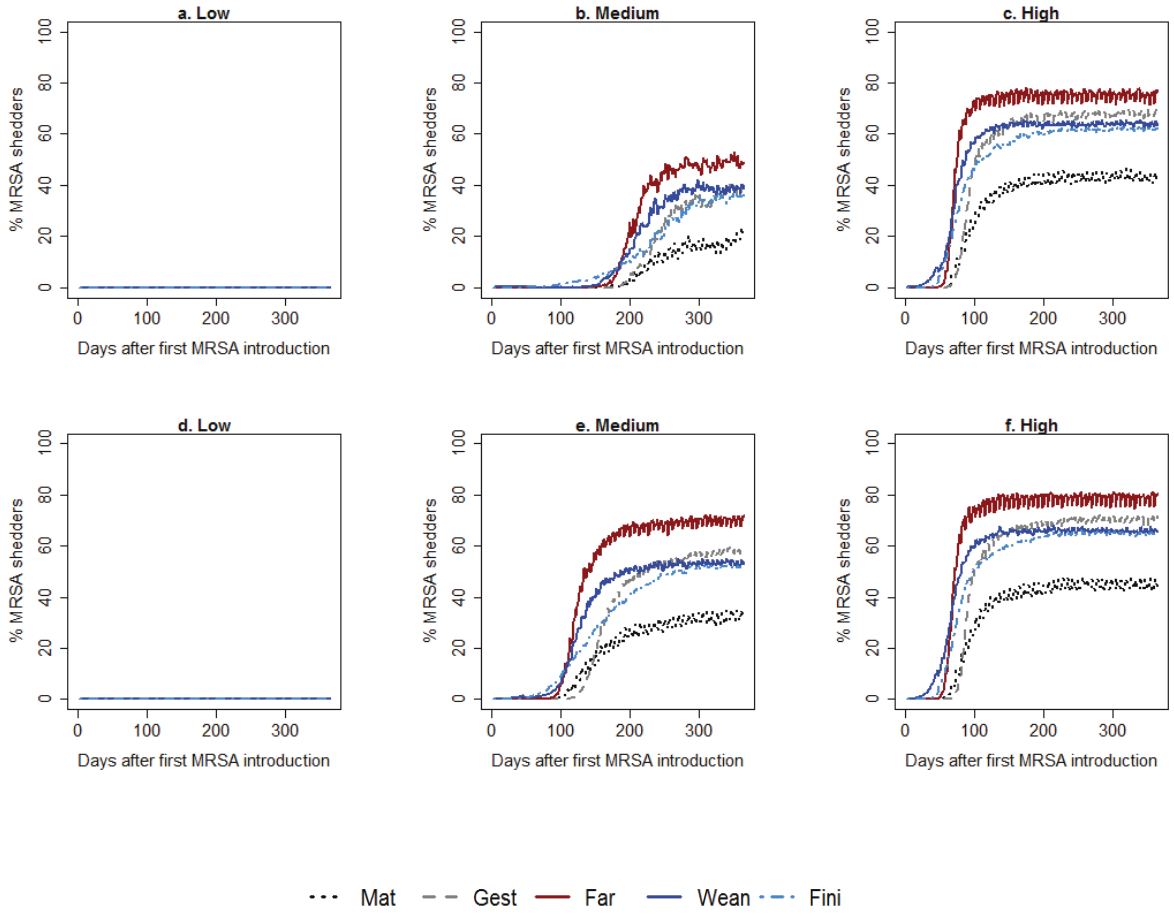
Assumed total duration from insertion in the mating stable to weaning in the farrowing stable = 147 days. It is assumed that sows are inseminated 5 days after arrival in the mating unit. The need for adding gilts to the sow teams are evaluated three days before the insemination. Three weeks after insemination, it is checked if re-isemination is needed for any sows (heat control). It is assumed that some of the pigs needing re-insemination will be culled. The sows reamin in the mating and control unit untill 4 weeks after insemination. The duration of the gestation period is assumed to be 114 days. Sows are moved to the farrowing unit 5 days before expected farrowing. Piglets will be weaned after 28 days. Stategic culling of sows will take place immediately after weaning (or after insemination failure has been observed). Emergency culling or deaths can occur anywhere in the cycle. Two-step nursery sows are selected among sows who farrowed 8 days ago or sows (step 1) and those whose own piglets have just been weaned (step 2).

Figure S2. Model output: Convergence after introduction of one intermittently shedding gilt



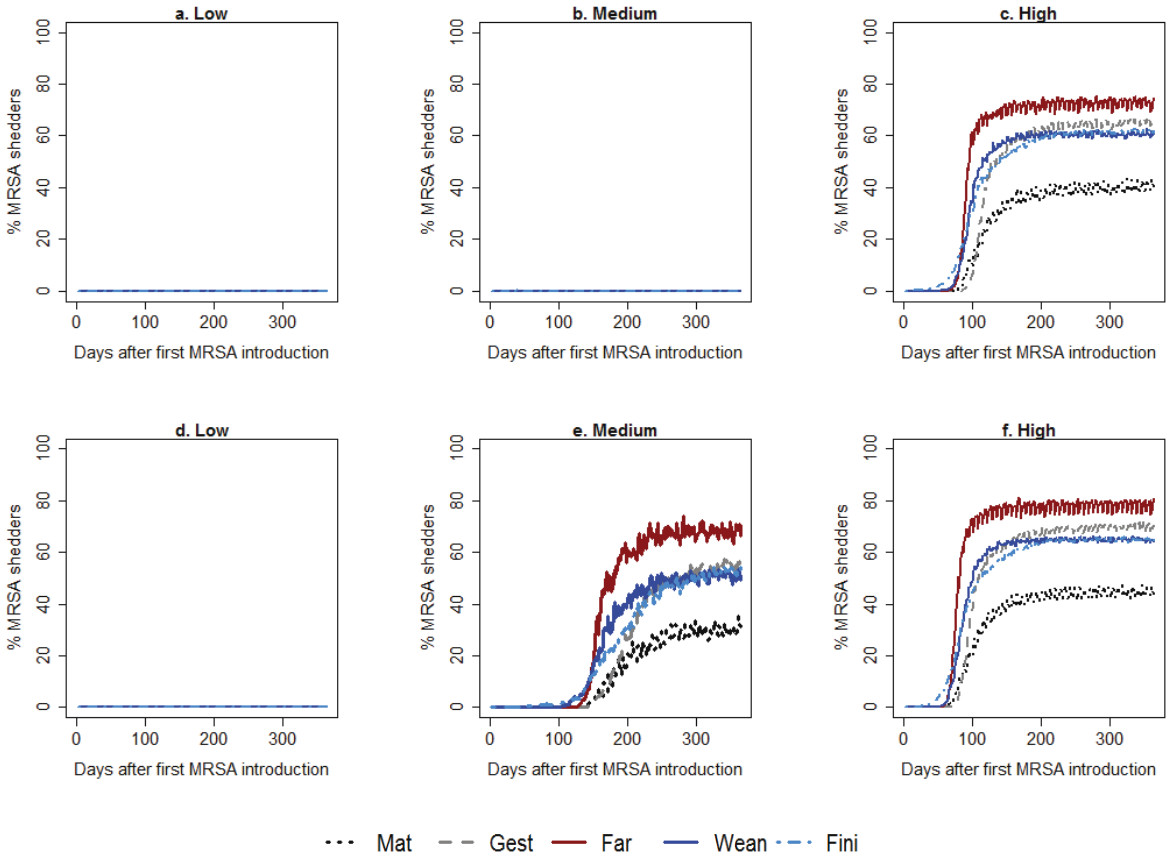
Note: All scenarios were run with 500 iterations. The number on the graphs to the left differ from 500, because iterations were MRSA faded out before the end of run were not included. It is assumed that convergence has been reached, when the variance stabilises.

Figure S3. Model output: Development in the median prevalence of MRSA shedders following introduction of one MRSA shedding weaner.



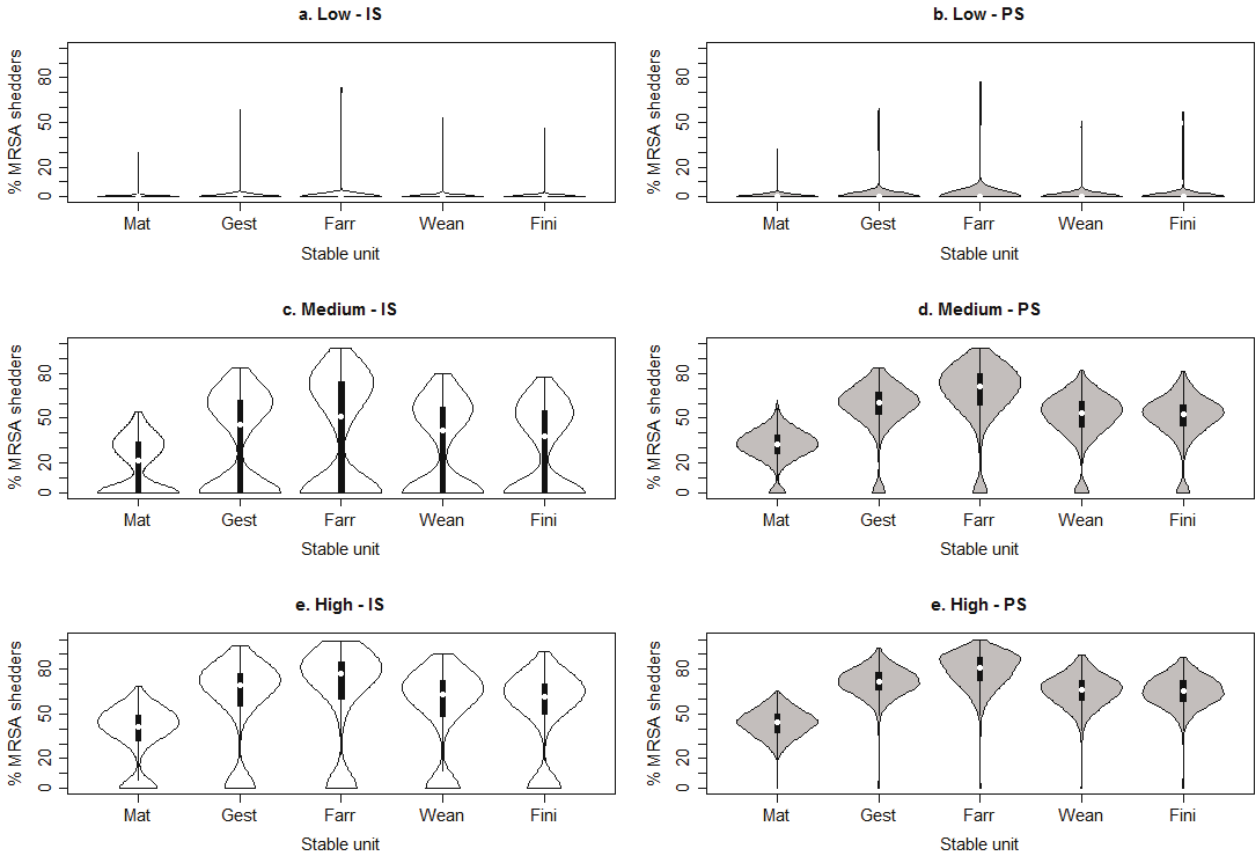
Predicted median prevalence over time following introduction of one intermittently (a-c) or persistently shedding weaner (d-f), when using low (a+d), medium (b+e) or high (c+f) transmission rates. Mat = Mating unit, Gest = Gestation unit, Farr = Farrowing unit, Wean = Weaner unit, Fini = Finisher unit. MRSA was introduced in the weaner section with the youngest pigs in order to ensure time for MRSA transmission to occur.

Figure S4. Model output: Development in the median prevalence of MRSA shedders following introduction of one MRSA shedding finisher



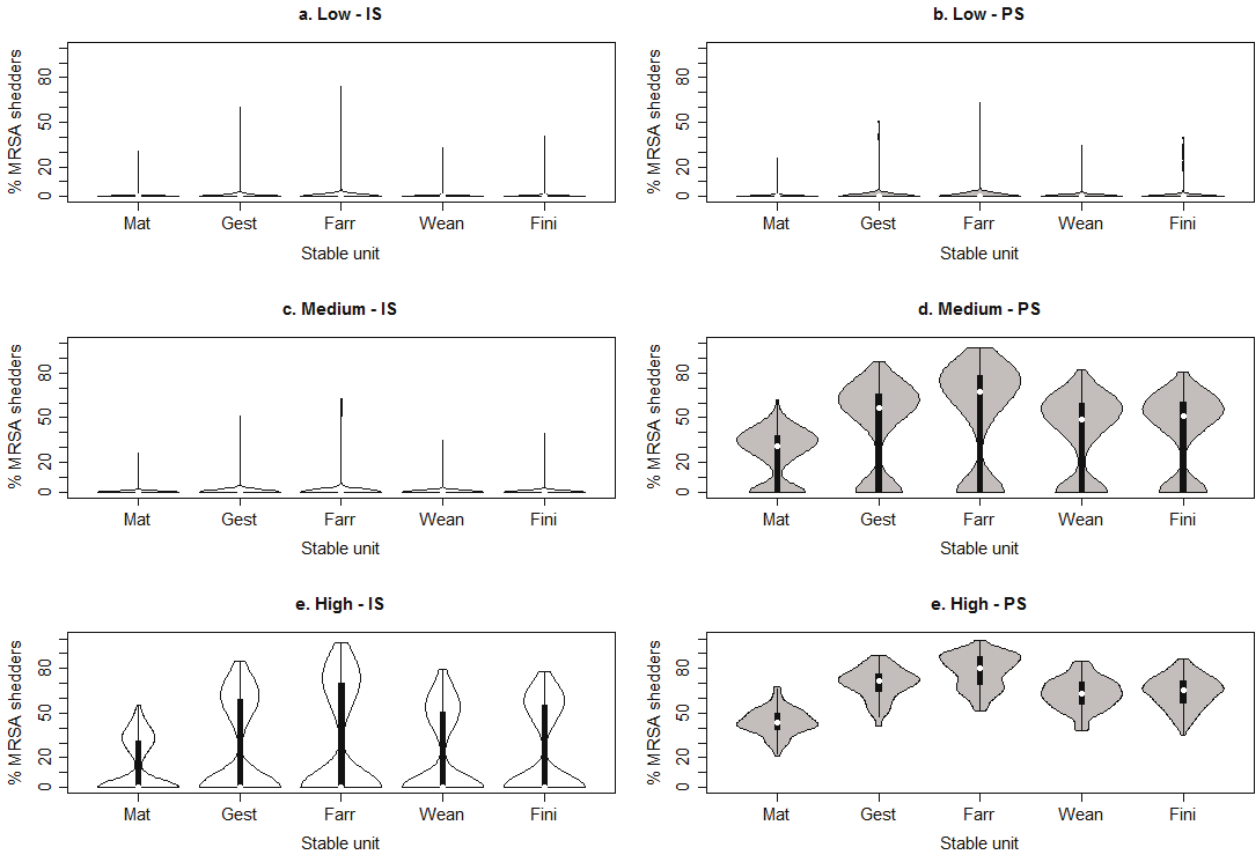
Predicted median prevalence over time following introduction of one intermittently (a-c) or one persistently shedding finisher (d-f), when using low (a+d), medium (b+e) or high (c+f) transmission rates. Mat = Mating unit, Gest = Gestation unit, Farr = Farrowing unit, Wean = Weaner unit, Fini = Finisher unit. MRSA was introduced in the section with the youngest pigs in order to ensure time for MRSA transmission to occur.

Figure S5. Model output: Violin plot of the prevalence following introduction of one weaner shedding MRSA intermittently or persistently.



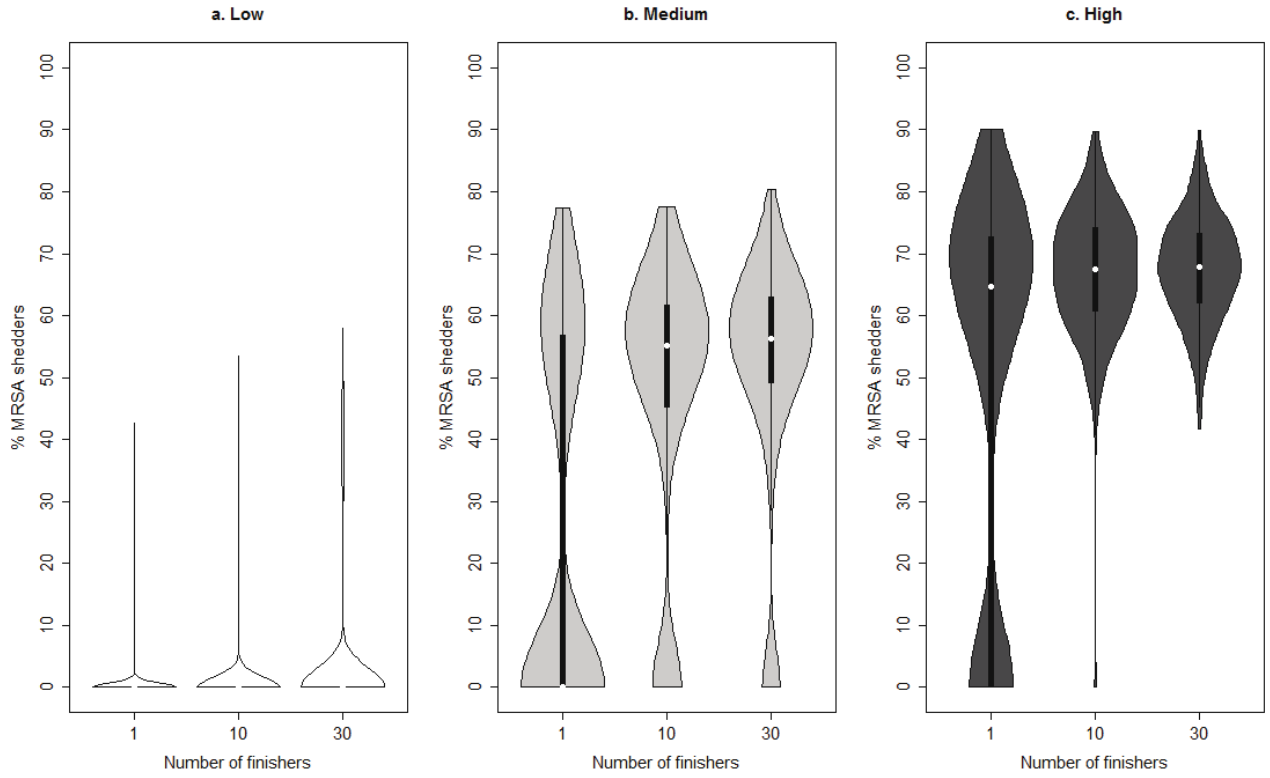
Predicted prevalence of MRSA shedders six years after introduction of a weaner shedding MRSA intermittently (left) or persistently (right) using low, medium or high transmission rates. (distribution of 500 iterations). The median prevalences are indicated by white dots. Mat = Mating unit, Gest = Gestation unit, Farr = Farrowing unit, Wean = Weaner unit, Fini = Finisher unit.

Figure S6. Model output: Violin plot of the prevalence following introduction of one finisher shedding MRSA intermittently or persistently.



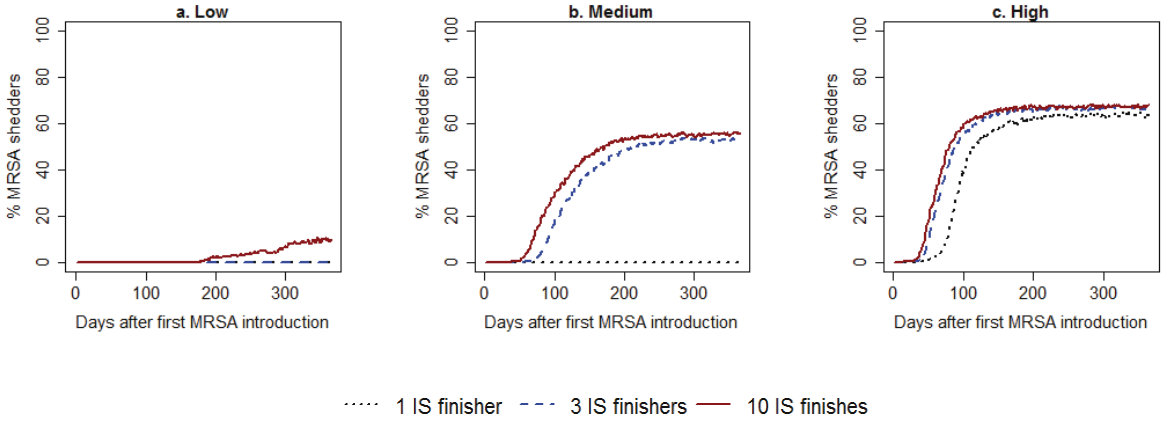
Predicted prevalence of MRSA shedders six years after introduction of a finisher shedding MRSA intermittently (left) or persistently (right) using low, medium or high transmission rates (distribution of 500 iterations). The median prevalences are indicated by white dots. Mat = Mating unit, Gest = Gestation unit, Farr = Farrowing unit, Wean = Weaner unit, Fini = Finisher unit.

Figure S7. Model output: Violin plot of the prevalence following introduction of one, ten or thirty finishers shedding MRSA intermittently.



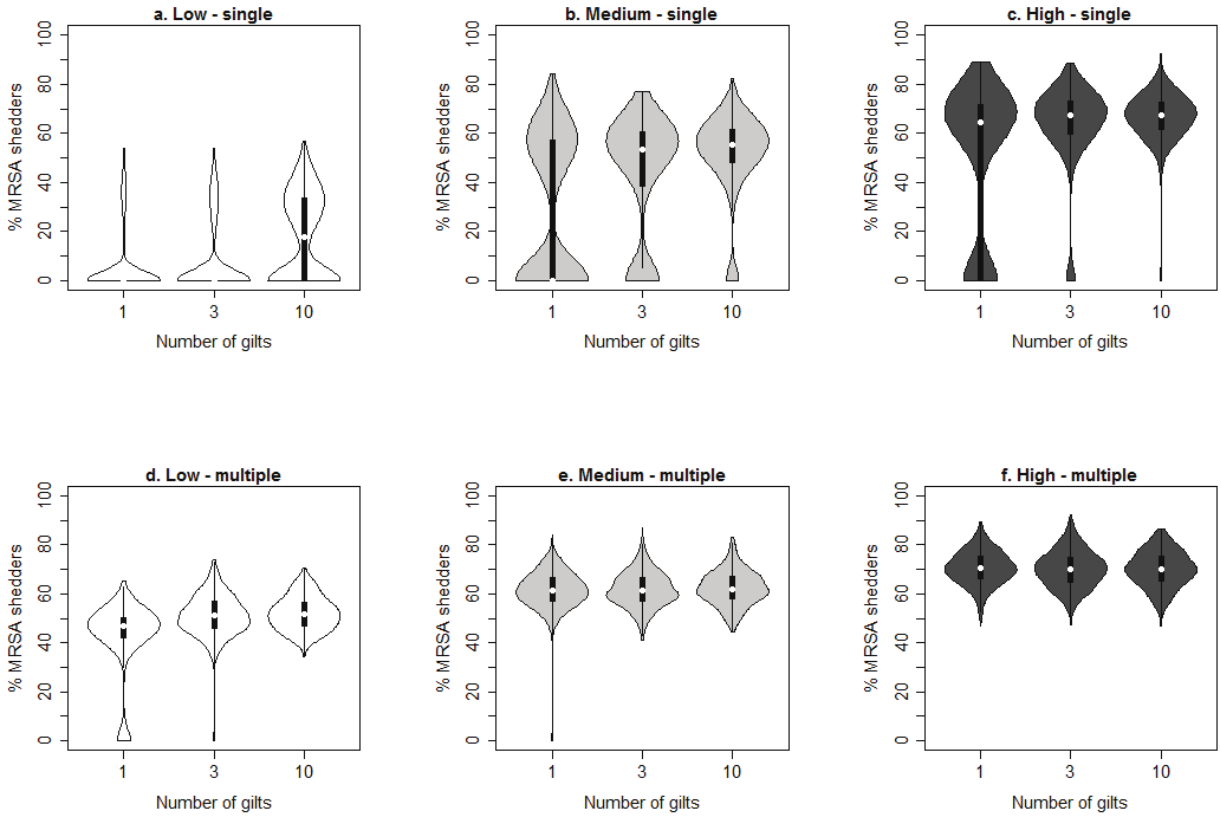
Predicted total prevalence of MRSA shedders in the herd six years after introduction of one, ten or thirty finishers shedding MRSA intermittently when low (a), medium (b) or high (c) transmission rates are used (distribution of 500 iterations). The median prevalences are indicated by white dots.

Figure S8. Model output: Development in the median prevalence of MRSA shedders following introduction of one, ten or thirty IS finishers.



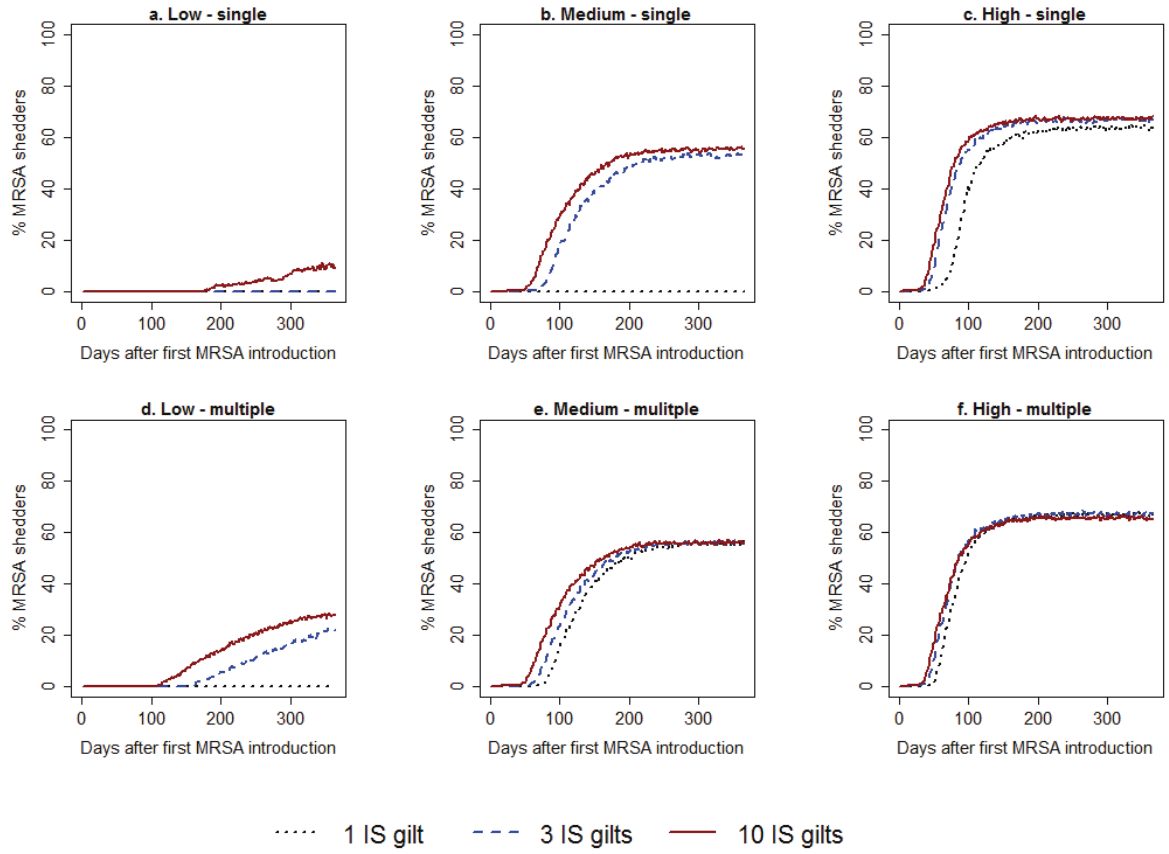
Predicted median prevalence over time when using low (a), medium (b) or high (c) transmission rates (distribution of 500 iterations).

Figure S9. Model output: Violin plot of the prevalence following introduction of one, three or ten gilt shedding MRSA intermittently every fortnight for three months.



Predicted total prevalence of MRSA shedders in the herd six years after introduction of one, three or ten gilts shedding MRSA intermittently every fortnight for three months, when low (a), medium (b) or high (c) transmission rates are used (distribution of 500 iterations). The median prevalences are indicated by white dots.

Figure S10. Model output: Development in the median prevalence of MRSA shedders following single or multiple introductions.



Predicted mean prevalence over time following single (a-c) or multiple (d-f) introductions of one, three or ten gilts shedding MRSA intermittently every fortnight for three months, when low (a+d), medium (b+e) or high (c+f) transmission was assumed.

6.2 Manuscript III

Mechanistic modelling of interventions against spread of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) within a Danish farrow-to-finish pig herd

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Submitted

Abstract

Knowledge on successful interventions against livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) within pig herds is sparse. In situations like this, a mechanistic simulation model can be a valuable tool for assessing the effect of potential intervention strategies, and prioritising which should be tested in the field. We have simulated on-farm interventions, with a previously published LA-MRSA spread model, within four different areas: 1) Reduced antimicrobial consumption, 2) Reduced number of pigs within each section, 3) Reduced mixing of pigs, and 4) Improved internal biosecurity. To model a decrease in the selective pressure, the transmission rates were reduced after LA-MRSA had become fully established within a herd, which resulted in a marked decrease in the prevalence within all stable units. However, LA-MRSA rarely disappeared completely from the herd; this was only observed in scenarios where the transmission rates were reduced to ≤ 30% of the original level. While changes in antimicrobial consumption patterns might be a very important step towards reducing the spread of LA-MRSA, the simulation results indicate that it may need to be paired with other preventive or intervention measures. Reducing the number of pigs within each section, reducing mixing of pigs, or improving internal biosecurity after LA-MRSA had become established within the herd only resulted in marginal changes in the median prevalence within the herd. However, these factors might be important in relation to being able to achieve or maintain a low level of antimicrobial consumption, and thus still indirectly influence the LA-MRSA prevalence within the herd. The results of a sensitivity analysis indicated the assumptions regarding the existence of pigs persistently shedding MRSA have a noticeable influence on the model results. The assumptions regarding transmission from sow to offspring at the day of birth also had a considerable influence on the MRSA prevalence within the farrowing unit but did not cause any marked changes in the simulated effect of interventions. Effects might differ between different farm types contaminated in different levels and this simulation study highlights a strong need for more knowledge from on-farm trials.

Introduction

Staphylococcus aureus is an opportunistic pathogen capable of causing a wide-range of diseases in humans and animals [1]. In 2005, findings of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) were reported for the first time in France and the Netherlands [2,3], and since then LA-MRSA has been detected in the pig population in many European countries [1].

The majority of LA-MRSA strains are resistant to tetracyclines [4] and use of these compounds is therefore expected to select for LA-MRSA. In a longitudinal study, where transmission rates of LA-MRSA between pigs were estimated both with and without the use of risk-antimicrobials (beta-lactams and tetracyclines), these were significantly different from each other [5], and in several studies group treatment with antimicrobials has been identified as a risk factor for pig farms becoming LA-MRSA contaminated [6–8]. Also, in an intervention study, where use of antimicrobials was reduced by 44%, this was associated with declining MRSA prevalence in pigs [9]. Thus, changing antimicrobial consumption patterns on the farms can be considered a relevant area of intervention.

LA-MRSA has been detected in high levels in air within stable units [10], and consequently pigs are exposed to LA-MRSA both through bacteria bound to dust particles suspended in the air, and through direct contact with their pen mates. Since the LA-MRSA contamination of the air is assumed to originate from pigs shedding LA-MRSA, a reduction in the number of pigs within a stable section might lead to decreased exposure, both through decreased concentrations in the air and through decreased direct contact to other pigs, provided that the within-pen stocking-density is also reduced. The number of direct contact events with other pigs during an animal's lifespan is dependent both on the stocking-density in the pens, and on how often mixing between pigs in different pens or batches occur. Both factors have been identified as risk factors for spread of other infectious agents [11,12].

In addition to in the air within stables, LA-MRSA has been detected in many different parts of the farm environment, including in dust, feed, faeces, and boot swabs of the service alley on contaminated farms [10]. Therefore, farm workers and equipment are also potential sources of spread of LA-MRSA between sections or stable units within the farm. Some units are more work intensive than others, e.g. the farrowing unit, and the work will involve more direct interaction between humans and pigs. Improved internal biosecurity, e.g. improved hand hygiene, change of boots between stables, fixed working order, having equipment dedicated to each unit etc., may reduce this spread.

On-farm studies showing successful interventions against spread of LA-MRSA, which do not involve emptying the farm and culling all animals, are sparse. Most of these have focused on the use of disinfectants, but the scope, study design, disinfection procedure and type of disinfectant applied varied,

and so did the results. In general, it has been shown possible to remove LA-MRSA entirely through disinfection in the absence of animals [13,14], or obtain a reduction in LA-MRSA levels in the air and bedding materials, when repeatedly applying disinfectant in the presence of LA-MRSA positive animals [15]. Other attempts at reducing the LA-MRSA contamination within farms, includes sow washing, where the original strain was detected in 64% of the animals again after washing [16], and use of an air cleaning system consisting of an air washer and a UV-irradiation system, which led to significantly reduced concentrations of LA-MRSA in the stable air [17].

In situations where the knowledge on successful interventions is limited, a mechanistic simulation model can be a valuable tool for assessing the effect of potential intervention strategies, and prioritising which one should be tested on real farms. One of the main challenges, when modelling spread of LA-MRSA is that the dynamics of infection in pigs are not clear, and assumptions regarding the existence of both intermittent shedders (IS) and persistent shedders (PS) might have a major impact on the results. In this paper, we use a previously published mechanistic individual-based model for spread of LA-MRSA within a pig herd [18] for simulating the outcome of implementing on-farm interventions within four different areas: 1) Reduced antimicrobial consumption, 2) Reduced number of pigs within each section, 3) Reduced mixing of pigs, and 4) Improved internal biosecurity. Using the Danish situation as an example, where LA-MRSA was isolated from 88% of 57 randomly selected pig herds tested in Denmark [19], we assume that LA-MRSA has already become fully established within the herd and reached a steady state prevalence in all farm units before the interventions are initiated. The aims of the study were to: 1) Assess the effect of the possible intervention strategies mentioned above and evaluate if it is possible to clear a farm from LA-MRSA, once it has become established by lowering the transmission, and 2) Assess the impact of assumptions and parameters on model predictions.

Materials and methods

Simulation model

All simulations and data analyses were carried out in R version 3.2.2 – "Fire Safety" [20]. The model used for the simulations is a mechanistic, stochastic, individual-based model with discrete time-steps of one day. All simulation scenarios were run for 500 iterations, except in the sensitivity analysis, where some simulations were run with 100 iterations as explained below in section: 'Sensitivity analysis'. The model consists of two main units, a herd model of a farrow-to-finish pig herd and an epidemic model for LA-MRSA. Both are briefly described below and a more detailed description of the full model can be found in Sørensen et al., 2017 [18], including a link to the model R-code: https://github.com/anvso/DTU-model.

Herd model

The model was designed to represent a typical Danish medium-sized farrow-to-finish herd (~ 500 sows, annual production: ~15,400 slaughter pigs). It was assumed that the herd used weekly batch production with 100% artificial insemination, and replacement gilts were purchased from other herds. The main processes in the model included: insemination, farrowing, slaughter, death/culling, re-insemination and use of two-step nurse sows. The farm consisted of five different stable units: the mating unit, the gestation unit, the farrowing unit, the weaner unit and the finisher unit.

Epidemic model

The epidemic model used for LA-MRSA was an SIS-model with two different infectious stages, since it was assumed that a pig could either be susceptible to LA-MRSA, or be an intermittent or persistent shedder of LA-MRSA. It was assumed that, as in humans, IS and PS formed two distinct groups [21], and therefore a pig could not go directly from being an IS to becoming a PS. Whether a pig became a PS was modelled to depend both on host-related factors and the degree of exposure to LA-MRSA. This was implemented by only assigning a certain fraction of the pigs the potential to become PS, with a probability of becoming PS upon exposure that changed depending on the prevalence within the section where the pig was housed being above or below a given limit [18]. The duration of shedding for IS varied from 1-26 days. The routes of transmission in the model included: within-pen, between-pen, between-section and between-stable transmission [18].

Interventions simulated

To allow enough time for LA-MRSA to become established in pigs within all stable units, the interventions were not initiated until 180 days after LA-MRSA had been introduced, reflecting the time needed for LA-MRSA to reach an endemic state. This is a relevant approach to testing interventions that could be useful if implemented in the endemic state currently found in Danish pig population. All scenarios were simulated with three different sets of transmission rates, referred to as ‘low’, ‘medium’ and ‘high’, except the scenarios related to reduced antimicrobial consumption, where the high rates were used as a baseline before intervention. The ‘low’ set of transmission rates were intended to represent a scenario with no use of beta-lactams or tetracyclines (rates based on [5]), whereas ‘high’ transmission represented a situation with high antimicrobial consumption (rates based on [5]). The ‘medium’ scenario represented a situation between the two extremes (rates were based on averages of ‘low’ and ‘high’) [18].

Reduced antimicrobial consumption

Changes in the antimicrobial consumption patterns, which lead to a decrease in the use of compounds selecting for LA-MRSA, are expected to decrease the rate of LA-MRSA transmission between pigs. To investigate if it was possible to clear a herd from LA-MRSA by decreasing transmission to a sufficiently low

level, all the transmissions rates used when assuming 'high' transmission, were reduced by 10% - 90% in steps of 10% each.

Reduced number of pigs within each section

In Denmark, it is common for farmers to sell pigs either immediately after weaning (weight ~7 kg) or after the nursery phase (weight ~30 kg), and therefore scenarios were specified with reductions in the number of pigs in each section within these two age groups, where we assumed that the farmer started selling pigs and gradually increased the proportion of every batch sold by 5%-steps every 6th month. The overall stocking density within a section was reduced, by either: 1) utilising less of the pens available within the section, or 2) reducing the number of pigs within each pen (reduced stocking density). It was assumed that a reduction in stocking density also would affect the transmission rate due to decreased contact rate, and thus the transmission rates were reduced stepwise with the same relative reduction per step as the relative reduction in density.

Reduced mixing

In the simulated herd, it was assumed that the farmer was using batch production i.e. in principle all-in/all-out on the section level. However, animals from different batches might in some cases be mixed. Regularly, some sows will be moved from one batch to another, either because of reproductive failure or because of being used as nurse sows (foster dams) for piglets born by sows in other batches. In a survey from 2016, 63% and 52% of the interviewed Danish pig herd owners, who used batch production, had a buffer section in their weaner or finisher unit, respectively (S1 table). Therefore, both the weaner and the finisher units were assumed to contain a buffer section for slower growing pigs that needed extra time in the unit before being ready to be moved to the finisher stable or before being sent for slaughter. It was assumed that the leftover pigs in the weaner unit could spend up to three weeks in the buffer section before being moved to the finisher unit and that these pigs would therefore be mixed with weaners from other batches. Mixing of pigs in the buffer section in the finisher unit was considered to only be of little importance, since pigs will not return from the buffer section, but instead be sent directly to slaughter from here. Mixing of pigs from different litters is common in Danish pig production herds, where pigs are frequently sorted according to size and assigned new pen mates when they are moved from one stable unit to another. In the baseline scenario, it was assumed that the pigs were sorted and assigned new pen mates at least twice: first when entering the weaner unit, and later when being moved from the weaner to the finisher unit. In practise, this was implemented in the model as random mixing at transition. In the present study, reduced mixing was simulated in three different ways: 1) No use of buffer sections and thus no possibility of mixing between pigs belonging to different batches, 2) No use of buffer stables along with reduced mixing (the pigs in each weaner pen were distributed in two pens, when being moved to the finisher unit, and as a result, these pigs only received new pen mates once, at entry in the weaner unit, when two litters would be put into one pen

together), 3) Keeping pigs from different litters separated all the way from farrowing and to slaughter. In practice, this also meant that the number of pigs within each pen was reduced considerably, because in the baseline scenario the maximum number of pigs per pen would be 30 in the weaner unit and 15 in finisher unit.

Improved internal biosecurity

The effect of increased internal biosecurity was modelled as a reduction in the transmission between sections, between stable units or both, and included the extreme cases of no between-section and/or between-stable transmission.

Sensitivity analysis

Many of the parameters used in the infection model originate from one study [5], and thus are subject to considerable uncertainty. In the first part of the sensitivity analysis, the effect of changes in within-pen and between-pen transmission rates relative to each other was investigated. This was intended to highlight which changes in model parameters that have the biggest impact on the outcome and therefore potential focus areas for intervention. Within-pen and between-pen transmission parameters were independently scaled from 0.3-1.1 times the baseline value in steps of 0.10, while the other parameters were kept constant. In the sensitivity analysis, the variation in these parameters was not included, and therefore simulations only required 100 iterations to generate stable estimates. Spread between-section and between-stable units were set to a fixed proportion of the between-pen spread, and therefore no separate sensitivity analysis was conducted on these. To assess the influence of intervening in one stable unit only, the within-pen and between-pen transmission rates were also changed individually in one unit at a time, where both rates were scaled with the same factor in each step.

The presence of pigs persistently shedding LA-MRSA is expected to have a considerable influence on the outcome of the intervention scenarios, and therefore the influence of the presence or absence of these was assessed in the second part of the sensitivity analysis. The assumptions regarding the probability of transmission from sow to offspring on the day of birth might also influence the interventions modelled and therefore this parameter was also subjected to sensitivity analysis. The effect of using values corresponding to 0%, 25%, 50 and 75% of the probabilities used in the standard parameterisation was investigated. The sensitivity analysis was conducted with only one set of transmission rates (‘high’ transmission).

Results

Reduced antimicrobial consumption

The median prevalence within the stable units over time decreased immediately after the reduction in transmission rates had been implemented, and then stabilised at a lower level, which depended on the proportion of reduction implemented (Fig 1.A-D and S1 Fig). Violin plots were used to illustrate the variation in the outcome of different iterations (Fig 1.E-H and S2 Fig). Generally, a bimodal distribution was observed with one proportion of the simulated prevalences clustering just above zero, and the more the transmission rates were reduced, the more iterations resulted in a prevalence of zero or just above zero (Fig 1.E-H and S2 Fig). Complete fade-out of LA-MRSA resulting from the introduced reduction in the transmission rate was only observed in the scenarios where the transmission rate was reduced to less than 30% of the initial level, and still this was a rare event (0.2%, 0.4% and 2.4% of iterations, when the transmission rates were reduced to 30%, 20% and 10% of the initial level, respectively (S2 Fig)).

Reduced number of pigs within each section

Reducing the number of pigs within each section in either way had only a marginal effect on the development in the simulated median prevalence over time, when assuming 'high' transmission (Fig 2), since a major effect was only observed, when enough time had elapsed for the number of pigs within each section to be reduced to level, that probably not will be realistic for farmers (>10% reduction). In the scenarios, where 'low' or 'medium' transmission was assumed, similar results were obtained (S3 Fig). There was slightly more effect, when the number of pigs was reduced within each pen and not only the number of pens in use within the section.

Reduced mixing

With the current parameterisation of the model, no effect was observed in any of the scenarios with reduced mixing, no matter if high (Fig 3), medium or low (S4 Fig) transmission was assumed.

Improved internal biosecurity

Reducing transmission between sections had no noticeable effect, when LA-MRSA had already become established within the herd (Fig 4). However, when 'low' transmission rates were used, a small temporary drop in the prevalence was observed immediately after intervention when it was assumed that the spread between section and between stables had been reduced to 25% of the initial level (S5 Fig).

Sensitivity analysis

The mean prevalence values after stabilisation in the five different stable units as well as the overall mean prevalence within the herd for different combinations of scaling of the transmission rates for within-pen

transmission and between-pen transmission are illustrated on Fig 5. The proportion of iterations where LA-MRSA faded out was the same for all units, since following introduction LA-MRSA either faded out in all units of the farm or became established within all units. In general, the highest prevalence was observed within the farrowing unit and the lowest within the mating unit. The changes in mean prevalence followed the same overall pattern within all stable units.

Gradually changing the parameter values for all transmission rates within one stable unit at a time resulted in a gradually changing mean prevalence within the stable unit, where the changes were applied. The changes did not markedly influence the mean prevalence in the other stable units, as the prevalence consistently remained lowest in the mating unit and highest in the farrowing unit (S6 Fig).

The influence of our assumptions about the existence of persistent shedders (PS) was assessed by running selected scenarios with no PS (S7 Fig). In general, the median prevalence stabilised at a lower level, when there was no PS, but only to a lesser degree in the farrowing unit, where there is a constant supply of new susceptible piglets and a high sow-to-offspring transmission (S7 Fig. B). The presence of PS in the model limited the possible decrease in prevalence following intervention. For reduced mixing and improved internal biosecurity, no effect was visible when simulating with the standard parameterisation that included the presence of PS, however, when running the scenario, where the pigs were kept together with their litter without PS, there was a marginal drop in the median prevalence within the weaner and finisher units (S7 Fig. E). When transmission between stables and sections were reduced by 50% in a scenario without PS, a small decrease was observed in all units immediately after intervention, except in the gestation unit (which is not separated into sections due to loose housing of the sows in larger groups), and in the farrowing unit (where sow to offspring transmission quickly generates new MRSA shedders) (S7 Fig. F). However, the effect observed was still far too small to be of any practical importance for field intervention.

The assumption regarding transmission from sow to offspring on the day of birth had a considerable influence on the prevalence within the farrowing unit (S8 Fig) but did not markedly alter the effect of any of the simulated interventions (S9 Fig).

Discussion

Reducing the transmission rates after LA-MRSA had become fully established within a herd to simulate a reduction in the selective pressure, resulted in a marked decrease in the prevalence within all stable units. However, LA-MRSA rarely disappeared completely from the herd and only in scenarios where the transmission rates were reduced to ≤ 30% of the original level. A reduction to ~40% of the original level corresponds to the transmission rates observed in a transmission study in the Netherlands, when no beta-

lactams or tetracyclines were used [5], but it must be expected that multiple factors related to management and the environment would affect transmission, and hence it remains unknown how large a reduction would be realistic. It has however been suggested that a reduction in the overall use of antimicrobials and especially those agents which co-select for LA-MRSA, might not result in a rapid decline in the occurrence of LA-MRSA; the effect will depend on the fitness cost of methicillin resistance for LA-MRSA and the impact of management and treatment procedures implemented to replace the current procedures [4]. Additionally, one could speculate that the high stability of tetracyclines and their ability to persist in the environment [22], might play a role.

In an intervention study of 36 Dutch pig farms, where the antimicrobial use decreased by 44% during the 18-month study, this decline was associated with a decreasing MRSA prevalence in pigs, despite tetracyclines and penicillins remaining the two most used drug types during the study period [9]. The observed decrease in prevalence did not occur as fast as those resulting from abrupt reduction of the transmission rates as in the present study, where an immediate rather than gradual reduction in the use of antimicrobials was assumed. Additionally, we assumed that the use of tetracyclines and penicillins would also be reduced. A reduction in transmission could also represent the effect of reducing the concentration of LA-MRSA in the air and the environment through for instance use of a disinfectant powder.

Reducing the number of pigs within each section after LA-MRSA had become established within the herd only resulted in marginal changes in the median prevalence within the herd, if the reduction should be kept within a range that is assumed to be economically feasible for the farmer (5-10%). These changes could all be attributed to the reduction in transmission rate implemented, rather than directly to the reduced number of animals within the section or pen. This could be caused by a weakness in the modelling approach, since we are not modelling the exposure through the air directly, even though it indirectly is included in the transmission rates. For density-dependent transmission, the transmission rates depend on the population size, and the estimate of transmission rate decrease with decreasing stocking-density are difficult to assess.

In the present study, no effect of modelling reduced mixing of pigs was observed. An investigation of the LA-MRSA status of piglets at the time of intervention, when the prevalence in the herd had stabilized at a high level, revealed that most piglets and litters were already LA-MRSA positive. The effect of reduced mixing between litters could intuitively not be observed when the majority of piglets were already shedders. However, even when applying lower transmission at day-one in the piglets' life in the sensitivity analysis, there was no apparent effect.

No environmental carryover effect was included in the LA-MRSA model used, i.e. we assumed perfect disinfection between batches [18]. We also assumed that LA-MRSA could quite easily be spread between

different compartments on the farm, if internal biosecurity procedures to avoid this were not practiced. LA-MRSA isolates originating from pig farms have been shown to be able to form robust biofilm under lab conditions [23], and thus may be able to survive on equipment for a long time. In the present study, no direct effect was observed as all units on the farm had already been contaminated, but this might still be important as a preventive measure in situations where LA-MRSA has not been introduced or in relation to keeping antimicrobial consumption low.

From the results of the sensitivity analysis, it became clear that our assumption regarding the existence of pigs persistently shedding LA-MRSA had a considerable influence on the results of the simulated interventions. The sensitivity analysis also revealed, that our assumption regarding transmission from sow to offspring at the day of birth, had a considerable influence on the general prevalence within the farrowing unit (S8 Fig), but not much influence on the effect of the simulated interventions (S9 Fig). The association between sow LA-MRSA status and the probability of piglets testing LA-MRSA positive have been confirmed in several studies [24–26], where the proportion of positive piglets in the days after farrowing were very different. We therefore expect the transmission on the day of birth might be dependent on the general infectious pressure on the farm, and therefore all the situations included in the sensitivity analysis could potentially be of practical relevance. Strongly decreased transmission at the day of birth could also represent the use of caesarean sections, as might be used to generate gnotobiotic pigs in nucleus breeding herds, e.g. if wanting to start a new LA-MRSA free production [27].

With the current parameterisation of the model, prevalences were in general highest within the farrowing unit, and lowest within the mating unit (S6 Fig), and thus the farrowing unit seems to be the area with the most potential for intervention. Also, changes within this unit seemed to have the most effect on the prevalence within the other units (S6 Fig).

When assessing the feasibility of the suggested interventions, practical and economic implications for the farmers should be considered, including any effects on health and growth rate of the pigs. Reducing antimicrobial consumption might be challenging, but the implementation of herd-specific interventions have in some cases been shown to reduce the use of antimicrobials without negative impact on overall economic and technical performance [28]. However, both the current antimicrobial consumption patterns and the reduction, that is possible to obtain, might of course vary considerable between farms, depending on management and current disease problems.
Also, while no direct effect of reducing the number of pigs within each section, reducing mixing or improving internal biosecurity were observed, these might all be important in relation to spread of other diseases, and consequently the antimicrobial consumption within the herd.

Preferably, there should be multiple benefits of the interventions, which require an investment from the farmer; either these should be a step toward not only reducing the occurrence of LA-MRSA, but also the occurrence of antimicrobial resistance in general or other problematic resistant bacteria such as extended-spectrum beta-lactamase producing bacteria (ESBL) or have a preventive effect on spread of disease within the herd in general. Also, it is crucial to obtain more knowledge on how to avoid MRSA being introduced or reintroduced in the herd.

Based on the results obtained from the present simulation study, it is unlikely that a highly contaminated farm can clear itself completely from LA-MRSA by only implementing interventions, which decrease transmission, e.g. reduced use of antimicrobials and zinc. However, this intervention did result in a marked decrease in the within-herd prevalence and might play an important role in preventing LA-MRSA in becoming established in a naïve herd. It is also important to keep in mind, that LA-MRSA has been found in organic [29] and antimicrobial-free herds [25], albeit much less frequently compared to in conventional herds (6% positive Danish organic herds in 2015 vs 68% positive Danish conventional herds in 2014). Therefore, while changes in antimicrobial consumption patterns might be an important step towards reducing the prevalence of LA-MRSA within a herd, it still needs to be supplemented by other preventive or intervention measures.

The results obtained are subject to uncertainty, due to the limitations of the model and the uncertainty of the parameters and the assumptions made. Especially, the assumption regarding PS has a noticeable influence on the results. Effects might differ between different farm types contaminated with LA-MRSA at different levels and this simulation study highlights a strong need for more knowledge from on-farm trials.

Conclusions

Reducing the transmission rates after LA-MRSA had become fully established within a herd, resulted in a marked decrease in the prevalence, but LA-MRSA only rarely disappeared completely. So, while changes in antimicrobial consumption patterns might be a very important step towards reducing the prevalence of LA-MRSA within a herd, it still needs to be supplemented by other preventive or intervention measures.

Slightly reducing the number of pigs within each section, reducing mixing of pigs, or improving internal biosecurity after LA-MRSA had become established within the herd only resulted in marginal changes in the median prevalence within the herd. However, these factors might be important in situations where LA-MRSA has not become established within the herd, or in relation to being able to achieve or maintain a low level of antimicrobial consumption.

The results of the sensitivity analysis indicated that the assumptions regarding the existence of pigs persistently shedding MRSA have a noticeable influence on the model results. The prevalence was in general, highest within the farrowing unit, and lowest within the mating unit, and thus the farrowing unit might be the area with most potential for intervention.

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References

1. Crombé F, Argudín MA, Vanderhaeghen W, Hermans K, Haesebrouck F, Butaye P. Transmission Dynamics of Methicillin-Resistant *Staphylococcus aureus* in Pigs. Front Microbiol. 2013;4: 57. doi:10.3389/fmicb.2013.00057

2. Armand-Lefevre L. Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. Emerg Infect Dis. 2005;11: 711–714.

3. Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg Infect Dis. 2005;11: 1965–1966. doi:10.3201/eid1112.050428

4. Guardabassi L, Larsen J, Weese JS, Butaye P, Battisti A, Kluytmans J, et al. Public health impact and antimicrobial selection of meticillin-resistant staphylococci in animals. Integr Med Res. 2013;1: 55–62. doi:10.1016/j.jgar.2013.03.011

5. Broens EM, Espinosa-Gongora C, Graat EAM, Vendrig N, Van Der Wolf PJ, Guardabassi L, et al. Longitudinal study on transmission of MRSA CC398 within pig herds. BMC Vet Res. 2012;8: 58. doi:10.1186/1746-6148-8-58

6. Alt K, Fetsch A, Schroeter A, Guerra B, Hammerl JA, Hertwig S, et al. Factors associated with the occurrence of MRSA CC398 in herds of fattening pigs in Germany. BMC Vet Res. 2011;7: 69. doi:10.1186/1746-6148-7-69

7. Fromm S, Beißwanger E, Käsbohrer A, Tenhagen B-A. Risk factors for MRSA in fattening pig herds – A meta-analysis using pooled data. Prev Vet Med. 2014;117: 180–188. doi:10.1016/j.prevetmed.2014.08.014

8. van Duijkeren E, Ikawaty R, Broekhuizen-Stins M, Spalburg E, de Neeling A, Allaart J, et al. Transmission of methicillin-resistant *Staphylococcus aureus* strains between different kinds of pig farms. Vet Microbiol. 2008;126: 383–389. doi:10.1016/j.vetmic.2007.07.021

9. Dorado-García, Alejandro Dohmen W, Bos MEH, Verstappen KM, Houben M, Wagenaar JA, Heederik DJJ. Dose-Response Relationship between Antimicrobial Drugs and Livestock-Associated MRSA in Pig Farming. Emerg Infect Dis. 2015;21: 950. doi:10.3201/eid2106.140706

10. Friese A, Schulz J, Hoehle L, Fetsch A, Tenhagen B-A, Hartung J, et al. Occurrence of MRSA in air and housing environment of pig barns. Vet Microbiol. 2012;158: 129–135. doi:10.1016/j.vetmic.2012.01.019

11. Lurette A, Belloc C, Touzeau S, Hoch T, Seegers H, Fourichon C. Modelling batch farrowing management within a farrow- to-finish pig herd : influence of management on contact structure and pig delivery to the slaughterhouse. Animal. 2008; 105–116. doi:10.1017/S1751731107000997

12. Cleveland-Nielsen A, Nielsen EO, Ersbøll AK. Chronic pleuritis in Danish slaughter pig herds. Prev Vet Med. 2002;55: 121–135.

13. Schmithausen RM, Schulze-Geisthoevel SV, Stemmer F, El-Jade M, Reif M, Hack S, et al. Analysis of Transmission of MRSA and ESBL-E among Pigs and Farm Personnel. PLoS One. 2015; doi:10.1371/journal.pone.0138173

14. Grøntvedt CA, Elstrøm P, Stegger M, Skov RL, Skytt Andersen P, Larssen KW, et al. Methicillin-Resistant *Staphylococcus aureus* CC398 in Humans and Pigs in Norway: A "One Health" Perspective on Introduction and Transmission. Clin Infect Dis. 2016;63: 1431–1438. doi:10.1093/cid/ciw552

15. Espinosa-Gongora C, Panduro P, Saxmose S. Effect of a disinfectant powder on methicillin-resistant *Staphylococcus aureus* in pigs, bedding and air samples under simulated farm conditions. APA; 2013;

16. Verhegghe M, Crombe F, De Man I, Haesebrouck F, Butaye P, Heyndrickx M, et al. Preliminary study of the effect of sow washing, as performed on the farm, on livestock-associated methicillin-resistant Staphylococcus aureus skin status and strain diversity. J Swine Heal Prod. 2013;21: 313–319.

17. Schulz J, Bao E, Clauß M, Hartung J. The potential of a new air cleaner to reduce airborne microorganisms in pig house air: preliminary results. Berl Munch Tierarztl Wochenschr. 2013;4: 143–148. doi:10.2376/0005-9366-126-143

18. Sørensen AIV, Toft N, Boklund A, Espinosa-Gongora C, Græsbøll K, Larsen J, et al. A mechanistic model for spread of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) within a pig herd. PLoS One. 2017;12: e0188429. doi:10.1371/journal.pone.0188429

19. Danish Veterinary and Food Administration. Resultaterne af screening for husdyr-MRSA i svin i 2016. (in Danish) [Internet]. 2017. pp. 1–2. Available: https://www.foedevarestyrelsen.dk/Nyheder/Aktuelt/Documents/MRSA ekspertgruppe - resultatene forekomst af husdyr-MRSA i svin 2016.pdf

20. R Core team [Internet]. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing , Vienna, Austria. http://www.R-project.org.

21. van Belkum A, Verkaik NJ, de Vogel CP, Boelens HA, Verveer J, Nouwen JL, et al. Reclassification of Staphylococcus aureus nasal carriage types. J Infect Dis. 2009;199: 1820–6. doi:10.1086/599119

22. Drogui R, P D. Tetracycline antibiotics in the environment : a review. Env Chem Lett. 2013;11: 209–227. doi:10.1007/s10311-013-0404-8

23. Nicholson TL, Shore SM, Smith TC, Fraena TS. Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) Isolates of Swine Origin Form Robust Biofilms. 2013; doi:10.1371/journal.pone.0073376

24. Burns A, Shore AC, Brennan GI, Coleman DC, Egan J, Fanning S, et al. A longitudinal study of *Staphylococcus aureus* colonization in pigs in Ireland. Vet Microbiol. Elsevier; 2014;174: 504–13. doi:10.1016/j.vetmic.2014.10.009

25. Weese JS, Zwambag A, Rosendal T, Reid-Smith R, Friendship R. Longitudinal Investigation of Methicillin-Resistant *Staphylococcus aureus* in Piglets. Zoonoses Public Health. 2011;58: 238–243. doi:10.1111/j.1863-2378.2010.01340.x

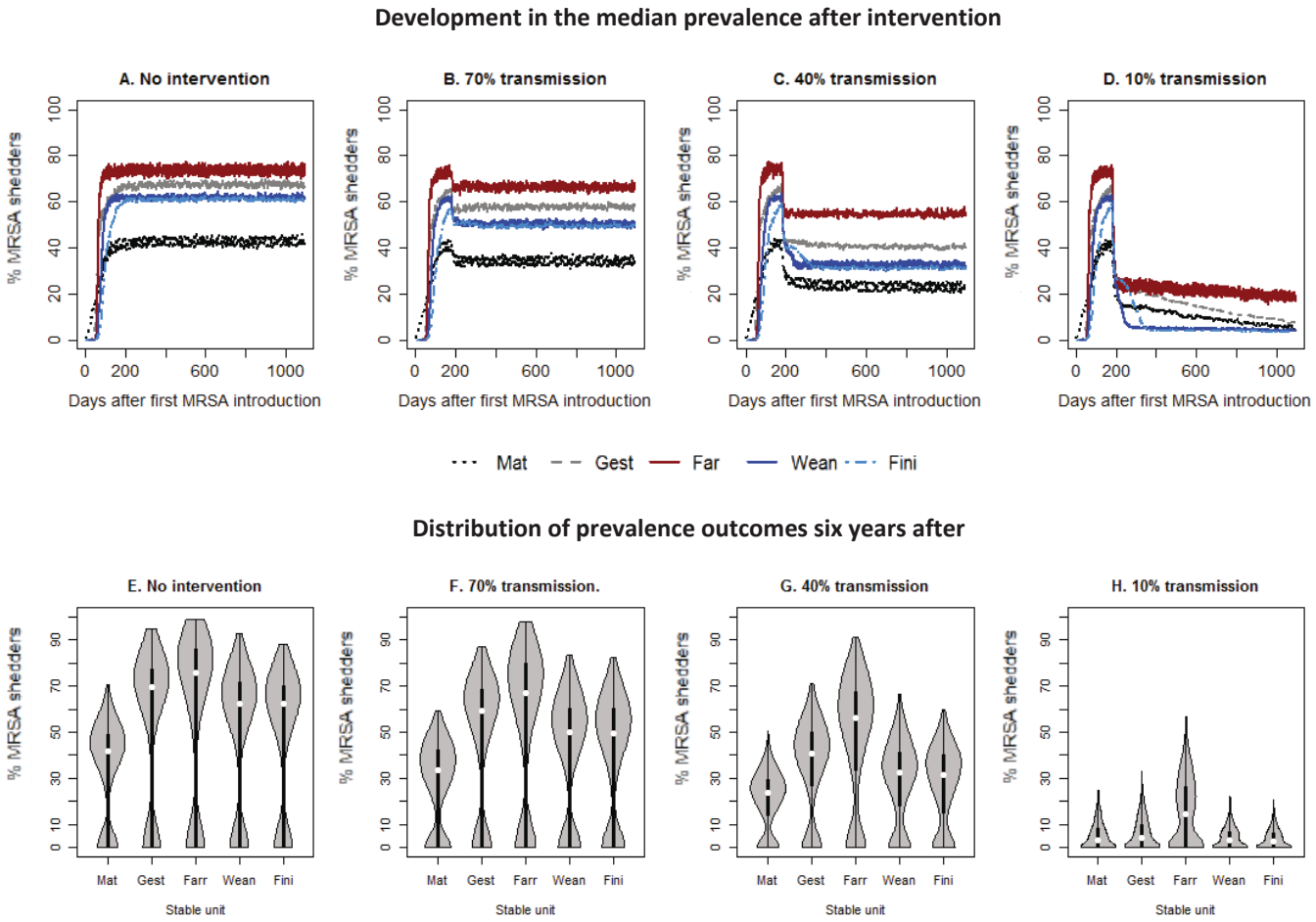
26. Verhegghe M, Pletinckx LJ, Crombé F, Weyenberg S Van, Haesebrouck F, Butaye P, et al. Cohort study for the presence of livestock-associated MRSA in piglets: Effect of sow status at farrowing and determination of the piglet colonization age. Vet Microbiol. 2013;162: 679–686. doi:10.1016/j.vetmic.2012.09.014

27. Olsen JV. Bilagsrapport om erhvervsøkonomiske analyser af omkostninger for håndtering og bekæmpelse af husdyr-MRSA i svin (in Danish) [Internet]. 2017. Available: https://curis.ku.dk/ws/files/182262661/IFRO_Udredning_2017_10b.pdf

28. Collineau L, Rojo-gimeno C, Léger A, Backhans A, Loesken S, Wauters E, et al. Herd-specific interventions to reduce antimicrobial usage in pig production without jeopardising technical and economic performance. Prev Vet Med. Elsevier B.V.; 2017;144: 167–178. doi:10.1016/j.prevetmed.2017.05.023

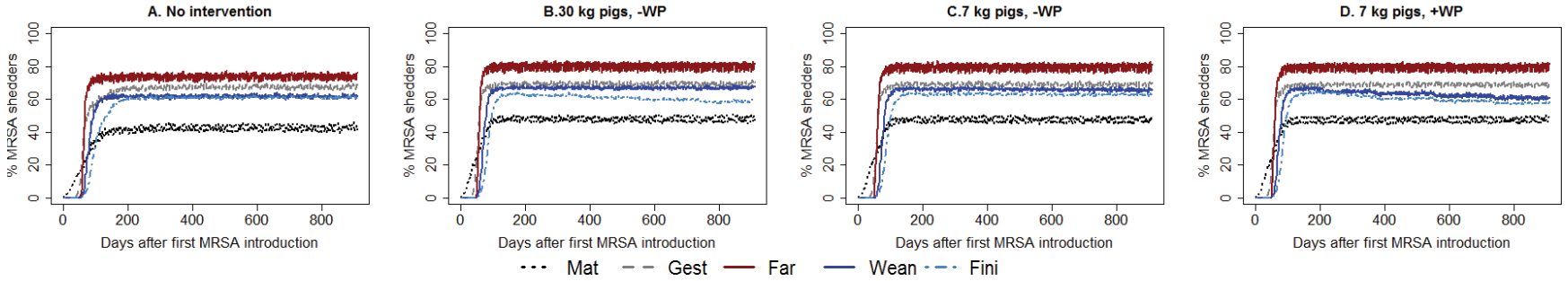
29. DANMAP. Danmap 2016 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark [Internet]. 2017. Available: www.danmap.org

Fig 1. Reduced transmission



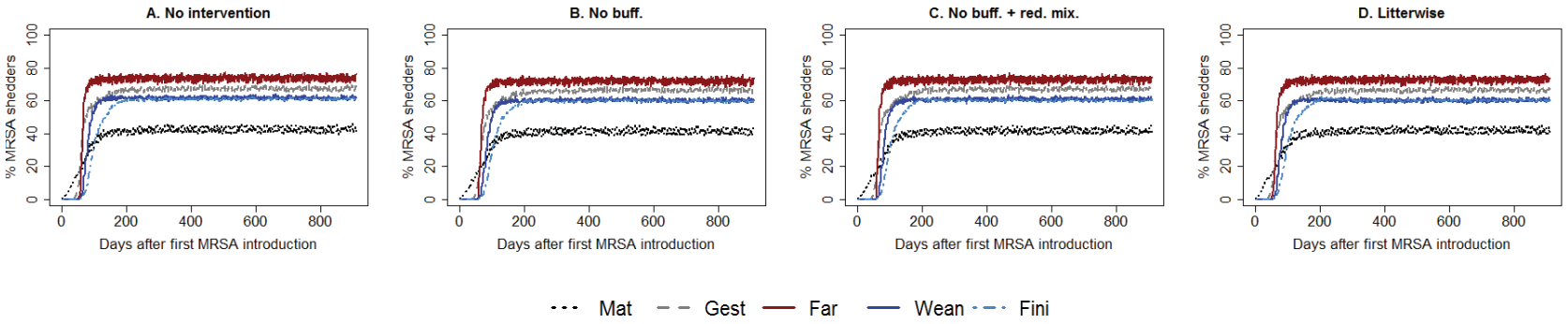
Note: Transmission was reduced 180 days after MRSA had been introduced. The percentages refer to the proportion of the 'high' set of transmission rates, the set of rates used after intervention at day 180 corresponds to.
Mat = mating unit, Gest = gestation unit, Farr = farrowing unit, Wean = weaner unit, Fini = finisher unit.

Fig 2. Reduced density.



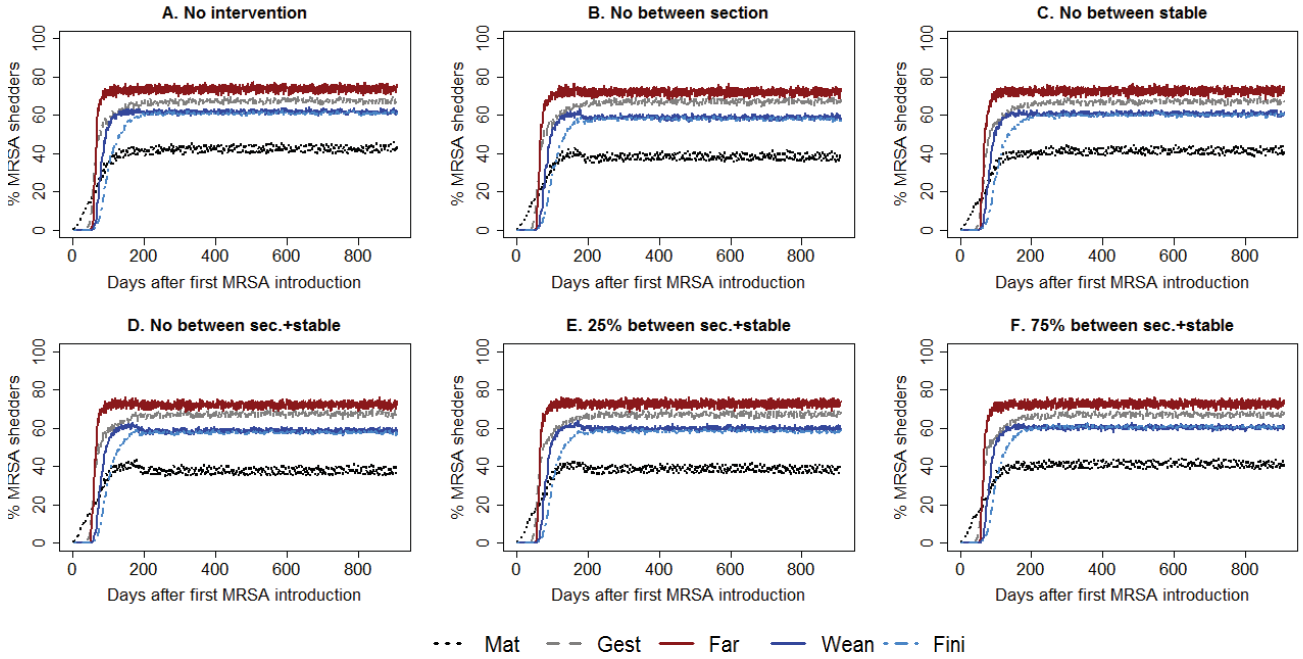
Note: Development in the median number and prevalence of MRSA shedders over time. High transmission.
Mat = mating unit, Gest = gestation unit, Far = farrowing unit, Wean = weaner unit, Fin = finisher unit.
The number of pigs within the relevant unit was gradually reduced by 5% every 6th month.
7 kg pigs/30 kg pigs refer to if the pigs are sold just after weaning (7 kg) or not until they reach approximately 30 kg, which also is the point, where they normally would be moved from the weaner to the finisher unit.
-/+ WP reduction refers if the within-pen density has also been reduced, or if some pens are just empty – the overall within-room density will be the same in both scenarios.

Fig 3. Reduced mixing.



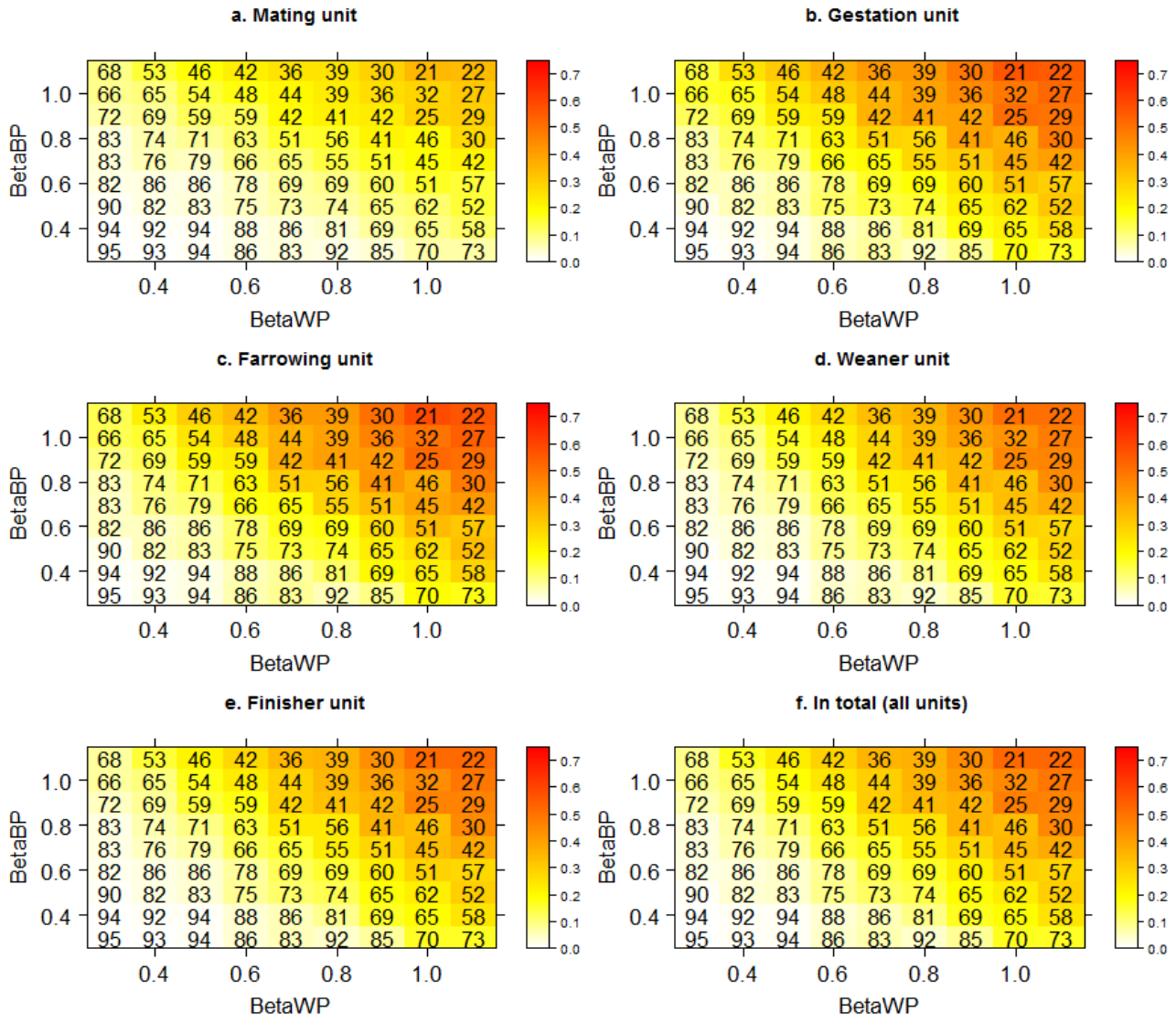
Note: Development in the median prevalence of MRSA shedders over time. High transmission.
Intervention was initiated 180 days after MRSA had been introduced.
Mat = mating unit, Gest = gestation unit, Far = farrowing unit, Wean = weaner unit, Fin = finisher unit.
No buff = no use of buffer sections.
Red. Mix = Reduced mixing – two litters are put into one pen together in the weaners unit, instead of random mixing of piglets.
Litterwise = weaners and finishers are only sharing pens with pigs from the same litter as themselves.

Fig 4. Improved internal biosecurity



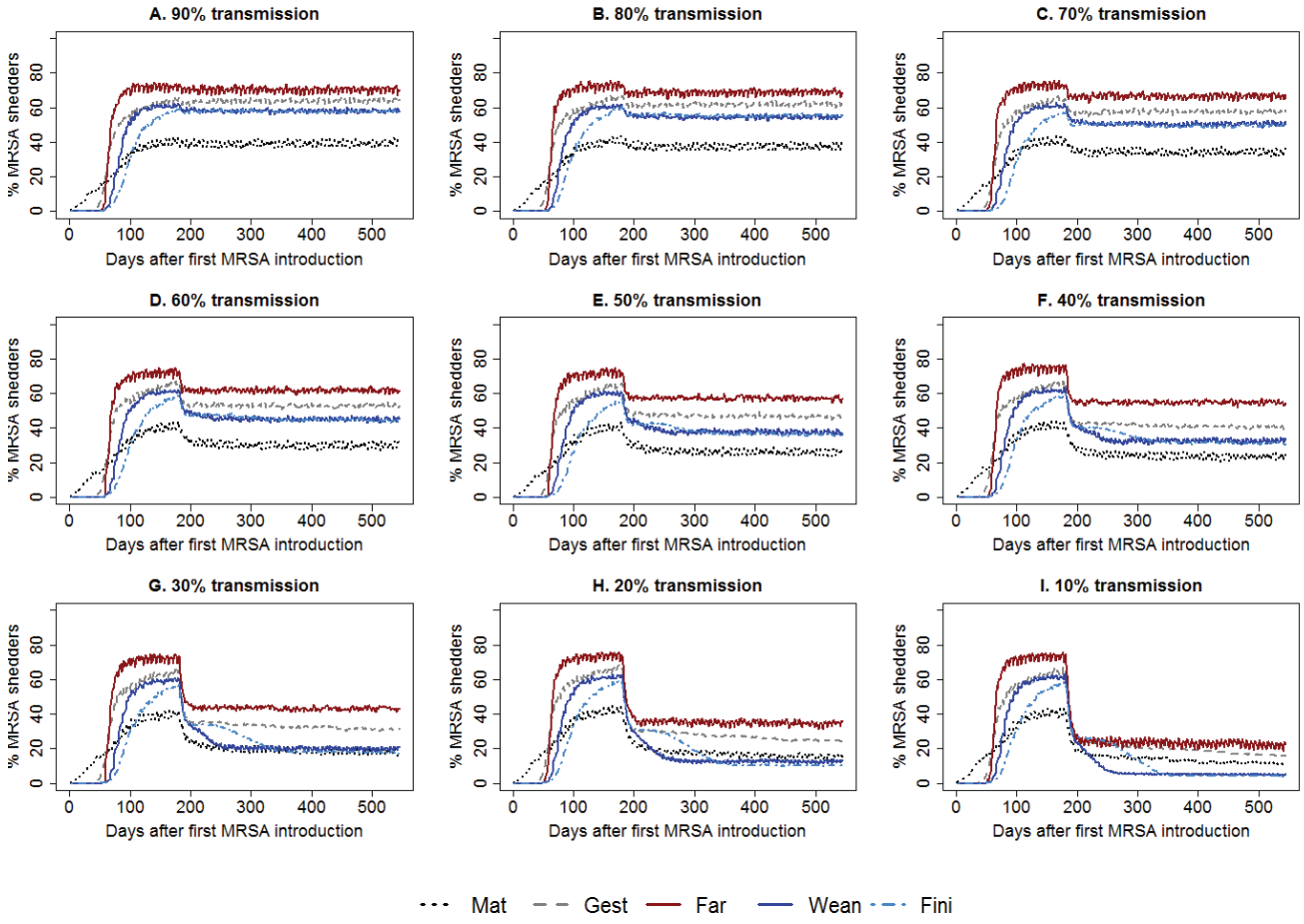
Note: Development in the median prevalence of MRSA shedders over time. High transmission.
Intervention was initiated 180 days after MRSA had been introduced.
Mat = mating unit, Gest = gestation unit, Far = farrowing unit, Wean = weaner unit, Fin = finisher unit.
Panel B-D illustrates the extreme cases of completely eliminating between compartment transmission, whereas E and F illustrate the influence of a reduction of the transmission between sections and stables to 25% (E) or 75% of the original value (F).

Fig 5. Mean prevalence in the stable units following changes in the within-pen and the between-pen transmission rate



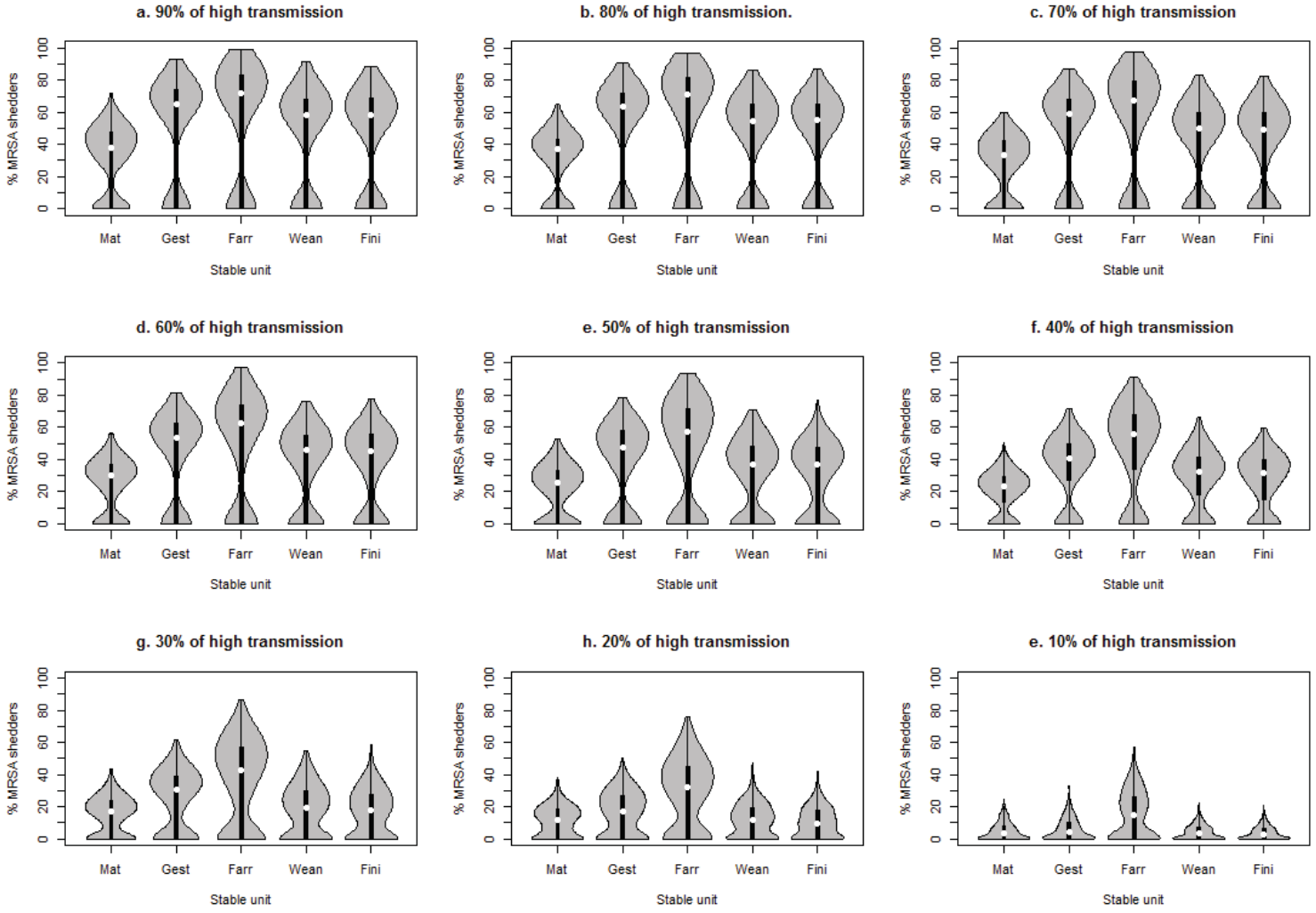
Note: The colour intensity represents the mean prevalence. The proportion of iterations where MRSA did not become established has been printed on each square.
BetaBP = between-pen transmission rate, scaled as indicated.
BetaWP = within-pen transmission rate, scaled as indicated

S1 Fig. Reduced transmission: Development in the median prevalence after intervention



Note: Mat = mating unit, Gest = gestation unit, Far = farrowing unit, Wean = weaner unit, Fin = finisher unit. Transmission was reduced 180 days after MRSA had been introduced. The percentages refer to the proportion of the 'high' set of transmission rates, the set of rates used after 180 days corresponds to.

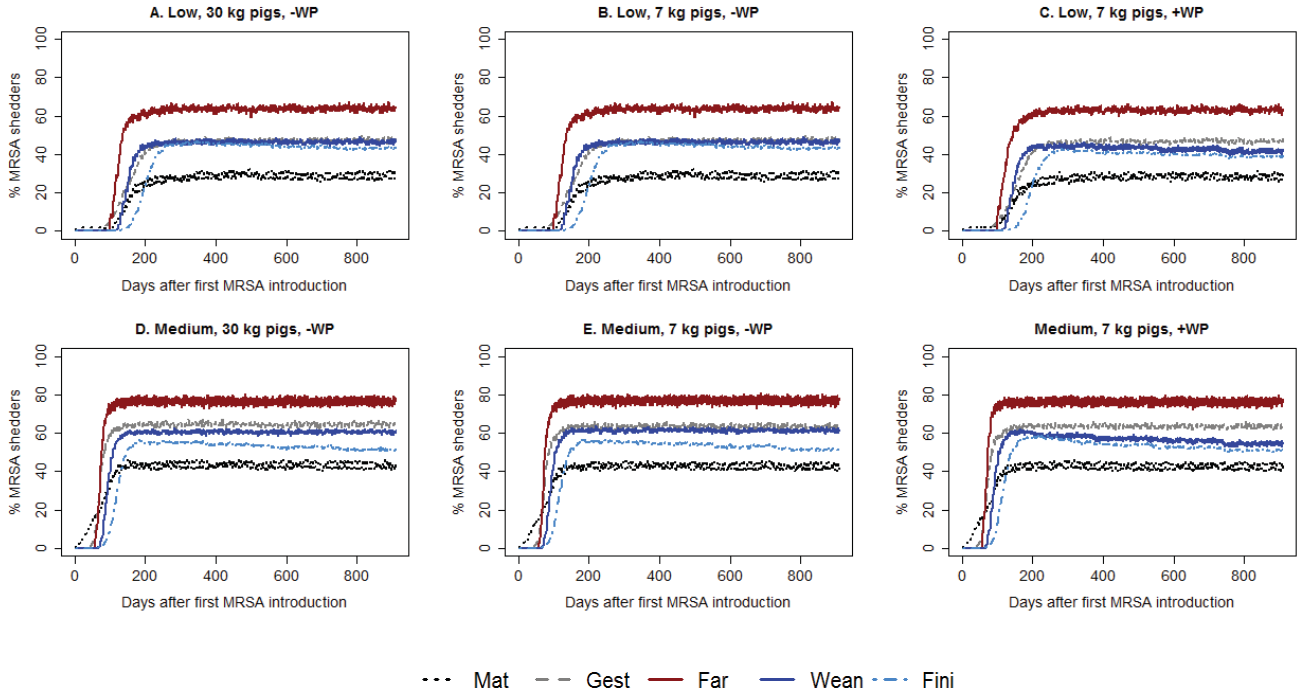
S2 Fig. Reduced transmission: prevalence in the stable units six years after introduction



Note: Mat = mating unit, Gest = gestation unit, Far = farrowing unit, Wean = weaner unit, Fin = finisher unit. Mat = mating unit, Gest = Transmission was reduced 180 days after MRSA had been introduced.

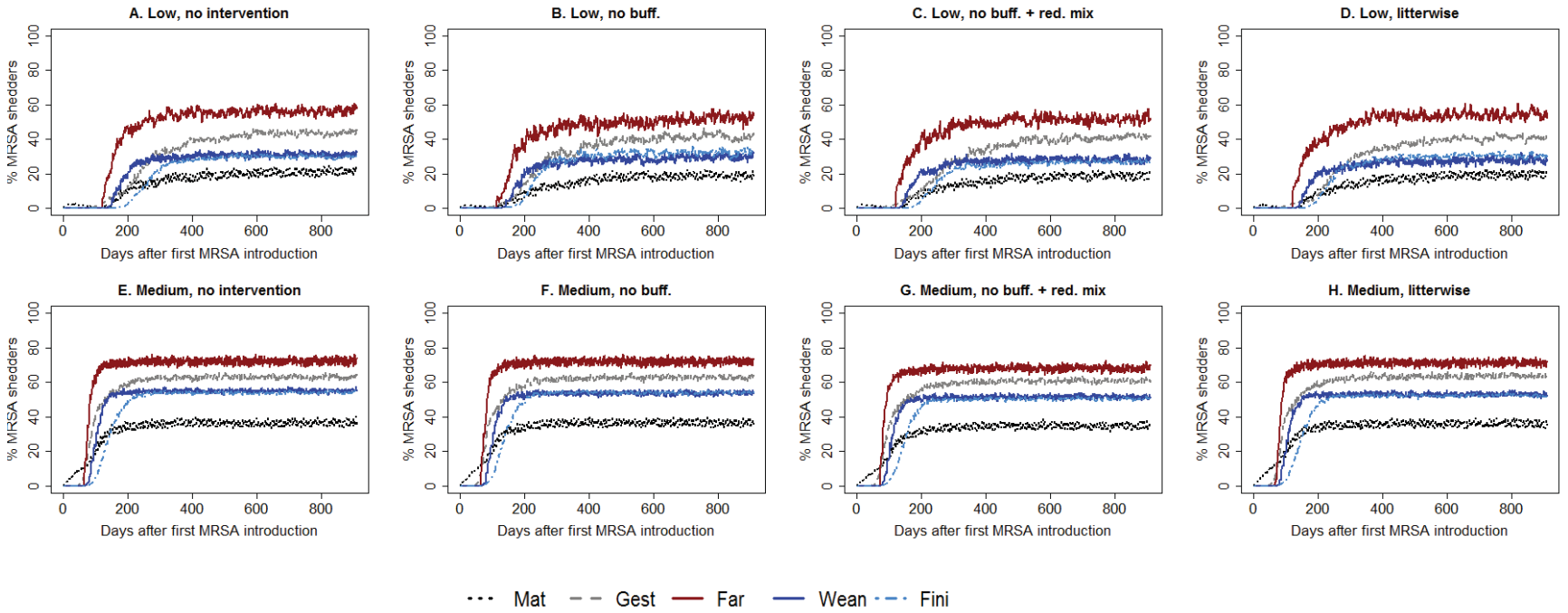
The widths of the violin plots illustrate the distribution of 500 iterations. The median prevalence is indicated by white dots.

S3 Fig. Reduced density: Low and medium transmission



Note: Development in the median number and prevalence of MRSA shedders over time (only includes iterations where MRSA became established). Intervention was initiated 180 days after MRSA had been introduced.
Mat = mating unit, Gest = gestation unit, Far = farrowing unit, Wean = weaner unit, Fin = finisher unit.
7 kg pigs/30 kg pigs refer to if the pigs are sold just after weaning (7 kg) or not until they reach approximately 30 kg, which also is the point, where they normally would be moved from the weaner to the finisher unit.
-/+ WP (within-pen reduction refers) if the within-pen density has also been reduced, or if some pens are just empty; the overall within-room density will be the same in both scenarios.

S4 Fig. Reduced mixing: Medium and low transmission

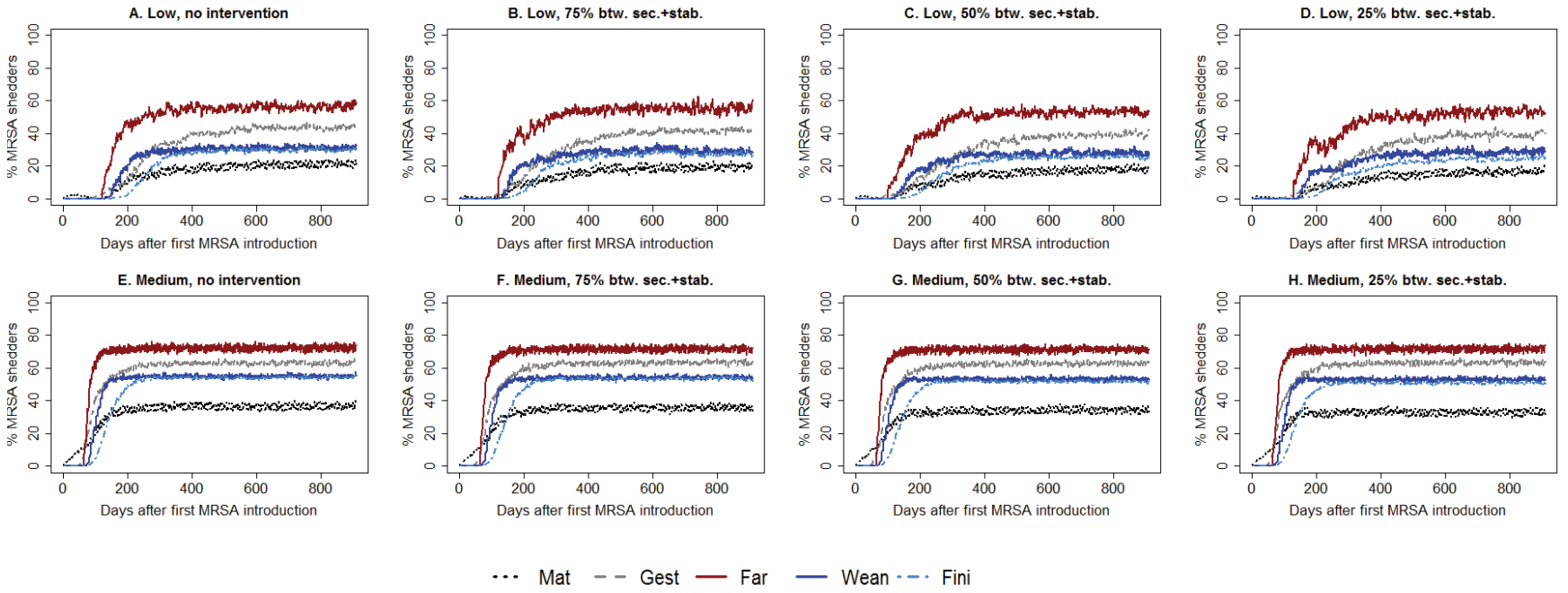


Note: Development in the median prevalence of MRSA shedders over time (includes only iterations where MRSA became established). Intervention was initiated 180 days after MRSA had been introduced.

Mat = mating unit, Gest = gestation unit, Far = farrowing unit, Wean = weaner unit, Fin = finisher unit.

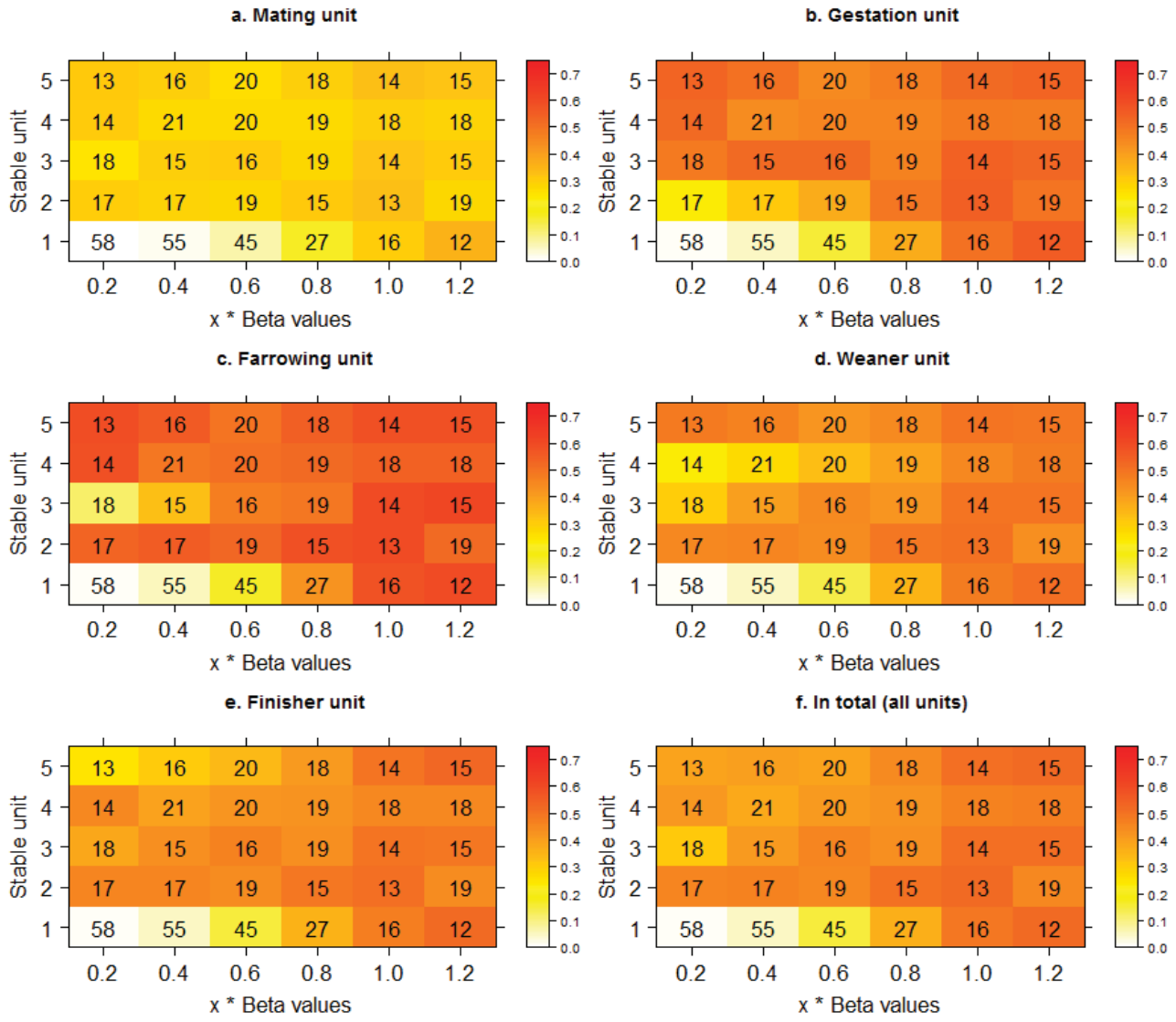
No buff = no use of buffer sections, Red. Mix = Reduced mixing – two litters are put into one pen together in the weaners unit, instead of random mixing of piglets, Litterwise = weaners and finishers are only sharing pens with pigs from the same litter as themselves.

S5 Fig. Improved internal biosecurity: Low and medium transmission.



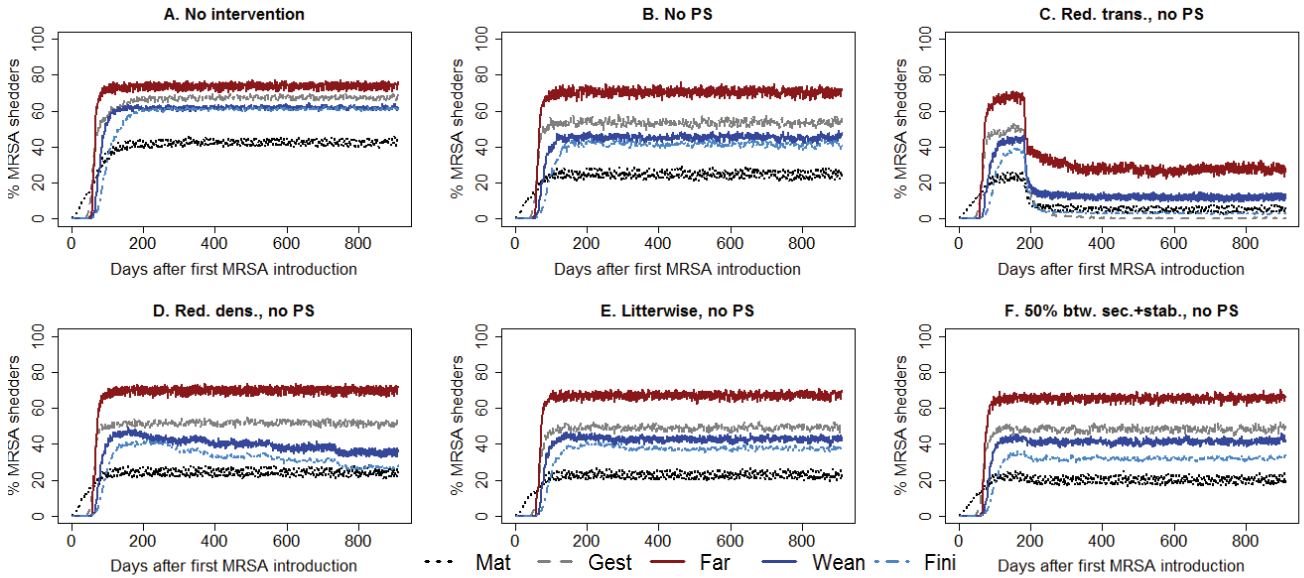
Note: Development in the median prevalence of MRSA shedders over time (includes only iterations where MRSA became established).
Mat = mating unit, Gest = gestation unit, Far = farrowing unit, Wean = weaner unit, Fin = finisher unit.
Intervention was initiated 180 days after MRSA had been introduced.
Panel B-D and F-H illustrate the influence of a gradual reduction of the transmission between sections and stables from 75% of the original value (B and F) to 25% of the original value (D and H).

S6 Fig. Mean prevalence following changes in transmission within one stable unit at a time.



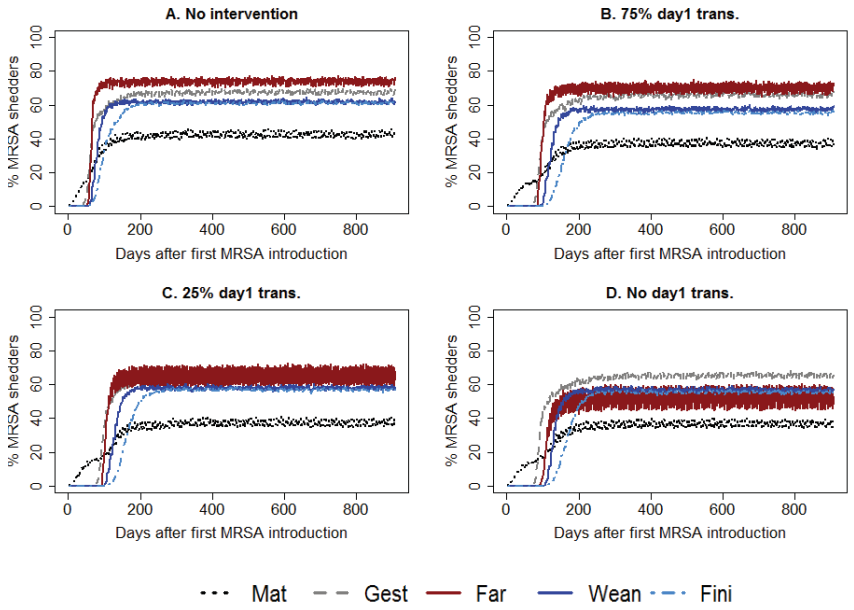
Note: The colour intensity represents the mean prevalence. The proportions of iterations where MRSA did not become established have been printed on each square. Stable units: 1. Mating unit, 2. Gestation unit, 3. Farrowing unit, 4. Weaner unit, 5. Finisher unit. x = proportion of the Beta values (transmission parameters) used. Beta values = all relevant transmission parameters applied within the unit.

S7 Fig. Sensitivity analysis: Persistent shedders.



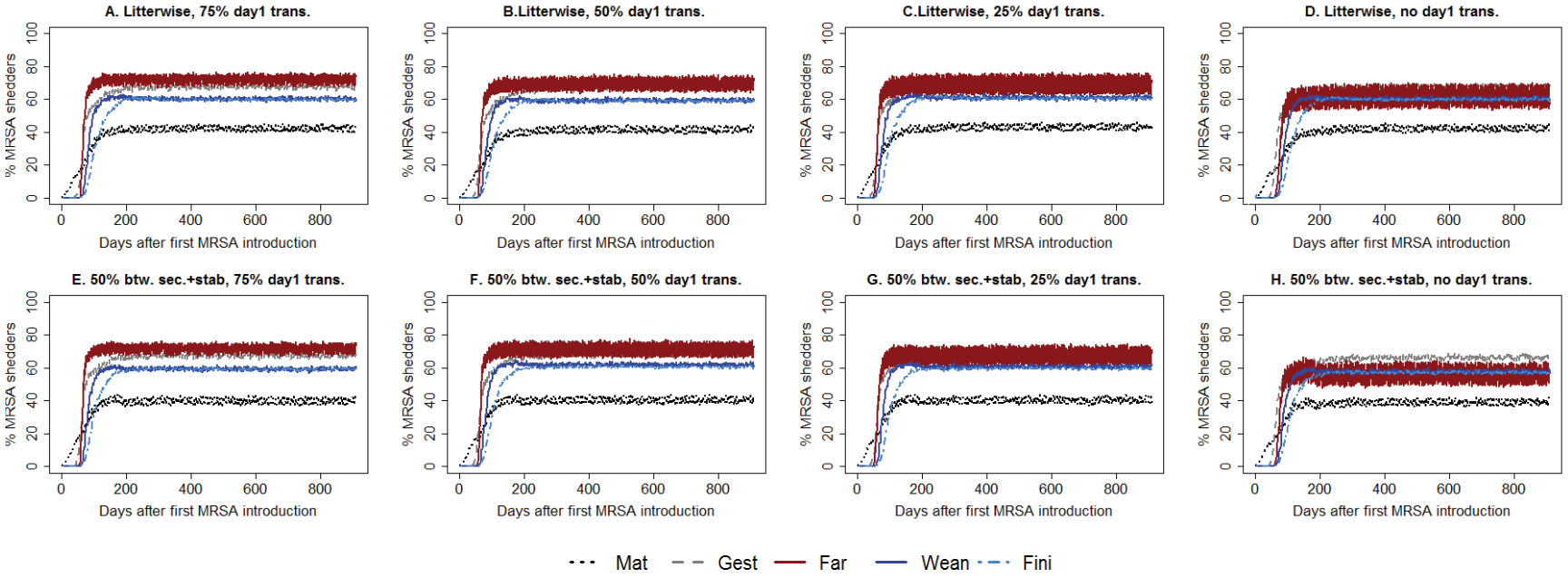
Note: Development in the median prevalence of MRSA shedders over time. High transmission.
No persistent shedders from the start of simulation. Intervention was initiated 180 days after MRSA had been introduced.
Mat = mating unit, Gest = gestation unit, Far = farrowing unit, Wean = weaner unit, Fin = finisher unit.
No PS = No existence of persistent shedders, it is assumed that all pigs will become intermittent shedders upon exposure.
Red. trans = transmission reduced to 40% of the initial level, red. dens = sale of 7 kg pigs and increasing reduction in within pen density.
Litterwise = weaners and finishers are only sharing pens with pigs from the same litters as themselves.
50% btw. sec. + stab = the transmission between sections and stables reduced by 50%, e.g. through improved biosecurity.

S8 Fig. Sensitivity analysis: Transmission on the day of birth.



Note: Development in the median prevalence of MRSA shedders over time. High transmission and no intervention.
Mat = mating unit, Gest = gestation unit, Far = farrowing unit, Wean = weaner unit, Fin = finisher unit.
Panel A-D illustrate the influence of a gradual reduction of transmission between sow and offspring on the day of birth from 75% of the original value (B) to 0% of the original value (D).

S9 Fig. Sensitivity analysis: Transmission on the day of birth - reduced mixing and increased biosecurity.



Note: Development in the median prevalence of MRSA shedders over time. Transmission on day one reduced from start of simulation. Mat = mating unit, Gest = gestation unit, Far = farrowing unit, Wean = weaner unit, Fin = finisher unit.
Panel A-E illustrate the influence of a gradual reduction of transmission between sow and offspring on the day of birth from 75% of the original value (A) to 0% of the original value (H), where transmission between sow and offspring on the day of birth is assumed to take place as described in Sørensen et al., 2017 (100%).

6.3. Discussion (Part B)

6.3.1 Selection of model structure

The simulation model for spread of LA-MRSA within a herd constructed in this part of the project was an individual-based mechanistic model (manuscript II). Building a mechanistic model including all major processes going on within the herd, enabled us to: 1) quantify the importance of the different mechanisms of LA-MRSA spread between animals and identify the main drivers of LA-MRSA spread, 2) propose and examine the effect of interventions in these processes, and 3) identify knowledge gaps and critical parameters. If possible, critical parameters should subsequently be estimated precisely from observed data. Furthermore, this approach enabled taking host-related factors regarding carriage into account, by only assigning specific pigs the potential to become persistent shedders.

Several alternative models for spread of diseases within pig herds exists, e.g. for African swine fever, porcine circovirus and *Salmonella* [185,200,201] or for modelling the effect of different management strategies [202,203]. Some of these models are not very detailed, whereas at least one of the management models [202] have a level of detail similar to the mechanistic model build in the present project and therefore in principle could have been adapted to this use. However, we needed a model where the parameterisation was based on current management practises in the Danish pig production, and which was built in a software, where we could also create the epidemic model.

When building a model, it is a balance to include just enough detail to describe all major processes properly without adding redundant details that read just as noise. In principle this could be tested by stepwise removing details from the model and comparing output from the reduced versions with the full model, however this exercise is seldom carried out, albeit the influence of some details, e.g. the existence of persistent shedders in the case of LA-MRSA, often will be included in sensitivity analysis. In the present model, many details e.g. use of two-step nursery sows (foster dams) were included in order to most accurately imitate the contact pattern among the pigs, since e.g. the risk of transmission to pen mates, were assumed to be higher than for pigs not housed within the same pen.

6.3.2 Other MRSA models

To our best knowledge, the model build in the present PhD project was the first mechanistic model for spread of LA-MRSA within a pig herd to be fully described in a scientific paper. For transparency, the source code of the model has been made freely available on the internet, where interested researchers can benefit from and further develop on it. The herd module could easily be incorporated in a model for spread of other bacteria or pathogens circulating within the pig population.

In addition to the model build in the present project, other types of models for spread of LA-MRSA do exist. This includes another model for spread of LA-MRSA within a pig herd [204], three models for spread of LA-MRSA between pig herds [6, T. Rosendal, personal communication; J. Schulz personal communication], as well as a meta-population model for spread of LA-MRSA into the general human population [205]. Currently a full description has only been published for two of these models, and therefore the models cannot be discussed in detail. One of the models under development is a model for spread of LA-MRSA in the Danish pig population [206], and this model also includes a within-herd module for spread of LA-MRSA. However due to the complexity of the modelled system, where focus is given to modelling the spread of LA-MRSA between herds, the within herd module was based on a relatively simple deterministic approach [J. Shulz & T. Halasa, personal communication]. It is the intention that the model build in the present PhD project will supplement this between-herd model.

Specifically for spread of LA-MRSA within a pig herd, currently at least one other model exist [204]. Based on the information available about this model, it also seems to be individual-based, whereas it is not known exactly what level of detail is included in the herd model. The epidemic model for LA-MRSA in this model includes two disease stages, susceptible and carrier. Interestingly, this model also included LA-MRSA load and growth of LA-MRSA on the pig, where the load affected transmission rate and duration of carriage.

In a meta-population model for spread of LA-MRSA from a farm and into the community, LA-MRSA is transmitted from a single pig farm and within and between different at-risk populations (five with direct or indirect contact to pigs and three without contact) [205]. Spread within the farm is not included in the model; it is just included as a place where farm workers, companion animals, veterinarians and transporters get exposed according to their frequency of visiting the farm [205]. The epidemic model used for MRSA in this study, included three different disease stages: susceptible/non-carrier, transient carrier or persistent carrier [205], similar to the stages in the model described in manuscript II.

Several models for spread of HA- or CA-MRSA within the hospital environment exist [207–212]. The type of models represented by these models range from relatively simple models [211] and to detailed agent-based models [207]. However, due to the huge differences in the environment modelled, these will not be described in further detail here. At least one model for spread of *S. aureus* in the hospital environment, that includes both antimicrobial resistant and sensitive strains exists [213], and MRSA models have been used as an example of models, where the structure (e.g. single strain / multi strain model) can heavily influence the inference drawn [214]. However, currently all described LA-MRSA models are single strain models, which might be a consequence of the lack of knowledge regarding the exact competition and selection dynamics between MSSA and MRSA.

6.3.3 Limitations and simplifications

The main limitations of the model are: the uncertainty caused by the many knowledge gaps regarding LA-MRSA; the large variation in reported R_0 values and thereby uncertainty related to the transmission rates; and a lack of validation by real-life LA-MRSA data for the within-farm LA-MRSA prevalence on Danish farms.

The transmission rates used are based on studies at four farms only, and these are expected to be highly-sensitive to farm-specific factors, such as antimicrobial use. The high uncertainty is illustrated by the wide range between the lowest and highest reported R_0 values from <1 to 52.5 [41,112,121]. The variation in R_0 values is expected to be caused by differences in age and antimicrobial consumption, as well as dose of exposure and possibly environmental factors. To take this challenge into account, the model was run with low, moderate and high transmission rates, in order to examine the impact of these rates on LA-MRSA dynamics. In all scenarios, once LA-MRSA becomes established in the herd, it will persist. The difference between the scenarios is the level of the prevalence within the herd and the proportion of iterations, where LA-MRSA becomes established in the herd. The higher the transmission rate, the larger number of iterations, where LA-MRSA becomes established, and the higher the within-herd prevalence.

Despite the lack of validation by real-life LA-MRSA, the relative distribution of the simulated prevalence within the different age groups is in reasonable agreement with what has been observed in other studies, albeit many observed the highest prevalence among weaners (Manuscript II, Table S11). With the current parameterisation this is not the case in the model, where the same post-weaning transmission rate estimates are applied for both weaners and finishers. The selective pressure in the early period in the weaner unit is often higher, as the pigs might suffer from weaning stress and diarrhoea and receive zinc or treatment with antimicrobials, which is not specifically taken into account in the model. For 18 Norwegian pig farms involved in LA-MRSA outbreaks, the time of LA-MRSA introduction on the farms have been estimated based on trade data [56]. In addition, the within-herd prevalence on these farms were estimated based on the proportion of positive sampling pools [56]. On the five farms, which were estimated to have been LA-MRSA positive for less than a month, the within-farm prevalence varied from 10-30%, while on the six farms that had been positive for approximately two months the within-herd prevalence varied from 11-50% [56]. It is not known, how many shedder pigs that were introduced on each farm, but the observed within-herd occurrences are in general within the range of outcomes observed in the simulations in the present project.

No separate environmental component was included in the LA-MRSA within-herd model, due to environmental spread already being included in the transmission rates used for within-pen and between-pen transmission [112]. However, findings of LA-MRSA in air [123,215] and the results of a study where naïve pigs were exposed to a contaminated environment [143], indicate that the environment might play an important role. Therefore intervention strategies aiming at reducing the occurrence of LA-MRSA in air

and dust have received some attention, but by not including a separate environmental component in the model, simulating the effect of this type of interventions has been made difficult. The advantage of interventions targeting LA-MRSA in the environment is the potential to reduce exposure to LA-MRSA for workers and visitors in the herd; whereas the disadvantage is that it is not removing LA-MRSA from the pigs, which are assumed to be the original source of LA-MRSA contamination. Currently, it is unknown if intermittently shedding pigs can clear themselves of LA-MRSA in the absence of infectious pressure from the environment. However, one would expect that any persistent carriers and/or truly colonised pigs would still be able to transmit LA-MRSA to their pen mates or be able to spread LA-MRSA to other farms through trade.

Several simplifications and prioritisations had to be made when building the model. For instance, movement of pigs to and from relief pens was not included in the model due to uncertainty about the frequency and duration of the use of relief pens. In addition, it was assumed that all piglets were weaned after 28 days; in reality this will usually vary depending on their individual weight gain. It was also assumed that the sows' hormonal cycles were synchronized, so that all sows farrowing at the same time would also come in heat again at the same time, and that their gestation periods would all be of the same duration. These simplifications were deemed acceptable, because minor variations in insemination time or day of farrowing would not cause sows to move teams and mix with other pigs than their team mates, and thus these simplifications would have limited impact on the results regarding spread of LA-MRSA. Use of cross-fostering was not included in the model, neither was pen location within a section. The risk of transmission from pigs in neighbouring pens is most likely larger than the risk of transmission between more distant pens. However, it must be kept in mind that there is a large diversity in stable design and management systems on farms, and the herd modelled is only one representation of how a herd might function.

6.3.4 Simulation of interventions

Results of modelling spread of LA-MRSA into the general human population indicate that a control policy that only targets human carriers will not be sufficient to control spread of LA-MRSA in the community, if it remains in the pig population [205]. Therefore, the effect of different farm-level control measures was modelled (manuscript III).

Reducing the transmission rates after LA-MRSA had become fully established within a herd, in an attempt at mimicking a decrease in antimicrobial consumption, resulted in a marked decrease in the within-herd prevalence of LA-MRSA shedders, but LA-MRSA was rarely completely eradicated. Improving internal biosecurity, reducing mixing of pigs, or slightly reducing the number of pigs within each section were only predicted to cause marginal changes in the median within-herd prevalence of LA-MRSA shedders.

The assumptions regarding effect of the interventions and the feasibility of implementing these can be discussed. For simplification, it was assumed that reducing antimicrobial consumption would immediately lead to a decline in the transmission rate. It has been demonstrated that under selective antibiotic pressure, the colonizing flora of hospitalised patients change already within 24 to 48 hours [216]. However, due to the level of dust and organic material in the stable environment, and the ability of tetracycline to remain in the environment for extended amounts of time [217], it remains unknown, whether it will have the same fast effect in pigs.

Additionally, the very sudden reduction in antimicrobial consumption in the modelled scenario might not be feasible. However, due to a lack of good data for the time realistically needed for such a drastic reduction in consumption, it was for simplification assumed to happen immediately. The current level of antimicrobial consumption will naturally vary between farms, as well as the potential for reduction of antimicrobial consumption. The possibilities for reducing antimicrobial consumption have been analysed at several occasions [31,218], and in general, it is believed that herds, where the animals are more resistant to disease, can be reached through focused optimization of: the animals' environment and surroundings; feed; breeding material; daily management and care [218]. However, very restricted antimicrobial use can also cause ethical dilemmas, since sick animals need to be treated or alternatively culled [31].

Originally, it was the intention that input data for modelling interventions should be harvested in other parts of the OHLAM project. However, at the time of submission of this thesis, these studies were still ongoing, and therefore a rather theoretical approach was taken. The availability of data for parameterisation from on-farm experiments would enable the modelling of interventions that currently cannot be modelled due to lack of data, e.g. effects of air filtration on spread of LA-MRSA within the herd, and thereby increase the usability of the model as a decision tool.

7. General discussion

7.1 Knowledge gaps regarding spread and control of LA-MRSA in pig herds

Several knowledge gaps or uncertainties regarding LA-MRSA were identified in this project, especially during conceptualisation and parameterisation of the model. These range from a lack of understanding of how LA-MRSA is introduced in herds, in cases where this cannot be attributed to trade, and to a general lack of knowledge regarding the biology, ecology and host-interactions of LA-MRSA. In some cases knowledge exist regarding *S. aureus* in humans, but it remains unknown how much of this can be extrapolated to LA-MRSA in pigs.

7.1.1 Introduction of LA-MRSA in pig herds by other means than trade

The results of two modelling studies [32, J. Schulz personal communication] suggest that with the fast spread of LA-MRSA in the Danish pig population, LA-MRSA might have been introduced in some herds by other means than trade. As mentioned before, human introduction of LA-MRSA and spread of LA-MRSA by trucks have been suspected in Norway [75]**.** At present, it is unknown to what degree spread through humans and the surrounding environment including air takes place, and spread through other vectors (e.g., rodents and insects) cannot be ruled out either.

Exactly how the transmission from humans to pigs took place in Norway has not been elucidated. Therefore, it is currently unknown, e.g. for how long a human need to be in pig barns and what level of contact with the pigs are needed in order for transmission of LA-MRSA to take place. Among humans there are numerous reports of transmission to household members [22,128,129,219], but in general it is unknown how close contact is needed for transmission. However, direct contact to humans or animals is still assumed to be the primary route of transmission [108], despite LA-MRSA being able to survive on contaminated surfaces for extended amounts of time [220].

7.1.2 Existence of persistent shedders/ supershedders in pigs

The results of the simulated interventions in manuscript III highlight the influence of the existence of pigs persistently shedding LA-MRSA. The existence of persistent *S. aureus* carriage in humans is well-established [11], but the situation might be different for LA-MRSA, as well as relevant physiological conditions in the nasal cavity might differ between humans and pigs. Also, if there is a genetic or host-related component involved, the situation in pigs might not be comparable to in humans, due to the often very close genetic-relatedness among pigs housed on the same farm.

The existence of *S. aureus* supershedders among pigs, i.e. pigs persistently carrying high loads of *S. aureus*, has been suggested [33]. Super-carriers is expected to only constitute a minority of the pigs, and in one study the occurrence did not seem to be linked to the overall contamination level at the farm, whereas this

seemed to be the case for ordinary persistent carriers. The latter could support the possibility of ordinary persistent carriers just being re-contaminated intermittent shedders [33]. Provided that supershedders or persistent shedders in general could be reliably identified, these animals would be an obvious target for interventions, assuming that more evidence regarding the role of these animals in the spread of LA-MRSA becomes available. In humans, a so-called 'culture rule' for the identification of persistent carriers of *S. aureus* have been derived, i.e. classification of human carriers based on the combined qualitative and quantitative results of two samples taken one week apart. However, because of the constant exposure of pigs to LA-MRSA in the air and surroundings on highly LA-MRSA contaminated farms and thereby a constant risk of re-contamination, in addition to possible differences in biology and host-interactions, it might be challenging to derive a similar rule for LA-MRSA in pigs. As mentioned previously, a locus associated with persistent nasal carriage of *S. aureus* carriage in pigs has been identified [36], and this might be a step on the way to finding a method for reliably distinguishing between pigs with different potentials for carriage.

7.1.3 Colonisation vs contamination

The question about the existence of persistent carriage in pigs is related to the question about whether pigs get truly colonised by LA-MRSA or only contaminated. It has been suggested that intermittent *S. aureus* carriage in humans might simply reflect exposure rather than actual colonisation [10], and similarly that only supershedder-pigs might be truly colonised [33]. In some animal experiments, problems with a lack of colonisation of pigs have been experienced [119,121]. So far this lack of colonisation has been attributed to unknown bacterial, host and environmental factors [10,119,121]. The mechanism behind *S. aureus* colonisation in humans is also not very well understood either, but a receptor in the nasal cavity believed to be involved in *S. aureus* colonisation has been identified [18].

7.1.4 Differences in the ability of pigs to pass on LA-MRSA to other pigs

In the model it was assumed that all pigs were equally likely to pass on LA-MRSA to other pigs, even though it is not known whether differences related to carrier type and or nasal load exists. In humans the *S. aureus* load found in the nasal cavity of persistent carriers are generally higher than in intermittent carriers [1,12,14], and a similar trend has been observed in pigs [33]. It has been showed that hospitalised patients harbouring high loads *S. aureus* in the nose shed more bacteria in their surroundings compared to patients harbouring lower loads [221]. However, currently it remains unknown, if and how this affects the risk of the individual passing on *S. aureus*. This question is also related to the dose of exposure needed for pigs to become carriers, which is highly relevant in relation to interventions that might reduce air concentrations in stables.

7.1.5 Relative influence of different routes of transmission to piglets

In relation to assessing the influence of management procedures, such as cross-fostering or use of nursery sows, it could be interesting to obtain more knowledge on factors influencing transmission of LA-MRSA to piglets. In a colonisation experiment, it was demonstrated that perinatal transmission of LA-MRSA is possible [119], but the same study, it was not possible to colonise piglets by direct nasal and gastrointestinal inoculation with the same mixture of strains [119]. More clarity within this area is relevant in relation to interventions, since the simulations indicated that many piglets become LA-MRSA positive already in the farrowing unit.

7.1.6 Duration of carriage

One of the most influential parameters in the sensitivity analysis in manuscript II was the duration of carriage for intermittent shedders. The values used originate from a transmission study carried out in animal experimental facilities [121]. However, at present it is unknown how duration of carriage is affected by infectious pressure in the surrounding environment or interference with other bacteria harboured in the nasal cavity. It also remains unknown, whether pigs persistently carrying LA-MRSA under some circumstances are able to clear themselves from LA-MRSA. Humans persistently carrying *S. aureus* have been known to carry the same strain for periods of 84 and 154 days [10,222], and in one study where 17 human persistent *S. aureus* carriers were re-sampled after eight years, 12 again tested positive for *S. aureus*, hereof three with a strain similar to the one isolated eight years previously [223]. In manuscript II, it was assumed that pigs persistently shedding LA-MRSA in general would continue to shed the bacteria throughout their lifetime, except for 1% of the persistent shedders, who were assigned the potential to undergo decolonisation after an extended amount of time.

7.1.7 Growth and survival of LA-MRSA in pigs and the surrounding environment

As mentioned in the discussion of part B, lack of direct inclusion of transmission of LA-MRSA through the environment is one of the limitations of the model. However many knowledge gaps remain within this area. Based on observations in humans [11,224], it is assumed that LA-MRSA is multiplying in the nasal cavity of pigs and possibly the throat and/or the skin. However, still much is not known regarding survival of LA-MRSA in the surrounding pig barn environment, and whether LA-MRSA might even be able to multiply in the environment due to the heavy contamination with biological material originating from the pigs.

7.2 Combined discussion

The study described in part A was the first LA-MRSA risk factor study conducted on Danish farms. It was the intention to cover as many potential risk factors as possible in one study, and therefore the number of factors included was fairly large. As mentioned previously, this resulted in confirmation of risk factors identified elsewhere, as well as identification of new potential risk factors, albeit many of these factors were also associated with herd size.

The findings in part B indicated that if at all possible, avoiding introduction of LA-MRSA in a herd is crucial, since based on model predictions, it is hard to prevent LA-MRSA from spreading and persisting within a herd, once it has been introduced. This is maybe hardly surprising, given findings in other studies. For example in the DVFA screening of Danish pig herds, all the re-tested herds, which tested positive in 2014 also tested positive in 2016. It is however not known whether any initiatives to get rid of LA-MRSA have been taken within any of these herds.

Among the interventions modelled in part B, only the reduction of antimicrobial consumption had a considerable effect on the within-farm prevalence. The risk factor studies in part A were not designed to measure within herd prevalence, and for overall LA-MRSA status of the farm no effect of any of the factors related to antimicrobial consumption was observed, except for peroral use of tetracycline for weaners on farms without sows in study 1 (assumed to represent group treatment with tetracycline) and use of group treatments administered through water in study 2. However, a marked reduction in antimicrobial consumption as simulated in manuscript III might also imply discontinuation of group treatments or limiting treatments to smaller groups of pigs, and thus one could argue that these results in part A support the results of the simulated interventions obtained in part B.

In the simulations (in part B), there was no marked effect of reducing mixing of pigs or improving internal biosecurity on a farm, where LA-MRSA had already become established. The simulation model predicted a reduction of the prevalence of MRSA, when the animal density within each section was drastically reduced, but this reduction was negligible, when only looking at realistic levels of reduction of the number of pigs within each unit. As mentioned previously, it is however still important to keep in mind, that the interventions modelled might still have an effect on farms, where LA-MRSA has not yet become established. In addition, these might also have a preventive impact on spread of other diseases within the herd and thereby help keeping the antimicrobial consumption low. In part A, where the questionnaire also included questions related to numbers of pigs within each section or each pen for the different age groups, only the typical number of weaners within each section was significantly associated with positive LA-MRSA status in univariable analysis. None of the variables related to mixing of pigs (use of buffer sections, sorting of pigs, cross-fostering or use of nursery sows) were significantly associated with LA-MRSA status.

8. Conclusions

The aims of the first part of this thesis were to identify herd-level risk factors for pig herds testing LA-MRSA positive (study 1) or more specifically for herds changing status from negative to positive during 2014-2016 (study 2). Based on these studies, it can be concluded:

- Previously identified risk factors for farms testing LA-MRSA positive (herd type, herd size, number of pig suppliers) were also associated with LA-MRSA status at Danish farms
- In univariable analysis a number of other factors were also associated with LA-MRSA status, however many of these factors were related to herd size as well, and thus it was not possible to conclude, whether herd size itself or these factors were the true risk factors.

The aims of the second part of this thesis were to build a stochastic model for spread of LA-MRSA within a pig herd to aid a better understanding of the dynamics of spread and persistence of LA-MRSA and subsequently use this model for studying the effectiveness of potential control strategies. Based on the simulations conducted with the current parameterization of the model, it can be concluded that:

- Once LA-MRSA has become established within a herd, it will spread to the whole herd, and be very hard to get rid of.
- Introduction of a low number of intermittently shedding pigs was predicted to frequently result in LA-MRSA not becoming established in the herd.
- Spread of LA-MRSA throughout the herd mainly followed the movement of pigs, and thus the later in the production process LA-MRSA was introduced, the longer it took to spread to the whole herd.
- After spread of LA-MRSA had reached a steady state, the prevalence was highest within the farrowing unit, and lowest within the mating unit, and thus the farrowing unit might be the area with most potential for intervention.
- When simulating interventions, reduced antimicrobial consumption resulted in a marked decrease in the prevalence, but LA-MRSA rarely disappeared completely. So, while changes in antimicrobial consumption patterns might be a very important step towards reducing the prevalence of LA-MRSA within a herd, it still needs to be supplemented by other measures.
- Reductions of mixing, reductions in the pig density within sections or improved within-herd biosecurity were predicted to only marginally change the median within-herd prevalence. However, in relation to being able to achieve a low level of antimicrobial consumption, these factors might still be of importance.
- The results of the sensitivity analysis indicated that the assumptions regarding the existence of pigs persistently shedding MRSA have a noticeable influence on the predicted effect of interventions.

9. Future perspectives

The results of the risk factor studies in part A confirmed that factors related to purchase of pigs, herd size and herd type seem to important determinants. As mentioned before several other factors, e.g. use of wet feed, might be worth further investigation, if it is possible to create a setup, where the effect of herd size can be excluded (using for instance a matched case-control study where matching is done on herd size), or where more herds with the factor in question present can be included, e.g. natural ventilation.

Regarding spread of LA-MRSA within a herd, several knowledge gaps related to infection dynamics were identified, including the influence of the environment, LA-MRSA load and persistent carriage. The model itself cannot be used as such for obtaining more knowledge on the existence of persistent carriers or supershedders, but it can be used for assessing the influence of different assumptions regarding the presence of these, as well as for simulating different hypothetical scenarios regarding assumed influence of LA-MRSA load. However, the accuracy of the outcomes when simulating different interventions would greatly benefit from more clarity regarding this.

Regarding control of LA-MRSA, the main problem is currently a lack of evidence for major effect of any type of intervention other than reducing antimicrobial consumption. However, if not many interventions efficient at directly reducing the carriage among pigs exist at present, another option could be to start looking at interventions targeting LA-MRSA in air and/or the barn environment in an attempt at limiting spread of LA-MRSA into the society. Including an environmental component in the model would in principle enable simulating this, but currently not much knowledge about this is available, and thus doing so might be associated with high uncertainty. It could also be interesting to add a component to the model to link within-herd prevalence to risk of infection for humans, albeit this also would be challenged by data gaps.

In order to provide a better basis for recommendations for prevention and control of LA-MRSA spread, future research should in general focus on filling the gaps identified in the general discussion section (section 7.1), as well as providing evidence for the effect of interventions. Thus there is still a big need for more data from observational studies and experimental trials, including data that can lead to more clarity regarding persistent shedders/supershedders and the influence of load in relation to infectiousness, as well as studies that can help clarify the role of the environment.

10. References

1. Vandenbergh M, Verbrugh H. Carriage of *Staphylococcus aureus:* epidemiology and clinical relevance. J Lab Clin Med. 1999;133: 525–534. doi:10.1067/mlc.2001.113504

2. O'Gara JP. Into the storm: Chasing the opportunistic pathogen *Staphylococcus aureus* from skin colonisation to life-threatening infections. Environ Microbiol. 2017;19: 3823–3833. doi:10.1111/1462-2920.13833

3. Clements MO, Foster SJ. Stress resistance in *Staphylococcus aureus*. Trends Microbiol. 1999;7: 458–462.

4. Crombé F, Argudín MA, Vanderhaeghen W, Hermans K, Haesebrouck F, Butaye P. Transmission Dynamics of Methicillin-Resistant *Staphylococcus aureus* in Pigs. Front Microbiol. 2013;4: 57. doi:10.3389/fmicb.2013.00057

5. Deurenberg RH, Stobberingh EE. The evolution of *Staphylococcus aureus*. Infect Genet Evol. 2008;8:747-763. doi:10.1016/j.meegid.2008.07.007

6. Armand-Lefevre L. Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. Emerg Infect Dis. 2005;11: 711–714.

7. Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg Infect Dis. 2005;11: 1965–1966. doi:10.3201/eid1112.050428

8. Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, et al. *Staphylococcus aureus* CC398: Host Adaptation and Emergence of Methicillin Resistance in Livestock. MBio. 2012;3. doi:10.1128/mBio.00305

9. Larsen J, Clasen J, Hansen JE, Paulander W, Petersen A, Larsen AR, et al. Co-presence of *tet(K)* and *tet(M)* in livestock-associated methicillin-resistant *Staphylococcus aureus* CC398 is associated with increased fitness during exposure to sub-lethal concentrations of tetracycline. Antimicrob Agents Chemother. 2016;60: AAC.00426-16. doi:10.1128/AAC.00426-16

10. van Belkum A, Verkaik NJ, de Vogel CP, Boelens HA, Verveer J, Nouwen JL, et al. Reclassification of *Staphylococcus aureus* nasal carriage types. J Infect Dis. 2009;199: 1820–6. doi:10.1086/599119

11. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh H a, et al. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis. 2005;5: 751–762. doi:10.1016/S1473-3099(05)70295-4

12. Eriksen N, Espersen F, Rosdahl V, Jensen K. Carriage of *Staphylococcus aureus* among 104 healthy persons during a 19-month period. Epidemiol Infect. 1995;115: 51–60. doi:10.1017/S0950268800058118

13. Hu L, Umeda A, Kondo S, Amako K. Typing of *Staphylococcus aureus* Colonising Human Nasal Carriers by Pulsed-Field Gel Electrophoresis. J Med Microbiol. 1995;42: 127–132. doi:10.1099/00222615-42-2-127

14. Verhoeven PO, Grattard F, Carricajo A, Lucht F, Cazorla C, Garraud O, et al. An algorithm based on one or two nasal samples is accurate to identify persistent nasal carriers of *Staphylococcus aureus*. Clin Microbiol Infect. 2012;18: 551–557. doi:10.1111/j.1469-0691.2011.03611.x

15. Ruimy R, Angebault CC, Lix Djossou F, Dupont C, Epelboin L, Jarraud S, et al. Are Host Genetics the Predominant Determinant of Persistent Nasal *Staphylococcus aureus* Carriage in Humans? J Infect Dis. 2010;202: 924–934. doi:10.1086/655901

16. Nouwen J, Boelens H, Belkum A Van, Verbrugh H. Human Factor in *Staphylococcus aureus* Nasal Carriage. Infect Immun. 2004;72: 5442–5448. doi:10.1128/IAI.72.11.6685

17. Andersen PS, Pedersen JK, Fode P, Skov RL, Jr VGF, Stegger M. Influence of Host Genetics and Environment on Nasal Carriage of *Staphylococcus aureus* in Danish Middle-Aged and Elderly Twins. J Infect Dis. 2012;206. doi:10.1093/infdis/jis491

18. Baur S, Rautenberg M, Faulstich M, Grau T, Severin Y, Unger C, et al. A Nasal Epithelial Receptor for *Staphylococcus aureus* WTA Governs Adhesion to Epithelial Cells and Modulates Nasal Colonization. PLoS Pathog. 2014;10. doi:10.1371/journal.ppat.1004089

19. Dall 'antonia M, Coen PG, Wilks M, Whiley A, Millar M. Competition between methicillin-sensitive and -resistant *Staphylococcus aureus* in the anterior nares. J Hosp Infect. 2005;61: 62–67. doi:10.1016/j.jhin.2005.01.008

20. Datta R, Quan V, Kim D, Peterson EM, Reynolds C, Meyers H, et al. Protective Effect of Methicillin-Susceptible *Staphylococcus aureus* Carriage against Methicillin-Resistant *S. aureus* Acquisition in Nursing Homes: A Prospective Cross-Sectional Study. Infect Control Hosp Epidemiol. 2014;35: 1257–1262. doi:10.1086/678062

21. Landelle C, Iten A, Uckay I, Sax H, Camus V, Cohen G, et al. Does colonization with methicillin-susceptible *Staphylococcus aureus* protect against nosocomial acquisition of methicillin-resistant *S. aureus*? Infect Control Hosp Epidemiol. 2014;35: 527–533. doi:10.1086/675825

22. Garcia-graells C, Cleef BAGL Van, Larsen J, Denis O, Skov R, Voss A. Dynamic of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* CC398 in Pig Farm Households: A Pilot Study. PLoS One. 2013;8: 6–11. doi:10.1371/journal.pone.0065512

23. Graveland H, Wagenaar JA, Bergs K, Heesterbeek H, Heederik D. Persistence of livestock associated MRSA CC398 in humans is dependent on intensity of animal contact. PLoS One. 2011;6. doi:10.1371/journal.pone.0016830

24. Köck R, Loth B, Köksal M, Schulte-Wülwer J, Harlizius J, Friedrich AW. Persistence of nasal colonization with livestock-associated methicillin-resistant *Staphylococcus aureus* in pig farmers after holidays from pig exposure. Appl Environ Microbiol. 2012;78: 4046–4047. doi:10.1128/AEM.00212-12

25. Ballhausen B, Kriegeskorte A, van Alen S, Jung P, Köck R, Peters G, et al. The pathogenicity and host adaptation of livestock-associated MRSA CC398. Vet Microbiol. Elsevier B.V.; 2017;200: 39–45. doi:10.1016/j.vetmic.2016.05.006

26. Mutters NT, Bieber CP, Hauck C, Reiner G, Malek V, Frank U. Comparison of livestock-associated and health care – associated MRSA — genes , virulence , and resistance. Diagn Microbiol Infect Dis. 2016;86: 417–421. doi:10.1016/j.diagmicrobio.2016.08.016

27. van de Sande-Bruinsma N, Leverstein van Hall MA, Janssen M, Nagtzaam N, Leenders S, de Greeff SC, et al. Impact of livestock-associated MRSA in a hospital setting. Antimicrob Resist Infect Control. 2015;4: 2–7. doi:10.1186/s13756-015-0053-8

28. DANMAP. DANMAP 2016 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark [Internet]. 2017. Available: www.danmap.org

29. Becker K, Ballhausen B, Kahl BC, K??ck R. The clinical impact of livestock-associated methicillin-resistant *Staphylococcus aureus* of the clonal complex 398 for humans. Vet Microbiol. Elsevier B.V.; 2015; doi:10.1016/j.vetmic.2015.11.013

30. Cuny C, Wieler L, Witte W. Livestock-Associated MRSA: The Impact on Humans. Antibiotics. 2015;4: 521–543. doi:10.3390/antibiotics4040521

31. Ministry of Environment and Food of Denmark. MRSA Risiko og håndtering, Rapport ved MRSA-ekspertgruppen (*in Danish*). 2017. Available: http://mfvm.dk/fileadmin/user_upload/MFVM/MRSA_rapport.pdf

32. Larsen J, Petersen A, Larsen AR, Sieber RN, Stegger M, Koch A, et al. Emergence of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* Bloodstream Infections in Denmark. Clin Infect Dis. 2017;65. doi:10.1093/cid/cix504

33. Espinosa-Gongora C, Dahl J, Elvstrøm A, van Wamel WJ, Guardabassi L. Individual predisposition to *Staphylococcus aureus* colonization in pigs on the basis of quantification, carriage dynamics, and serological profiles. Appl Environ Microbiol. 2015;81: 1251–6. doi:10.1128/AEM.03392-14

34. Bangerter PD, Sidler X, Perreten V, Overesch G. Longitudinal study on the colonisation and transmission of methicillin-resistant *Staphylococcus aureus* in pig farms. Vet Microbiol. Elsevier B.V.; 2016;183: 125–134. doi:10.1016/j.vetmic.2015.12.007

35. Weese JS, Zwambag A, Rosendal T, Reid-Smith R, Friendship R. Longitudinal Investigation of Methicillin-Resistant *Staphylococcus aureus* in Piglets. Zoonoses Public Health. 2011;58: 238–243. doi:10.1111/j.1863-2378.2010.01340.x

36. Skallerup P, Espinosa-Gongora C, Jørgensen CB, Guardabassi L, Fredholm M. Genome-wide association study reveals a locus for nasal carriage of *Staphylococcus aureus* in Danish crossbred pigs. BMC Vet Res. 2015; doi:10.1186/s12917-015-0599-y

37. Verstappen KM, Willems E, Fluit AC, Duim B, Martens M, Wagenaar JA. *Staphylococcus aureus* Nasal Colonization Differs among Pig Lineages and Is Associated with the Presence of Other Staphylococcal Species. Front Vet Sci. 2017;4: 1–6. doi:10.3389/fvets.2017.00097

38. Espinosa-Gongora C, Larsen N, Schønning K, Fredholm M, Guardabassi L. Differential Analysis of the Nasal Microbiome of Pig Carriers or Non-Carriers of *Staphylococcus aureus*. PLoS One. 2016;11: e0160331. doi:10.1371/journal.pone.0160331

39. Fetsch A, Roesler U, Kraushaar B, Friese A. Co-colonization and clonal diversity of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* in sows. Vet Microbiol. Elsevier B.V.; 2016;185: 7–14. doi:10.1016/j.vetmic.2016.01.011

40. Weese JS, Slifierz M, Jalali M, Friendship R. Evaluation of the nasal microbiota in slaughter-age pigs and the impact on nasal methicillin-resistant *Staphylococcus aureus* (MRSA) carriage. BMC Vet Res. 2014;10: 1–10. doi:10.1186/1746-6148-10-69

41. Crombé F, Vanderhaeghen W, Dewulf J, Hermans K, Haesebrouck F, Butaye P. Colonization and transmission of methicillin-resistant *Staphylococcus aureus* ST398 in nursery piglets. Appl Environ Microbiol. 2012;78: 1631–1634. doi:10.1128/AEM.07356-11

42. Verhegghe M, Crombé F, Pletinckx LJ, Haesebrouck F, Butaye P, Herman L, et al. Genetic diversity of livestock-associated MRSA isolates obtained from piglets from farrowing until slaughter age on four farrow-to-finish farms. Vet Res. BioMed Central Ltd.; 2014;45: 89. doi:10.1186/s13567-014-0089-4

43. Dorado-García, Alejandro Dohmen W, Bos MEH, Verstappen KM, Houben M, Wagenaar JA, Heederik DJJ. Dose-Response Relationship between Antimicrobial Drugs and Livestock-Associated MRSA in Pig Farming. Emerg Infect Dis. 2015;21: 950. doi:10.3201/eid2106.140706

44. Fromm S, Beißwanger E, Käsbohrer A, Tenhagen B-A. Risk factors for MRSA in fattening pig herds – A meta-analysis using pooled data. Prev Vet Med. 2014;117: 180–188. doi:10.1016/j.prevetmed.2014.08.014

45. Jouy E, Le Roux A, Ké Ranflec ’h A, Granier SA, Laurent F, Kempf I, et al. Methicillin-resistant *Staphylococcus aureus* ST398 contamination and transmission in pigs after a low dose inoculation. Lett Appl Microbiol. 2012;54: 518–523. doi:10.1111/j.1472-765X.2012.03239.x

46. Szabó I, Beck B, Friese A, Fetsch A, Tenhagen B-A, Roesler U. Colonization kinetics of different methicillin-resistant *Staphylococcus aureus* sequence types in pigs and host susceptibilities. Appl Environ Microbiol. 2012; doi:10.1128/AEM.05327-11

47. Foster AP. Staphylococcal skin disease in livestock. Vet Dermatol. 2012;23:342-e63. doi:10.1111/j.1365-3164.2012.01093.x

48. Schwarz S, Kadlec K, Strommenger B. Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* detected in the BfT-GermVet monitoring programme 2004 – 2006 in Germany. J Antimicrob Chemother. 2008;61: 282–285. doi:10.1093/jac/dkm487

49. Meemken D, Blaha T, Tegeler R, Tenhagen B-A, Guerra B, Hammerl JA, et al. Livestock Associated Methicillin-Resistant *Staphylococcus aureus* (LaMRSA) Isolated from Lesions of Pigs at Necropsy in Northwest Germany Between 2004 and 2007. Zoonoses Public Health. 2010;57: e143–e148. doi:10.1111/j.1863-2378.2009.01313.x

50. Ministry of Environment and Food of Denmark. Handlingsplan for husdyr-MRSA (*in Danish*). 2015. Available: http://mfvm.dk/nyheder/nyhed/nyhed/handlingsplan-til-bekaempelse-af-husdyr-mrsa-1/

51. Sørensen AL. Oplevelse af sammenhæng hos bærere af Meticillin Resistente *Staphylococcus aureus* (MRSA). Et kvalitativt studie (*in Danish*). MPH thesis. Nordic School of Public Health, Göteborg, Sweden. 2012. Available: http://www.diva-portal.org/smash/record.jsf?pid=diva2%3A707758&dswid=mainwindow

52. Rump B, De Boer M, Reis R, Wassenberg M, Van Steenbergen J. Signs of stigma and poor mental health among carriers of MRSA. J Hosp Infect. Elsevier Ltd; 2017;95: 268–274. doi:10.1016/j.jhin.2016.09.010

53. Lindberg M, Carlsson M, Högman M, Skytt B. Suffering from meticillin-resistant *Staphylococcus aureus*: experiences and understandings of colonisation. J Hosp Infect. 2009;73: 271–277. doi:10.1016/j.jhin.2009.07.002

54. Mozzillo KL, Ortiz N, Miller LG. Patients with Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infection - 21st Century Lepers. J Hosp Infect. 2010;75: 132–134. doi:10.1016/j.jhin.2009.10.031.

55. The Danish Council on Ethics. Antibiotikaresistens – sociale aspekter (*in Danish*). 2014. Available: http://www.etiskraad.dk/~/media/Etisk-Raad/Etiske-Temaer/Sundhedsvaesenet/Publikationer/Antibiotikaresistens-arbejdspapirer-2014/Antibiotikaresistens-sociale-aspekter.pdf?la=da

56. Kristoffersen AB, Grøntved CA, Tavornpanich S, Elström P, Norström M. Spredningsmodell og samfunnsøkonomisk analyse av tiltak mot LA-MRSA tiltak mot LA-MRSA (*in Norwegian*). 2016. Available: https://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2016/spredningsmodell-og-samfunnsokonomisk-analyse-av-tiltak-mot-la-mrsa

57. Höjgaard S, Aspevall O, Bengtsson B, Hæggman S, Lindberg M, Mieziewska K, et al. Preventing introduction of livestock associated MRSA in a pig population - Benefits, costs, and knowledge gaps from the Swedish perspective. PLoS One. 2015;10. doi:10.1371/journal.pone.0122875

58. Danish Agriculture and Food Council. Statistik 2016 svinekød (*in Danish*). 2017. Available: https://www.google.dk/url?sa=t&rct=j&q=&esrc=s&source=web&cd=3&ved=0ahUKEwiuu7rz-_jYAhUD1ywKHWaPCkMQFggxMAI&url=http%3A%2F%2Fwww.lf.dk%2F~%2Fmedia%2Flf%2Ftal-og-analyser%2Faarsstatistikker%2Fstatistik-svin%2F2016%2F22204-038-2017-a5-statistik-svin-2016-dk

59. Chuang Y, Huang Y. International Journal of Antimicrobial Agents Livestock-associated meticillin-resistant *Staphylococcus aureus* in Asia: An emerging issue? Int J Antimicrob Agents. 2015;45: 334–340. doi:10.1016/j.ijantimicag.2014.12.007

60. Sahibzada S, Abrah S, Coombs GW, Pang S, Jordan D, Heller J. Transmission of highly virulent community-associated MRSA ST93 and livestock-associated MRSA ST398 between humans and pigs in Australia. Sci Rep. 2017; 1–11. doi:10.1038/s41598-017-04789-0

61. Khanna T, Friendship R, Dewey C, Weese JS. Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. Vet Microbiol. 2008;128: 298–303. doi:10.1016/j.vetmic.2007.10.006

62. Harper AL, Ferguson DD, Larson KRL, Hanson BM, Male MJ, Donham KJ, et al. An overview of livestock-associated MRSA in agriculture. J Agromedicine. 2010;15: 101–104. doi:10.1080/10599241003627110

63. Brennan GI, Abbott Y, Burns A, Leonard F, Mcmanus A, Connell BO, et al. The Emergence and Spread of Multiple Livestock-Associated Clonal Complex 398 Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus* Strains among Animals and Humans in the Republic of Ireland , 2010 – 2014. PLoS One. 2016; 1–11. doi:10.1371/journal.pone.0149396

64. Overesch G, Büttner S, Rossano A, Perreten V. The increase of methicillin-resistant *Staphylococcus aureus* (MRSA) and the presence of an unusual sequence type ST49 in slaughter pigs in Switzerland. BMC Vet Res. 2011;7.

65. Kraemer JG, Pires J, Kueffer M, Semaani E, Endimiani A, Hilty M, et al. Prevalence of extended-spectrum β -lactamase-producing Enterobacteriaceae and Methicillin-Resistant *Staphylococcus aureus* in pig farms in Switzerland. Sci Total Environ. Elsevier B.V.; 2017;604: 401–405. doi:10.1016/j.scitotenv.2017.06.110

66. Hartley H, Watson C, Nugent P, Beggs N, Dickson E, Kearns A. Confirmation of LA-MRSA in pigs in the UK. Vet Rec. 2014;175: 74–76. doi:10.1136/vr.g4620

67. Ivbule M, Miklasevics E, Cupane L, Berzina L, Balins A, Valdovska A. Presence of methicillin-resistant *Staphylococcus aureus* in slaughterhouse environment, pigs, carcasses, and workers. J Vet Res. 2017;61: 267–277. doi:10.1515/jvetres-2017-0037

68. Sarrou S, Liakopoulos A, Chasioti M, Foka A, Fthenakis G, Billinis C, et al. Dissemination of Methicillin-Susceptible CC398 *Staphylococcus aureus* Strains in a Rural Greek Area. PLoS One. 2015; 1–8. doi:10.1371/journal.pone.0122761

69. Huang E, Gurzau AE, Hanson BM, Kates AE, Smith TC, Pettigrew MM, et al. Detection of livestock-associated methicillin-resistant *Staphylococcus aureus* among swine workers in Romania. J Infect Public Health. King Saud Bin Abdulaziz University for Health Sciences; 2014;7: 323–332. doi:10.1016/j.jiph.2014.03.008

70. Habrun B, Račić I, Beck R, Budimir A, Benić M, Kompes G, et al. The presence of methicillin-resistant *Staphylococcus aureus* on large pig breeding farms in Croatia [Internet]. Acta Veterinaria Hungarica. 2011. pp. 419–425. doi:10.1556/AVet.2011.028

71. Broens EM, Graat EAM, Engel B, Oosterom RAA Van, Giessen AW Van De, Wolf PJ Van Der. Comparison of sampling methods used for MRSA-classification of herds with breeding pigs. Vet Microbiol. Elsevier B.V.; 2011;147: 440–444. doi:10.1016/j.vetmic.2010.07.021

72. European Food Safety Authority. Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in methicillin-resistant *Staphylococcus aureus* in food-producing animals and food. 2012. doi:10.2903/j.efsa.2012.2897. Available: https://www.efsa.europa.eu/en/efsajournal/pub/2897

73. European Food Safety Authority. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015. 2017. doi:10.2903/j.efsa.2017.4694. Available: https://www.efsa.europa.eu/en/efsajournal/pub/4694

74. European Food Safety Authority. Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008. Part A: MRSA prevalence estimates. 2009. Available: http://www.efsa.europa.eu/en/efsajournal/pub/1376

75. Grøntvedt CA, Elstrøm P, Stegger M, Skov RL, Skytt Andersen P, Larssen KW, et al. Methicillin-Resistant *Staphylococcus aureus* CC398 in Humans and Pigs in Norway: A “One Health” Perspective on Introduction and Transmission. Clin Infect Dis. 2016;63: 1431–1438. doi:10.1093/cid/ciw552

76. Agersø Y, Hasman H, Cavaco LM, Pedersen K, Aarestrup FM. Study of methicillin resistant *Staphylococcus aureus* (MRSA) in Danish pigs at slaughter and in imported retail meat reveals a novel MRSA type in slaughter pigs. Vet Microbiol. 2012;157: 246–250. doi:10.1016/j.vetmic.2011.12.023

77. Smith TC. Livestock-Associated *Staphylococcus aureus*: The United States Experience. PLOS Pathog. 2015;11: 1–8. doi:10.1371/journal.ppat.1004564

78. Arriola CS, Güere ME, Larsen J, Skov RL, Gilman RH, Armando E, et al. Presence of Methicillin-Resistant *Staphylococcus aureus* in Pigs in Peru. PLoS One. 2011;6: 2009–2011. doi:10.1371/journal.pone.0028529

79. Takeuti KL, Malgarin CM, Amaral AF, de Barcellos DESN. Frequency of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Fattening Pigs in the State of presence in finishing pigs in the State of Rio Grande do Sul, Brazil. Acta Scinetiae Vet. 2016;44: 1–4.

80. Fall C, Seck A, Richard V, Ndour M, Sembene M, Laurent F, et al. Epidemiology of *Staphylococcus aureus* in Pigs and Farmers in the Largest Farm in Dakar, Senegal. Foodborne Pathog Dis. 2012;9. doi:10.1089/fpd.2012.1197

81. Graveland H, Wagenaar JA, Heesterbeek H, Mevius D, van Duijkeren E, Heederik D. Methicillin resistant *Staphylococcus aureus* ST398 in veal calf farming: Human MRSA carriage related with animal antimicrobial usage and farm hygiene. PLoS One. 2010;5: 4–9. doi:10.1371/journal.pone.0010990

82. Friese A, Schulz J, Zimmermann K, Tenhagen B-A, Fetsch A, Hartung J, et al. Occurrence of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* in Turkey and Broiler Barns and Contamination of Air and Soil Surfaces in Their Vicinity. Appl Environ Microbiol. 2013;79: 2759–2766. doi:10.1128/AEM.03939-12

83. Leonard FC, Markey BK. Meticillin-resistant *Staphylococcus aureus* in animals: A review. Veterinary Journal. 2008. pp. 27–36. doi:10.1016/j.tvjl.2006.11.008

84. Nemeghaire S, Roelandt S, Argudín MA, Haesebrouck F, Butaye P. Characterization of methicillin-resistant *Staphylococcus aureus* from healthy carrier chickens. Avian Pathol. 2013;42: 342–6. doi:10.1080/03079457.2013.805183

85. van de Giessen AW, van Santen-Verheuvel MG, Hengeveld PD, Bosch T, Broens EM, Reusken CBEM. Occurrence of methicillin-resistant *Staphylococcus aureus* in rats living on pig farms. Prev Vet Med. 2009;91: 270–273. doi:10.1016/j.prevetmed.2009.05.016

86. Agnoletti F, Mazzolini E, Bacchin C, Bano L, Berto G, Rigoli R, et al. First reporting of methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 in an industrial rabbit holding and in farm-related people. Vet Microbiol. 2014;170: 172–177. doi:10.1016/j.vetmic.2014.01.035

87. Hansen JE, Larsen AR, Skov RL, Chriél M, Larsen G, Angen Ø, et al. Livestock-associated methicillin-resistant *Staphylococcus aureus* is widespread in farmed mink (*Neovison vison*). Vet Microbiol. 2017;207: 44–49. doi:10.1016/j.vetmic.2017.05.027

88. Guardabassi L, Stegger M, Skov R. Retrospective detection of methicillin resistant and susceptible *Staphylococcus aureus* ST398 in Danish slaughter pigs. Vet Microbiol. 2007;122: 384–6. doi:10.1016/j.vetmic.2007.03.021

89. Bagcigil FA, Moodley A, Baptiste KE, Jensen VF, Guardabassi L. Occurrence, species distribution, antimicrobial resistance and clonality of methicillin-and erythromycin-resistant staphylococci in the nasal cavity of domestic animals. Vet Microbiol. 2007;121: 307–315. doi:10.1016/j.vetmic.2006.12.007

90. DANMAP. DANMAP 2010 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. 2011. Available: www.danmap.org

91. DANMAP. DANMAP 2011 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. 2012. Available: www.danmap.org

92. van de Vijver LPL, Tulinski P, Bondt N, Mevius D, Verwer C. Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) in organic pig herds in the Netherlands. Zoonoses Public Health. 2014;61: 338–345. doi:10.1111/zph.12076

93. Danish Veterinary and Food Administration. MRSA screeningsundersøgelser 2015 (*in Danish*). 2015. Available: https://www.foedevarestyrelsen.dk/SiteCollectionDocuments/Foder- og foedevaresikkerhed/Slutrapporter/Rapport-om-MRSA-screeningsundersoegelser-2015.pdf

94. DANMAP. DANMAP 2009 - Use of antimicrobial agents and occurrence of antimicrobial resistance in batceria from food animals, food and humans in Denmark. 2010. Available: www.danmap.org

95. DANMAP. DANMAP 2012 - Use of antimicrobial agents and occurrence of antimicrobial resistance in batceria from food animals, food and humans in Denmark. 2013. Available: www.danmap.org

96. Broens EM, Graat E a M, Van Der Wolf PJ, Van De Giessen AW, De Jong MCM. Transmission of methicillin resistant *Staphylococcus aureus* among pigs during transportation from farm to abattoir. Vet J. Elsevier Ltd; 2011;189: 302–305. doi:10.1016/j.tvjl.2010.08.003

97. Eriksson J, Espinosa-Gongora C, Stamphøj I, Larsen AR, Guardabassi L. Carriage frequency, diversity and methicillin resistance of *Staphylococcus aureus* in Danish small ruminants. Vet Microbiol. 2013;163: 110–115. doi:10.1016/j.vetmic.2012.12.006

98. Islam MZ, Espinosa-Gongora C, Damborg P, Sieber RN, Munk R, Husted L, et al. Horses in Denmark Are a Reservoir of Diverse Clones of Methicillin-Resistant and -Susceptible S*taphylococcus aureus*. Front Microbiol. 2017;8:543. doi:10.3389/fmicb.2017.00543

99. Catry B, Van Duijkeren E, Pomba MC, Greko C, Moreno MA, Pyörälä S, et al. Reflection paper on MRSA in food-producing and companion animals: epidemiology and control options for human and animal health. Epidemiol Infect. 2010;138: 626–44. doi:10.1017/S0950268810000014

100. Hansen JE, Sørensen AIV, Espinosa-Gongora C, Larsen AR, Larsen J, Skov R, et al. Assessment of Methods to Quantify Livestock Associated MRSA in Pig Herds. The Danish Microbiological Society Annual Congress 2015 Programme & Abstracts. 2015. p. 29.

101. Larsen J, Stegger M, Andersen PS, Petersen A, Larsen AR, Westh H, et al. Evidence for Human Adaptation and Foodborne Transmission of Livestock- Associated Methicillin-Resistant *Staphylococcus aureus*. Clin Infect Dis. 2016;63. doi:10.1093/cid/ciw532

102. Larsen J, Petersen A, Sørum M, Stegger M, van Alphen L, Valentiner-Branth P, et al. Meticillin-resistant *Staphylococcus aureus* CC398 is an increasing cause of disease in people with no livestock contact in Denmark, 1999 to 2011. Eurosurveillance. 2015;20: 5–13. doi:10.2807/1560-7917.es.2015.20.37.30021

103. DANMAP. DANMAP 2015 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. 2016. Available: www.danmap.org

104. Statens Serum Institut. MRSA, laboratorieanmeldelsespligtige sygdomme - Statens Serum Institut (*webpage in Danish*). 2018 [cited 25 Jan 2018]. Available: https://www.ssi.dk/Smitteberedskab/Sygdomsovervaagning/Sygdomsdata.aspx?sygdomskode=MRSA&aar=2007%7C2018&stype=10&xaxis=Aar&yaxis=Total&show=Table&datatype=Laboratory&extendedfilters=True#).

105. Skov R. LA-MRSA = Husdyr associeret MRSA (*in Danish*). 2010. Available: http://www.ft.dk/samling/20101/almdel/flf/bilag/171/955815/index.htm

106. DANMAP. DANMAP 2013 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark [Internet]. 2014. Available: www.danmap.org

107. DANMAP. DANMAP 2014 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. 2015. Available: http://www.danmap.org/~/media/projekt sites/danmap/danmap reports/danmap 2014/danmap_2014.ashx

108. Danish Health Authority. Guidance on Preventing the Spread of MRSA. 2016. Available: https://www.sst.dk/da/sygdom-og-behandling/smitsomme-sygdomme/mrsa/~/media/F3F52EC1C6A94C6080F50F435DA02E59.ashx

109. European Centre for Disease Prevention and Control. Surveillance of antimicrobial resistance in Europe 2016. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). 2017. Available: https://ecdc.europa.eu/sites/portal/files/documents/AMR-surveillance-Europe-2016.pdf

110. Böcher S, Skov RL, Knudsen MA, Guardabassi L, Mølbak K, Schouenborg P, et al. The search and destroy strategy prevents spread and long-term carriage of methicillin-resistant *Staphylococcus aureus*: Results from the follow-up screening of a large ST22 (E-MRSA 15) outbreak in Denmark. Clin Microbiol Infect. 2010;16: 1427–1434. doi:10.1111/j.1469-0691.2010.03137.x

111. Ciccolini M, Dahl J, Chase-Topping ME, Woolhouse MEJ. Disease transmission on fragmented contact networks: Livestock-associated Methicillin-resistant *Staphylococcus aureus* in the Danish pig-industry. Epidemics. 2012;4: 171–178. doi:10.1016/j.epidem.2012.09.001

112. Broens EM, Espinosa-Gongora C, Graat EAM, Vendrig N, Van Der Wolf PJ, Guardabassi L, et al. Longitudinal study on transmission of MRSA CC398 within pig herds. BMC Vet Res. 2012;8: 58. doi:10.1186/1746-6148-8-58

113. Dewaele I, Messens W, Man I De, Delputte P, Herman L, Butaye P, et al. Sampling, prevalence and characterization of methicillin-resistant *Staphylococcus aureus* on two Belgian pig farms. Vet Sci Dev. 2011;1: 4–8. doi:10.4081/vsd.2011.e1

114. Merialdi G, Galletti E, Rugna G, Granito G, Franco A, Battisti A, et al. Longitudinal study on MRSA nasal colonization in a farrow to finish pig herd. 4th European Symposium of Porcine Health Management. 2012. p. 1.

115. Merialdi G, Galletti E, Guazzetti S, Rosignoli C, Alborali G, Battisti A, et al. Environmental methicillin-resistant *Staphylococcus aureus* contamination in pig herds in relation to the productive phase and application of cleaning and disinfection. Res Vet Sci. Elsevier Ltd; 2013;94: 425–427. doi:10.1016/j.rvsc.2012.10.020

116. Nathaus R, Blaha T, Tegeler R, Meemken D. *Staphylococcus aureus* in zwei Schweine-zuchtbestanden (Intra-herd prevalence and colonisation dynamics of Methicillin-resistant Staphylococcus aureus (MRSA) in two pig breeding herds) (*In German with abstract in English*). Berl Munch Tierarztl Wochenschr. 2010;1236: 221–228. doi:10.2376/0005-9366-123-221

117. Pletinckx LJ, Verhegghe M, Crombé F, Dewulf J, De Bleecker Y, Rasschaert G, et al. Evidence of possible methicillin-resistant *Staphylococcus aureus* ST398 spread between pigs and other animals and people residing on the same farm. Prev Vet Med. 2013;109: 293–303. doi:10.1016/j.prevetmed.2012.10.019

118. Verhegghe M, Pletinckx LJ, Crombé F, Weyenberg S Van, Haesebrouck F, Butaye P, et al. Cohort study for the presence of livestock-associated MRSA in piglets: Effect of sow status at farrowing and determination of the piglet colonization age. Vet Microbiol. 2013;162: 679–686. doi:10.1016/j.vetmic.2012.09.014

119. Moodley A, Latronico F, Guardabassi L. Experimental colonization of pigs with methicillin-resistant *Staphylococcus aureus* (MRSA): insights into the colonization and transmission of livestock-associated MRSA. Epidemiol Infect. 2011;139: 1594–1600. doi:10.1017/S0950268810002888

120. Moodley A, Nielsen SS, Guardabassi L. Effects of tetracycline and zinc on selection of methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type 398 in pigs. Vet Microbiol. 2011;152: 420–423. doi:10.1016/j.vetmic.2011.05.025

121. Broens EM, Graat E a M, van de Giessen AW, Broekhuizen-Stins MJ, de Jong MCM. Quantification of transmission of livestock-associated methicillin resistant *Staphylococcus aureus* in pigs. Vet Microbiol. 2012;155: 381–388. doi:10.1016/j.vetmic.2011.09.010

122. van den Broek IVF, van Cleef BAGL, Haenen A, Broens EM, van der Wolf PJ, van den Broek MJM, et al. Methicillin-resistant *Staphylococcus aureus* in people living and working in pig farms. Epidemiol Infect. 2009;137: 700–708. doi:10.1017/S0950268808001507

123. Bos MEH, Verstappen KM, van Cleef B a GL, Dohmen W, Dorado-García A, Graveland H, et al. Transmission through air as a possible route of exposure for MRSA. J Expo Sci Environ Epidemiol. 2016;26: 263–269. doi:10.1038/jes.2014.85

124. Angen Ø, Feld L, Larsen J, Rostgaard K, Skov R, Madsen AM, et al. Transmission of Methicillin-Resistant *Staphylococcus aureus* to Human Volunteers Visiting a Swine Farm. Appl Environ Microbiol. 2017;83: 1–10.

125. Van Cleef BAGL, Graveland H, Haenen APJ, Van De Giessen AW, Heederik D, Wagenaar JA, et al. Persistence of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* in Field Workers

after Short-Term Occupational Exposure to Pigs and Veal Calves. J Clin Microbiol. 2011;49: 1030–1033. doi:10.1128/JCM.00493-10

126. Frana TS, Beahm AR, Hanson BM, Kinyon JM, Layman LL, Karriker LA, et al. Isolation and Characterization of Methicillin-Resistant *Staphylococcus aureus* from Pork Farms and Visiting Veterinary Students. PLoS One. 2013;8. doi:10.1371/journal.pone.0053738

127. Liu W, Liu Z, Yao Z, Fan Y, Ye X, Chen S. The prevalence and influencing factors of methicillin-resistant *Staphylococcus aureus* carriage in people in contact with livestock : A systematic review. Am J Infect Control. Elsevier Inc; 2015;43: 469–475. doi:10.1016/j.ajic.2014.12.009

128. Cuny C, Nathaus R, Layer F, Strommenger B, Altmann D, Witte W. Nasal Colonization of Humans with Methicillin-Resistant *Staphylococcus aureus* (MRSA) CC398 with and without Exposure to Pigs. PLoS One. 2009;4: 1–6. doi:10.1371/journal.pone.0006800

129. Verkade E, Bergh MK Den, Benthem B Van, Cleef B Van. Transmission of Methicillin-Resistant *Staphylococcus aureus* CC398 from Livestock Veterinarians to Their Household Members. PLoS One. 2014;9. doi:10.1371/journal.pone.0100823

130. Walter J, Espelage W, Adlhoch C, Cuny C, Schink S, Jansen A, et al. Persistence of nasal colonisation with methicillin resistant *Staphylococcus aureus* CC398 among participants of veterinary conferences and occurrence among their household members : A prospective cohort study , Germany 2008 – 2014. Vet Microbiol. Elsevier B.V.; 2017;200: 13–18. doi:10.1016/j.vetmic.2016.03.015

131. Feingold BJ, Silbergeld EK, Curriero FC, van Cleef BAGL, Heck MEOC, Kluytmans JAJW. Livestock density as risk factor for livestock-associated methicillin-resistant *Staphylococcus aureus*, the Netherlands. Emerg Infect Dis. 2012;18: 1841–9. doi:10.3201/eid1811.111850

132. van Rijen MML, Bosch T, Verkade EJM, Schouls L, Kluytmans JAJW. Livestock-Associated MRSA Carriage in Patients without Direct Contact with Livestock. 2014;9: 2–7. doi:10.1371/journal.pone.0100294

133. Anker JCH, Koch A, Ethelberg S, Mølbak K, Larsen J, Jepsen MR. Distance to pig farms as risk factor for community-onset livestock-associated MRSA CC398 infection in persons without known contact to pig farms-A nationwide study. Zoonoses Public Health. 2018;2016: 1–9. doi:10.1111/zph.12441

134. Hetem DJ, Bootsma MCJ, Troelstra A, Bonten MJM. Transmissibility of livestock-associated methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis. 2013;19: 1797–1802. doi:10.3201/eid1911.121085

135. Verkade E, Bosch T, Hendriks Y, Kluytmans J. Outbreak of Methicillin-Resistant *Staphylococcus aureus* ST398 in a Dutch Nursing Home. Infect Control Hosp Epidemiol. 2012;33: 624–625.

136. Wulf MWH, Markestein A, Linden FT Van Der, Voss A, Klaassen C, Verduin CM. First outbreak of methicillin-resistant *Staphylococcus aureus* ST398 in a Dutch hospital, june 2007. Eurosurveillance. 2008;13: 2007–2008. doi:10.1111/j.1469-0691.2007.01927.x

137. Feld L, Bay H, Angen Ø, Larsen AR, Madsen AM. Survival of LA-MRSA in Dust from Swine Farms. Ann Work Expo Heal. 2018; 1–10. doi:10.1093/annweh/wxx108

138. Agersø Y, Vigre H, Cavaco LM, Josefsen AMH. Comparison of air samples, nasal swabs, ear-skin swabs and environmental dust samples for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in pig herds. Epidemiol. Infect. 2014;142: 1727–1736. doi:10.1017/S095026881300280X

139. Friese A, Schulz J, Hoehle L, Fetsch A, Tenhagen BA, Hartung J, et al. Occurrence of MRSA in air and housing environment of pig barns. Vet Microbiol. 2012;158: 129–135. doi:10.1016/j.vetmic.2012.01.019

140. Nicholson TL, Shore SM, Smith TC, Fraena TS. Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) Isolates of Swine Origin Form Robust Biofilms. PLoS One. 2013;8: e73376. doi:10.1371/journal.pone.0073376

141. Hansen JE, Astrup LB, Pedersen K. Livestock-associated MRSA CC398 survival in manure. Poster. 17th International Symposium on Staphylococci and Staphylococcal Infections Seoul Republic of Korea. 2016.

142. Casey J, Curriero F, Cosgrove S, Nachman K, Schwartz B. High-Density Livestock Operations, Crop Field Application of Manure, and Risk of Community-Associated Methicillin-Resistant *Staphylococcus aureus* Infection in Pennsylvania. Jama Intern Med. 2013;173: 1980–1990. doi:10.1001/jamainternmed.2013.10408

143. Gibbons JF, Markey BK, Jahns H, Boland F, Abbott Y, Burns A, et al. Investigation of the persistence and transmission of MRSA CC 5 in pigs following intra-nasal inoculation. Vet Microbiol. 2013;162: 771–778. doi:10.1016/j.vetmic.2012.10.001

144. Tulinski P, Fluit AC, Wagenaar JA, Mevius D, Vijver L Van De, Duim B. Methicillin-Resistant Coagulase-Negative Staphylococci on Pig Farms as a Reservoir of Heterogeneous Staphylococcal Cassette Chromosome mec Elements. Appl Environ Microbiol. 2012; 299–304. doi:10.1128/AEM.05594-11

145. Espinosa-Gongora C, Broens EM, Moodley a., Nielsen JP, Guardabassi L. Transmission of MRSA CC398 strains between pig farms related by trade of animals. Vet Rec. 2012;170: 564–564. doi:10.1136/vr.100704

146. van Duijkeren E, Ikawaty R, Broekhuizen-Stins M, Spalburg E, de Neeling A, Allaart J, et al. Transmission of methicillin-resistant *Staphylococcus aureus* strains between different kinds of pig farms. Vet Microbiol. 2008;126: 383–389. doi:10.1016/j.vetmic.2007.07.021

147. Broens EM, Graat EAM, Van der Wolf PJ, Van de Giessen AW, Van Duijkeren E, Wagenaar JA, et al. MRSA CC398 in the pig production chain. Prev Vet Med. Elsevier B.V.; 2011;98: 182–189. doi:10.1016/j.prevetmed.2010.10.010

148. Tenhagen B, Fetsch A, Alt K, Käsbohrer A, Bräunig J, Appel B. MRSA in herds of fattening pigs in Germany - Associated risk factors. Safe pork 2009 - Québec city, Québec, Canada. 2009. pp. 124–127.

149. European Food Safety Authority. Analysis of the baseline survey on the prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in holding with breeding pigs, in the EU, 2008. Part B: factors associated with MRSA contamination of holdings. 2010. Available: https://www.efsa.europa.eu/en/efsajournal/pub/1597

150. Becker K, Ballhausen B, Köck R, Kriegeskorte A. Methicillin resistance in *Staphylococcus* isolates: The "*mec* alphabet" with specific consideration of *mecC*, a *mec* homolog associated with zoonotic *S. aureus* lineages. Int J Med Microbiol. 2014;304: 794–804. doi:10.1016/j.ijmm.2014.06.007

151. Kadlec K, Feßler AT, Hauschild T, Schwarz S. Novel and uncommon antimicrobial resistance genes in livestock-associated methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect. 2012;18: 745–755. doi:10.1111/j.1469-0691.2012.03842.x

152. Verhegghe M, Pletinckx LJ, Crombé F, Vandersmissen T, Haesebrouck F, Butaye P, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 in pig farms and multispecies farms. Zoonoses Public Health. 2013;60: 366–74. doi:10.1111/zph.12007

153. Broens EM, Graat EAM, Van PJ, Wolf D, Van De Giessen AW, De Jong MCM. Prevalence and risk factor analysis of livestock associated MRSA-positive pig herds in The Netherlands. Prev Vet Med. 2011;102: 41–49. doi:10.1016/j.prevetmed.2011.06.005

154. Dorado-García A, Graveland H, Bos MEH, Verstappen KM, Van Cleef BAGL, Kluytmans JAJW, et al. Effects of Reducing Antimicrobial Use and Applying a Cleaning and Disinfection Program in Veal Calf Farming: Experiences from an Intervention Study to Control Livestock-Associated MRSA. PLoS One. 2015; doi:10.1371/journal.pone.0135826

155. Maribo H: Zink (ZN) (*In Danish*). Danish Pig Research Centre; 2012. Available: http://vsp.lf.dk/Viden/Foder/Naeringsstoffer/Mineraler/Zink.aspx

156. Danmap. Updated erratum DANMAP 2015. 2017. Available: www.danmap.org

157. Aarestrup FM, Cavaco L, Hasman H. Decreased susceptibility to zinc chloride is associated with methicillin resistant *Staphylococcus aureus* CC398 in Danish swine. Vet Microbiol. 2010;142: 455–7. doi:10.1016/j.vetmic.2009.10.021

158. Cavaco LM, Hasman H, Stegger M, Andersen PS, Skov R, Fluit AC, et al. Cloning and occurrence of czrC, a gene conferring cadmium and zinc resistance in methicillin-resistant *Staphylococcus aureus* CC398 isolates. Antimicrob Agents Chemother. 2010;54: 3605–3608. doi:10.1128/AAC.00058-10

159. Cavaco LM, Hasman H, Aarestrup FM. Zinc resistance of *Staphylococcus aureus* of animal origin is strongly associated with methicillin resistance. Vet Microbiol. 2011;150: 344–348. doi:10.1016/j.vetmic.2011.02.014

160. Amachawadi RG, Scott HM, Nitikanchana S, Vinasco J, Tokach MD, Dritz SS, et al. Nasal Carriage of *mecA*-Positive Methicillin-Resistant *Staphylococcus aureus* in Pigs Exhibits Dose-Response to Zinc Supplementation. Foodborne Pathog Dis. 2014;12: 1–5. doi:10.1089/fpd.2014.1851

161. Slifierz MJ, Friendship RM, Scott Weese J. Methicillin-resistant *Staphylococcus aureus* in commercial swine herds is associated with disinfectant and zinc usage. Appl Environ Microbiol. 2015;81: 2690–2695. doi:10.1128/AEM.00036-15

162. Slifierz MJ, Friendship R, Weese JS. Zinc oxide therapy increases prevalence and persistence of methicillin-resistant *Staphylococcus aureus* in pigs: A randomized controlled trial. Zoonoses Public Health. 2015;62: 301–308. doi:10.1111/zph.12150

163. European Commission. Commision Implemented Regulation (EU) 2017/1145 of 8 June 2017 on the withdrawal from the market of certain feed additives authorised pursuant to Council Directives 70/524/EEC and 82/471/EEC and repealing the obsolete provisions authorising those feed. European Commision; 2017. pp. 1–21. Available: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R1145&rid=8

164. Alt K, Fetsch A, Schroeter A, Guerra B, Hammerl JA, Hertwig S, et al. Factors associated with the occurrence of MRSA CC398 in herds of fattening pigs in Germany. BMC Vet Res. 2011;7: 69. doi:10.1186/1746-6148-7-69

165. Gardner IA, Willeberg P, Mousing J. Empirical and theoretical evidence for herd size as a risk factor for swine diseases. Anim Heal Res Rev. 2002;3. doi:10.1079/AHRR200239

166. Seier-petersen MA, Nielsen LN, Ingmer H, Aarestrup FM, Agersø Y. Biocide Susceptibility of *Staphylococcus aureus* CC398 and CC30 Isolates from Pigs and Identification of the Biocide Resistance Genes, qacG and qacC. Microb Drug Resist. 2015;21: 527–536. doi:10.1089/mdr.2014.0215

167. Sidhu MS, Heir E, Sørum H, Holck A. Genetic Linkage Between Resistance to Quaternary Ammonium Compounds and β-Lactam Antibiotics in Food-Related *Staphylococcus spp* . Microb Drug Resist. 2001;7.

168. Ministry of Environment and Food of Denmark. MRSA. Facts about livestock-associated MRSA and about livestock-associated MRSA in Denmark in relation to Danish pigs and pork. 2018 [cited 8 Feb 2018]. Available: https://www.foedevarestyrelsen.dk/english/Animal/MRSA/Pages/default.aspx

169. Ministry of Environment and Food of Denmark. BEK 1319. Bekendtgørelse om særlige foranstaltninger til nedbringelse af antibiotikaforbruget i svinebesætninger (*in Danish*). 2010. Available: https://www.retsinformation.dk/pdfPrint.aspx?id=134559

170. Jensen VF, de Knegt L V., Andersen VD, Wingstrand A. Temporal relationship between decrease in antimicrobial prescription for Danish pigs and the “Yellow Card” legal intervention directed at reduction of antimicrobial use. Prev Vet Med. 2014;117: 554–564. doi:10.1016/j.prevetmed.2014.08.006

171. Ministry of Environment and Food of Denmark. Vægtede ADD'er (Weighted Animal Daily Dose). 2017 [cited 8 Feb 2018]. Available: https://www.foedevarestyrelsen.dk/Leksikon/Sider/Vægtede-ADDer.aspx

172. Ministry of Environment and Food of Denmark. Bekendtgørelse om obligatorisk hygiejnekursus for visse personer, der håndterer levende svin (*in Danish*). 2017 [cited 8 Feb 2018] p. 2018. Available: https://www.retsinformation.dk/Forms/R0710.aspx?id=195127

173. Olsen J V, Calvo-Artavia FF, Sandøe P, Toft N. Cost of LA-MRSA eradication from Danish pig herds (poster). SVEPM. Talinn, Estonia; 2018. Available: http://www.svepm.org.uk/posters.html

174. Schmithausen RM, Schulze-Geisthoevel SV, Stemmer F, El-Jade M, Reif M, Hack S, et al. Analysis of Transmission of MRSA and ESBL-E among Pigs and Farm Personnel. PLoS One. 2015; doi:10.1371/journal.pone.0138173

175. Luyckx K, Millet S, Weyenberg S Van, Herman L, Heyndrickx M, Dewulf J, et al. A 10-day vacancy period after cleaning and disinfection has no effect on the bacterial load in pig nursery units. BMC Vet Res. BMC Veterinary Research; 2016;12:236: 1–6. doi:10.1186/s12917-016-0850-1

176. Espinosa C, Panduro P, Saxmose S. Effect of a disinfectant powder on methicillin-resistant *Staphylococcus aureus* in pigs, bedding and air samples under simulated farm conditions. Pig J. APA; 2013;68: 13–18.

177. Verhegghe M, Crombe F, De Man I, Haesebrouck F, Butaye P, Heyndrickx M, et al. Preliminary study of the effect of sow washing, as performed on the farm, on livestock-associated methicillin-resistant *Staphylococcus aureus* skin status and strain diversity. J Swine Heal Prod. 2013;21: 313–319.

178. Schulz J, Bao E, Clauß M, Hartung J. The potential of a new air cleaner to reduce airborne microorganisms in pig house air: preliminary results. Berl Munch Tierarztl Wochenschr. 2013;4: 143–148. doi:10.2376/0005-9366-126-143

179. Luyckx K, Millet S, Weyenberg S Van, Herman L, Heyndrickx M, Dewulf J, et al. Comparison of competitive exclusion with classical cleaning and disinfection on bacterial load in pig nursery units. BMC Vet Res. BMC Veterinary Research; 2016;12: 1–10. doi:10.1186/s12917-016-0810-9

180. Verstappen KM, Duim B, van Nes A, Snijders S, van Wamel WJB, Wagenaar J a. Experimental nasal colonization of piglets with methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. Vet Microbiol. 2014; doi:10.1016/j.vetmic.2014.09.019

181. Karska-Wysocki B, Bazo M, Smoragiewicz W. Antibacterial activity of *Lactobacillus acidophilus* and *Lactobacillus casei* against methicillin- resistant *Staphylococcus aureus* (MRSA). Microbiol Res. 2010;165: 674–686. doi:10.1016/j.micres.2009.11.008

182. Heesterbeek H, Anderson RM, Andreasen V, Bansal S, Angelis D De, Dye C, et al. Modeling infectious disease dynamics in the complex landscape of global health. Science. 2015;347. doi:10.1126/science.aaa4339

183. Halasa T, Willeberg P, Christiansen LE, Boklund A, Alkhamis M, Perez A, et al. Decisions on control of foot-and-mouth disease informed using model predictions. Prev Vet Med. 2013;112: 194–202. doi:10.1016/j.prevetmed.2013.09.003

184. Halasa T, Bøtner A, Mortensen S, Christensen H, Toft N, Boklund A. Simulating the epidemiological and economic effects of an African swine fever epidemic in industrialized swine populations. Vet Microbiol. Elsevier B.V.; 2016;193: 7–16. doi:10.1016/j.vetmic.2016.08.004

185. Hill A, Snary E, Arnold M, Alban L, Cook A. Dynamics of *Salmonella* transmission on a British pig grower-finisher farm: a stochastic model. Epidemiol Infect. 2008;136: 320–333. doi:10.1017/S0950268807008485

186. Kirkeby C, Græsbøll K, Nielsen SS, Christiansen LE, Toft N, Rattenborg E, et al. Simulating the Epidemiological and Economic Impact of Paratuberculosis Control Actions in Dairy Cattle. Front Vet Sci. 2016;3: 1–13. doi:10.3389/fvets.2016.00090

187. Keeling MJ, Woolhouse MEJ, Shaw DJ, Matthews L, Chase-topping M, Haydon DT, et al. Dynamics of the 2001 UK Foot and Mouth Epidemic: Stochastic Dispersal in a Heterogeneous Landscape. Science. 2001;294: 813–818.

188. Keeling MJ, Woolhouse MEJ, May RM, Davies G, Grenfell BT. Modelling vaccination strategies against foot-and-mouth disease. Nature. 2003;421.

189. Kitching RP. Predictive models and FMD: the emperor's new clothes? Vet J. 2004;167: 127–128. doi:10.1016/j.tvjl.2003.10.014

190. Christley RM, Mort M, Wynne B, Wastling JM, Heathwaite AL, Pickup R, et al. "Wrong, but Useful": Negotiating Uncertainty in Infectious Disease Modelling. PLoS One. 2013;8. doi:10.1371/journal.pone.0076277

191. Boden LA, McKendrick IJ. Model-Based Policymaking: A Framework to Promote Ethical "Good Practice" in Mathematical Modeling for Public Health Policymaking. Front Public Heal. 2017;5: 1–7. doi:10.3389/fpubh.2017.00068

192. Grimm V, Berger U, Deangelis DL, Polhill JG, Giske J, Railsback SF. The ODD protocol: A review and first update. Ecol Modell. Elsevier B.V.; 2010;221: 2760–2768. doi:10.1016/j.ecolmodel.2010.08.019

193. Grimm V, Berger U, Bastiansen F, Eliassen S, Ginot V, Giske J, et al. A standard protocol for describing individual-based and agent-based models. Ecol Modell. 2006;198: 115–126. doi:10.1016/j.ecolmodel.2006.04.023

194. Schmolke A, Thorbek P, DeAngelis DL, Grimm V. Ecological models supporting environmental decision making: A strategy for the future. Trends Ecol Evol. 2010;25: 479–486. doi:10.1016/j.tree.2010.05.001

195. Grimm V, Augusiak J, Focks A, Frank BM, Gabsi F, Johnston ASA, et al. Towards better modelling and decision support: Documenting model development , testing , and analysis using TRACE. Ecol Modell. Elsevier B.V.; 2014;280: 129–139. doi:10.1016/j.ecolmodel.2014.01.018

196. Solle D, Hitzmann B, Herwig C, Remelhe MP, Ulonska S, Wuerth L, et al. Between the Poles of Data-Driven and Mechanistic Modeling for Process Operation. Chemie Ing Tech. 2017;89: 542–561. doi:10.1002/cite.201600175

197. Lazer D, Kennedy R, King G, Vespignani A. The Parable of Google Fly: Traps in Big Data Analysis. Science. 2014;343: 1203–1206. Available: http://gking.harvard.edu/files/gking/files/0314policyforumff.pdf

198. Venkatramanan S, Lewis B, Chen J, Higdon D, Vullikanti A, Marathe M. Using data-driven agent-based models for forecasting emerging infectious diseases. Epidemics. The Author(s); 2017;Article in. doi:10.1016/j.epidem.2017.02.010

199. Vynnycky E, White RG. An introduction to infectious disease modelling. New York, NY: Oxford University Press; 2010.

200. Nielsen JP, Larsen TS, Halasa T, Christiansen LE. Estimation of the transmission dynamics of African swine fever virus within a swine house. Epidemiol Infect. 2017; 1–10. doi:10.1017/S0950268817001613

201. Andraud M, Rose N, Grasland B, Pierre JS, Jestin A, Madec F. Influence of husbandry and control measures on porcine circovirus type 2 (PCV-2) dynamics within a farrow-to-finish pig farm: a modelling approach. Prev Vet Med. 2009;92: 38–51. doi:10.1016/j.prevetmed.2009.07.009

202. Lurette A, Belloc C, Touzeau S, Hoch T, Seegers H, Fourichon C. Modelling batch farrowing management within a farrow-to-finish pig herd: influence of management on contact structure and pig delivery to the slaughterhouse. animal. 2008; doi:10.1017/S1751731107000997

203. Jørgensen E. Stochastic Modelling of Pig Production. 1998. Report No.: No. 73. Available: http://www.jbs.agrsci.dk/~ejo/dinapig/growth.ps

204. Porphyre T, Giotis ES, Lloyd DH, Stärk KDC. Assessing control measures against MRSA ST398 in farrow-to-finish pig farm: a modelling approach (poster). ISVEE. Maastrict; 2012. p. 398.

205. Porphyre T, Giotis ES, Lloyd DH, Stärk KDC. A Metapopulation Model to Assess the Capacity of Spread of Meticillin-Resistant *Staphylococcus aureus* ST398 in Humans. PLoS One. 2012;7. doi:10.1371/journal.pone.0047504

206. Schulz J, Toft N, Boklund A, Larsen J, Halasa T. Towards control of LA-MRSA - Simulation modeling of LA-MRSA spread between pig farms (poster). SVEPM. Elsingore, Denmark; 2016. Available: http://www.svepm.org.uk/posters.html

207. Barnes S, Golden B, Wasil E. MRSA Transmission Reduction Using Agent-Based Modeling and Simulation. Informs J Comput. 2010;22: 635–646. doi:10.1287/ijoc.1100.0386

208. Gurieva T V, Bootsma MC, Bonten MJ. Decolonization of patients and health care workers to control nosocomial spread of methicillin-resistant *Staphylococcus aureus*: a simulation study. BMC Infect Dis. 2012;12.

209. McBryde ES, Pettitt AN, McElwain DLS. A stochastic mathematical model of methicillin resistant *Staphylococcus aureus* transmission in an intensive care unit: Predicting the impact of interventions. J Theor Biol. 2007;245: 470–481. doi:10.1016/j.jtbi.2006.11.008

210. Macal CM, North MJ, Collier N, Dukic VM, Lauderdale DS, David MZ, et al. Modeling the spread of community-associated MRSA. Proceedings Title: Proceedings of the 2012 Winter Simulation Conference (WSC). 2012. doi:10.1109/WSC.2012.6465271

211. Lipsitch M, Bergstrom CT, Levin BR. The epidemiology of antibiotic resistance in hospitals: paradoxes and prescriptions. Proc Natl Acad Sci U S A. 2000;97: 1938–1943. doi:10.1073/pnas.97.4.1938

212. Bootsma MCJ, Diekmann O, Bonten MJM, Levin SA. Controlling methicillin-resistant *Staphylococcus aureus*: Quantifying the effects of interventions and rapid diagnostic testing. Proc Natl Acad Sci U S A. 2006;103: 5620–5625.

213. Bergstrom CT, Lo M, Lipsitch M. Ecological theory suggests that antimicrobial cycling will not reduce antimicrobial resistance in hospitals.

214. Spicknall IH, Foxman B, Marrs CF, Eisenberg JNS. A modeling framework for the evolution and spread of antibiotic resistance: Literature review and model categorization. Am J Epidemiol. 2013;178: 508–520. doi:10.1093/aje/kwt017

215. Friese A, Schulz J, Hoehle L, Fetsch A, Tenhagen BA, Hartung J, et al. Occurrence of MRSA in air and housing environment of pig barns. Vet Microbiol. 2012;158: 129–135. doi:10.1016/j.vetmic.2012.01.019

216. Schentag JJ, Hyatt JM, Carr JR, Paladino JA, Birmingham MC, Zimmer GS, et al. Genesis of Methicillin-Resistant *Staphylococcus aureus* (MRSA), How Treatment of MRSA Infections Has Selected for Vancomycin-Resistant Enterococcus faecium , and the Importance of Antibiotic Management and Infection Control. Clin Infect Dis. 1998;26: 1204–1214.

217. Drogui R, P D. Tetracycline antibiotics in the environment: a review. Env Chem Lett. 2013;11: 209–227. doi:10.1007/s10311-013-0404-8

218. Vaarst M, Sørensen JT. Muligheder for antibiotikafri produktion af økologisk mælk og svinekød i Danmark (*in Danish*). 2017.

219. Van Cleef BAGL, Van Benthem BHB, Verkade EJM, Van Rijen MML, Kluytmans-Van Den Bergh MFQ, Graveland H, et al. Livestock-Associated MRSA in Household Members of Pig Farmers: Transmission and Dynamics of Carriage, A Prospective Cohort Study. 2015; doi:10.1371/journal.pone.0127190

220. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. doi:10.1186/1471-2334-6-130

221. White A. Relation between quantitative nasal cultures and dissemination of staphylococci. J Lab Clin Med. 1961;58: 273–277.

222. Nouwen JL, Ott A, Kluytmans-Vandenbergh MF, Boelens HA, Hofman A, van Belkum A, et al. Predicting the *Staphylococcus aureus* nasal carrier state: derivation and validation of a "culture rule." Clin Infect Dis. 2004;39: 806–811. doi:10.1086/423376

223. Vandenbergh MFQ, Yzerman EPF, Van A, Boelens H a M, Sijmons M, Henri A. Follow-Up of *Staphylococcus aureus* Nasal Carriage after 8 Years: Redefining the Persistent Carrier State. J Clin Microbiol. 1999;37: 3133–3140.

224. Mertz D, Frei R, Periat N, Zimmerli M, Battegay M, Flückiger U, et al. Exclusive *Staphylococcus aureus* Throat Carriage. Arch Intern Med. 2009;169: 692–706.e4. doi:10.1016/B978-0-323-40181-4.00115-8

225. Burns A, Shore AC, Brennan GI, Coleman DC, Egan J, Fanning S, et al. A longitudinal study of *Staphylococcus aureus* colonization in pigs in Ireland. Vet Microbiol. 2014;174: 504–513. doi:10.1016/j.vetmic.2014.10.009

Appendices

Appendix I. Transmission overview tables

Appendix II. Questionnaire

Appendix I. Table 1: Transmission from sow to offspring. Summary of transmission studies. Page 1 of 2

Agegroup		Sow to offspring		
Reference		Verhegghe et al., 2013 [118]	Weese et al., 2011 [35]	Burns et al., 2014 [225]
Study description	**Objective**	To investigate: *The effect of sow status on MRSA status of the piglets. * The age at which piglets become colonized	To investigate: *The effect of sow status on MRSA status of the piglets. *Longitudinal MRSA colonization in piglets	To investigate: *The effect of sow status on MRSA status of the piglets. * If pigs carry the same SA strain throughout the production
	Study population	48 sows (12 sows at each of 4 farms) + 10 piglets from each sow (or others if littersize<10)	10 litters at one farm	6 sows + offspring (73 piglets) at one big farm included based on sow colonization status: 2x (nasally pos. + vaginally pos.), 2x (nasally pos. + vaginally neg.), 2x (nasally neg. + vaginally neg.)
	Own/foster dam	Own dam + 11 extra piglets from other sows	Own dam	Own dam
	Sample types	Nasal (both nares)	Nasal	Nasal + vaginal
	No. of samplings	Approximately 10	9 for sows, 8 for piglets	2 for sows, 7 times for piglets
	Sampling times	Piglets: 1h and Day = {1,3,5,7,17} On one farm piglets were sampled only three times (day 3,7 and 23)	Piglets: Day={1,3,7,14,21,28,42, 56,70} Sows: 14 d prior to farrowing + at farrowing + same times as piglets	Piglets: Day={2,17,21,45,49,96,100} Sows: 7 days prior to farrowing + 2 days after
	MRSA introduction	Naturally contaminated	Naturally contaminated	Naturally contaminated
Management	**Weaning time**	20-27 days	24-28 days	21 days (two diff weaning stages)
	Use of antimicrobials	All the farms (A-D) routinely medicated piglets upon entering the growing unit: A + C: Promycin and amoxicillin, B: Trimethoprim and sulfadiazine, D: Amoxicillin	No treatment of sows nor piglets	In-feed batch treatment as follows: Week 3-5: Tilmicosin 1kg/t Week 5-7: Trimethoprim/ Sulphadiazine 2 kg/t Week 7-14: as above for the first 4 days
	Use of Zinc	No data	No data	No data
	Human handling	Fixed route in the stable om farm A&B, not on farm C&D	No data	No data (other than the 30 staff members all tended to a specific age group).
Env. sampling	**Parallel air samples**	Yes, air surrounding the pigs + outgoing air	No	No
	Parallel environmental swabs	Yes (wall + floor)	No	No

Appendix I. Table 1: Transmission from sow to offspring. Summary of transmission studies, continued. Page 2 of 2

Agegroup		Sow to offspring		
Reference		Verhegghe et al., 2013 [118]	Weese et al., 2011 [35]	Burns et al., 2014 [225]
Results	**Overall conclusions#**	Two different trends observed: At two farms the colonization percentages were high from the beginning and finally reached 100%. On the other two farms colonization remained low in the nursing unit, but increased at the end of their stay in the growing unit. Average colonization age for piglets: 17.8 days [95% CI: 15.3-20.2]. Ranged from 0.1 days at farms B and C to 46.6 days at farm A.	*Transmission and high colonization rates possible in the absence of antimicrobial use. *Sow status have an effect on piglets' status: 100% of piglets from pos sows and 84% from neg. sows were pos. at least once. *Sig. variations in MRSA colonization in piglets over time *Age significantly associated with probability of colonization * The authors speculate that inherent difference in susceptibility to MRSA might exist	*Odds of being SA pos., 12xhigher for piglets born from nasally positive sows, *…and even three times higher (than mentioned above) for piglets born from sows being both nasally and vaginally positive.
	Probability of a sow infecting its piglets	Mean predicted probability at various time points: After 1 h: From pos. sows: 0.65 From neg. sows: 0.26 After 1 day: From pos. sows: 0.75 From neg. sows: 0.35	100% of piglets from pos. sows and 84% from neg. sows were pos. at least once before weaning, but only 1/100 piglets tested pos. on day 1. Fig 2 shows the predicted probability over time	At day 2, MRSA only: OR=13.037 for piglets from sows being nasally pos.+vaginally neg. vs nasally neg.+vaginally neg. OR=20.444 for piglets from sows being nasally pos. + vaginally pos. vs nasally neg. +vaginally neg.
	Duration of carriage	Too short duration of study to assess this (17 or 23 days, depending on the farm)	No data for individual pigs. Sampling frequency would not have been high enough to determine accurately.	-
	Proportion of persistent /intermittent carriers	No data. The authors suggest that the existence of intermittent carriers among the sows could be the reason for piglets born by sows tested negative being positive	Data presented on aggregated form. Only fig.1+2 to judge from. Potentially max. 1/100 testing positive at all occasions, but not fair to include samplings prior to colonization	Only one piglet tested positive on all occasions (but with different strains). The authors suggest that piglets get transiently rather than persistently colonized from their dam.

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Appendix I. Table 2: Transmission between piglets + all. Summary of transmission studies. Page 1 of 2

Agegroup		Piglets		All
Reference		Verstappen et al., 2014 [180]	Crómbe et al., 2012 [41]	Broens et al., 2012a [112]
Study description	**Objective**	To quantitatively study: *The colonization of MRSA *The co-colonization of MSSA and MRSA	* To investigate the spread of ST398 in nursery piglets.	*To quantify transmission within pig herds incl. routes *To identify factors affecting transmission between pigs
	Study population	8 piglets from a single sow	31 piglets (3 wk old) from 4 litters in the same MRSA-free herd	2 DK + 4 NL farms: 63 sows + offspring sampled during one production cycle
	Own/foster dam	-	-	Own dam
	Sample types	Nasal	Nasal, skin, and swabs of the perineum	Nasal, vaginal (sows only), and rectal (newborn piglets only)
	No. of samplings	13 (according to M&M, but 14 on fig.1 in results section)	21 samplings	6 samplings
	Sampling times	Piglets: Day={1,2,3,5,6,7,8,9,10,11, 12,13, 15, 17}	Every second day in the interval 0-42 days post-inoculum.	Piglets: Day={3,21,42,70,175} Sows: 7 days prior to farrowing + same times as piglets until weaning
	MRSA introduction	Nasal inoculation with 3.5 * 10^8 CFU/ animal when 6 days old	Inoculation with 3*10^8 CFU in each nare + behind each ear with 1.5 * 10^8 CFU at day 28 → total dose = 9*10^8 CFU/animal	Naturally contaminated
	Sow status recorded	No (piglets in isolators)	No	Yes
Management	**Weaning time**	Housed in isolators with milking system after being delivered by caesarian	21 days (not completely clear)	Not stated, but the sampling 3 wk after farrowing is called “just before weaning”
	Use of antimicrobials	No piglets or sows received ab treatment during the study period.	No treatment	Recorded for each pen, as yes/no to use of risk antimicrobials (Tetracyclines and beta-lactams) for at least one pig within the pen.
	Use of Zinc	No data	No data	Used in the 2 DK herds (but all pigs were already positive before entering the weaner unit), not in the 4 NL herds
	Human handling	No data	No data	No data

Appendix I. Table 2: Transmission between piglets + all. Summary of transmission studies. Page 2 of 2

Agegroup		Piglets		All
Reference		Verstappen et al., 2014 [180]	Crómbe et al., 2012 [41]	Broens et al., 2012a [112]
	Parallel air samples	No	No	No
	Parallel env. swabs	No	Yes	Yes
Results	**Overall conclusions#**	*Nasal colonization succesfull in all piglets with stable numbers of *S. aureus* between 10^4-10^6 CFU. *MSSA and MRSA were able to co-colonize.	*R_0 between 3.92 and 52.54 in the three experimental groups. *The authors suggest that: "…a pen housing a MRSA negative piglets will upon introduction of a MRSA positive animal become a "dynamic system" of interacting reservoirs, regardless of whether the entities of the system are truly colonized or not.	* Rapid increase in prevalence after weaning in one herd, despite no use of risk antimicrobials *R_0 varied between 3.7 and 4.3 and was significantly above 1, indicating a high probability of persistence of LA- MRSA,
	Transmission rates	-	3 parallel groups: β (95% CI) 1) 0.89 (0.51-1.54) 2) 1.14 (0.78-1.66) 3) 2.69 (1.30-5.57)	Many different rates listed, depending on if DK, NL or DK+NL, post- or pre-weaning, with/or without use of antimicrobials and the infection pressure in the surroundings (pigs in neighboring pens + env. swabs).
	Probability of a sow infecting its piglets	-	-	After 3 days: NL herds: 84% from pos. sows and 48% from neg. DK herds:78% from pos. sows and 73% from neg.
	Duration of carriage	Persistent colonization throughout the duration of the experiment (2 weeks)	3 parallel groups: Mean(SD, median, min-max, n) in days: 1) 13.00 (10.53, 15, 1-25, 15) 2) 3.44 (2.99, 2, 1-13, 32) 3) 19.53 (11.45, 13, 5-39, 15)	17.4 days from Broens et al., 2012a, were used when calculating R_0's. No new data (too long interval btw. samplings to allow this?)
	Proportion of persistent /intermittent carriers	Persistent colonization throughout the duration of the experiment (2 weeks)	It is assumed that all pigs are intermittent carriers (SIS-model).	No individual pig data reported, and the interval between samplings are probably too big for data to be useful for this

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Appendix I. Table 3: Transmission between weaners. Summary of transmission studies. Page 1 of 2

Agegroup		Weaners		
Reference		Szabó et al., 2012 [46]	Jouy et al., 2011 [45]	Broens et al., 2012b [121]
Study description	**Objective**	To investigate: *Kinetics of colonization, host susceptibility and transmissibility for ST398, ST8 or ST9 *Transmission to uninoculated pigs.	To investigate: *Contamination and transmission after low dose inoculation	To: *Colonize pigs *Quantify transmission btw. pigs (based on transmission between 1st and 2nd contact pigs)
	Study population	57 weaned piglets from 10 litters	12 weaners (8 wk old) from an SPF farm	Ex.1: 5 female weaners (6 wk old) from a conventional MRSA negative farm Ex.2: 15 castrated male pigs (6 wk old)
	Own/foster dam	-	-	-
	Sample types	Nasal, skin, faecal, conjunctiva and organs	Nasal, faecal	Ex.1: Nasal, rectal and vaginal Ex. 2: Nasal and rectal
	No. of samplings	9 or more depending on group	8 samplings	Ex. 1: 22 samplings Ex. 2: 19 samplings
	Sampling times	5 piglets were sacrified at day 3 p.i, the rest on day 21 and tested on day 1,2,3,7,10,14,17 and 21) except the sentinel group (6 inoculated + 6 non-ino.), which was sacrified on day 42 p.i.	Every second day in the interval 0-14	Sampling twice a week in both experiments (but seems to be more samplings in the tables)
	MRSA introduction	Nasal inoculation with $5.0*10^8$ CFU/animal	Nasal inoculation with $2*10^4$ CFU/animal	Ex. 1: Nasal inoculation with 10^8 CFU in each nare (Ex. 1) (2 wk after inoculation with 10^8 CFU MSSA in each nare). Ex. 2: Oral inoculation with $5*10^9$ CFU.
Management	**Weaning time**	-	-	-
	Use of antimicrobials	No information (but it is stated that no pigs showed clinical signs of infection during the experiment)	No treatment during the experiment	No treatment during the experiment
	Use of Zinc	No data	No data	No data
	Human handling	All staff was neg. during the exp. Disinfection and complete change of clothing required before entering a pen	The 5 persons handling the pigs wore protective gloves, goggles and masks. All neg. before and after the experimental trial	No data

Appendix I. Table 3: Transmission between weaners. Summary of transmission studies. Page 2 of 2

Agegroup		Weaners		
Reference		Szabó et al., 2012 [46]	Jouy et al., 2011 [45]	Broens et al., 2012a [121]
	Parallel air samples	Yes, exhaust and supply air in air shafts	No	No
	Parallel env. swabs	Yes	Yes	No
Results	**Overall conclusions#**		*Low dose led to transmission between pigs. *Faecal execretion not needed for env. contamination. *The authors conclude that the min. inocul. dose needed for pers.colonization seems to be not $<10^8$ CFU per animal (based on this and other studies)	*R_0= 3.7-4.3 for direct con tact btw. Pigs (within pen transmission) *Duration of infection: Under the assumption that all pigs are intermittent carriers mean duration of infection = 17.4 (a pig defined as pos after one pos test) or 10.3 days (a pig defined as pos when testing pos at two consecutive samplings). *MRSA conc seems to decrease initially after which it increases and then stabilizes, which might indicate that environmental transmission does play a role
	Transmission rates	-	-	Scenario 1 (defined as pos after one pos test): β = 0.42 (95% CI: 0.25-0.66) Scenario 2 (defined as pos when testing pos at two consecutive samplings): β = 0.21 (95% CI: 0.12-0.38)
	Probability of a sow infecting its piglets	-	-	-
	Duration of carriage	Not estimated (Total sampling period probably a bit too short)	Only 2/12 pigs tested positive at two consecutive samplings 2 days apart (but low dose inoculation)	Scenario 1: 10.3 days (SD=7.7; median=7.5, min-max=1-26, n=24) Scenario 2: 17.4 days (SD=7.9; median=18, min-max: 6-29, n=15) (See scenario explanation under transmission rates)
	Proportion of persistent /intermittent carriers	-	No persistent carriers (but low dose inoculation)	Not mentioned in the text. But based on table 2: Two of the five 2nd contact pigs tested positive at all occasions (day 37,41,48,51,55,58,62) once they had been contaminated (exposed since day 15)

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Appendix II – Questionnaire

MRSA spørgeskema (*in Danish*)

A. Generelle oplysninger: **Kode:**

1. CHR nr: (Sæt nul foran 5-cifrede CHR numre)							**2. Interviewdato:** (dd-mm-åååååå)		**3. Interviewer:** (initialer)		
Talt med (navn):											
Besætningsejer: (bekræftes)											
CHR nr. adresse:									Fastnet		
									Mobil		

Produktionstype	**Ja**	**Nej**	**Hvis Ja, specificér venligst**
4. Har du specialproduktion? (f.eks. UK, Øko)			**Type:**
5. Er besætningen en SPF besætning?			**Rød:** ☐ **Blå:** ☐ **Grøn:** ☐

Besætningsstørrelse (kun dyr på det CHR nr, hvor der er udtaget prøver)		
6. Antal slagtesvin/år (årsleverance):	**7. Antal årssøer i besætningen:**	**8. Årlig produktion af smågrise:**
9. Race/krydsning: (for smågrise/slagtesvin)	**LYD: ____ %, LYDH: ____ %, LYH: ____ %, LYY: ____ %, Andet: ____ %**	

Indkøb af svin	**Fra hvor mange besætninger indenfor det seneste år**
10. Smågrise (7-30 kg)	
11. Ungsvin (30-45 kg)	
12. Sopolte/søer	
13. Orner	

B. Kontakt til andre dyr

14. Er der andre husdyr end svin på CHR-nummeret?				Ja: ☐ Nej: ☐		**Kommentar**	
Hvis Ja:	**15. Hvilke? (sæt et eller flere krydser)**	Kvæg:	Hest:	Mink:	Får/ged:	Fjerkræ:	Andet (nævn dyreart):

Hvor tæt anslår du at besætningens svin er på nærmeste… (i km):	**16. Spredning af gylle eller gødning fra andre besætninger?**	**17. Heste**	**18. Svin på fold** (hvis =< 5 km)

Hund		**Ja**	**Nej**	**Usikker**	**Kommentar**
19. Færdes der hund/hunde på ejendommen?					
Hvis Ja: Har hunde adgang til:	**20. Besætningsområder med svin?**				
	21. Oplagret halm, andet stibundsmateriale eller hø?				
	22. Oplagret svinefoder?				

Kat		**Ja**	**Nej**	**Usikker**	**Kommentar**
23. Er der tamme/vilde katte på ejendommen?					
Hvis Ja:	**24. Er der tale om staldkatte/(SPF katte), der aldrig kommer ud af staldene?**				
Hvis Nej i 24: Har katte adgang til:	**25. Besætningsområder med svin?**				
	26. Oplagret halm, andet stibundsmateriale eller hø?				
	27. Oplagret svinefoder?				

Vilde fugle		**Ja**	**Nej**	**Usikker**	**Kommentar**
Har vilde fugle adgang til:	**28. Besætningsområder med svin?**				
	29. Oplagret halm, andet stibundsmateriale eller hø?				
	30. Oplagret svinefoder?				

Gnavere (mus/rotter)	**Type**	**Lille**	**Nogen**	**Stor**	**Meget stor**	**Usikker**	**Kommentar**
31. Hvor stor vurderer du, at gnaverforekomsten i besætningen er?	Mus						
	Rotter						
Hvem står for gnaverbekæmpelsen i besætningen (sæt kryds)?							
32. Det gør vi selv:	**33. Kommune/Region:**		**34. Firma (kontrakt):**			**35. Pt. intet behov for bekæmpelse:**	

C. Søer (Hvis besætningen ikke har søer, gå til spørgsmål E, side 205)

C1. Opstaldning – Drægtighedsstald

36. Hvor gammel er din drægtighedsstald?	Antal år:			
37. Hvilken gulvtype anvendes til drægtige søer?	Drænet gulv:☐ Delvist spaltegulv:☐ Fast gulv:☐ Andet:☐ Kommentar:			
38. Hvilken strøelse anvendes der i drægtighedsstalden?	Ingen:☐ Halm i begrænset mængde:☐ Halm som dybstrøelse:☐ Spåner:☐ Andet: ☐			
39. Hvor mange m^2 er der pr. so på INDENDØRS arealet?	<2:☐	2-2,5:☐	>2,5:☐	Kommentar

		Ja	Nej	Usikker	Kommentar
40. Er drægtighedsstalden sektioneret?		Nej:☐ Vaskemure:☐ Fuldsektionering:☐			
Hvis Ja:	**41. Hvor mange drægtige søer er der pr. sektion?**	Typisk: Min: Max:			
42. Bruger du konsekvent holddrift i drægtighedsstalden (rengøring til gødnings-frit niveau mellem alle hold)?					
Hvis Ja:	**43. Betyder dette at dyr aldrig flyttes ml. holdene?**	Altid :☐ For det meste:☐ Et par flytninger pr. hold: ☐			
44. Bruger du desinfektionsmiddel i forbindelse med rengøring?					
Hvis Ja:	**45. Hvordan gør du?** (her tænkes på form (gas/væske/pulver) og procedure)				
46. Tørrer du drægtighedsstalden ud inden indsætning af nye dyr (hvidtør)?					
Hvis Ja:	**47. Hvor lang tomperiode/tid til udtørring har du ml. holdene (i dg.)?**	Typisk: Min: Max:			
48. Hvor mange drægtige søer har du i hver sti?		Typisk: Min: Max:			
49. Har du syge-/aflastningsstier inde i selve drægtighedsstalden?					

C2. Opstaldning – Farestald

50. Hvor gammel er din farestald?	Antal år:			
51. Hvilken gulvtype anvendes til diegivende søer?	Fuldspaltegulv:☐ Delvist spaltegulv:☐ Fast gulv:☐ Drænet gulv:☐ Andet:☐ Kommentar:			
52. Hvilken strøelse anvendes der i farestalden?	Ingen:☐ Halm i begrænset mængde:☐ Halm som dybstrøelse:☐ Spåner: ☐ Andet: ☐			
53. Hvor mange m^2 er der pr. so på INDENDØRS arealet?	<2:☐	2-2,5:☐	>2,5:☐	Kommentar

		Ja	Nej	Usikker	Kommentar
54. Er farestalden sektioneret?		Nej: ☐ Vaskemure: ☐ Fuldsektionering: ☐			
Hvis Ja:	**55. Hvor mange diegivende søer er der pr. sektion?**	Typisk: Min: Max:			
56. Bruger du konsekvent holddrift i farestalden (rengøring til gødnings-frit niveau mellem alle hold)?					
Hvis Ja:	**57. Betyder dette at dyr aldrig flyttes ml. holdene?**	Altid : ☐ For det meste: ☐ Et par flytninger pr. hold: ☐			
58. Bruger du desinfektionsmiddel efter rengøring?					
Hvis Ja:	**59. Hvordan gør du?** (her tænkes på form (gas/væske/pulver) og procedure)				
60. Tørrer du farestalden ud inden indsætning af nye dyr (hvidtør)?					
Hvis Ja:	**61. Hvor lang tomperiode/tid til udtørring har du ml. holdene (i dg.)?**	Typisk: Min: Max:			
62. Har du syge-/aflastningsstier inde i selve farestalden?					

C3. Fodring – Drægtige søer

	Ja	Andel af søer	Nej	Usikker	Kommentar
63. Bruger du pelleteret færdigfoder til drægtige søer?		%			
64. Bruger du hjemmeblandet foder til drægtige søer?		%			
Hvis Ja: **65. Bruger du færdigt tilskudsfoder?**		%			
66. Bruger du tør- eller vådfodring til drægtige søer?	Tørfodring: ☐ Vådfodring: ☐				

	Ja	Andel af søer	Nej	Usikker	Kommentar
67. Har drægtige søer adgang til halm andet end som stibunds- eller rodemateriale?		%			

C4. Fodring – Diegivende søer

	Ja	Andel af søer	Nej	Usikker	Kommentar
68. Bruger du pelleteret færdigfoder til diegivende søer?		%			
69. Bruger du hjemmeblandet foder til diegivende søer?		%			
Hvis Ja: **70. Bruger du færdigt tilskudsfoder?**		%			
71. Bruger du tør- eller vådfodring til diegivende søer?	Tørfodring: ☐ Vådfodring: ☐				

	Ja	Andel af søer	Nej	Usikker	Kommentar
72. Har diegivende søer adgang til halm andet end som stibunds- eller rodemateriale?		%			

D. So-insemination og pattegrise

73. Benyttes der orne eller KS? (angiv andel)		Orne: ___% KS: ___%	**Kommentar**
Ved KS:	**74. Har søerne trynekontakt med orne før insemination?**	Ja:☐ Nej:☐	

Hvordan håndteres store kuld pattegrise:		**Ja**	**Nej**	**Af og til**	**Kommentar**
75. Bruger du ammesøer?					
Hvis Ja:	**76. Et- eller to-trins ammesøer eller andet system?**	Et:	To:	Andet:	Specificer:
	77. Til hvilke pattegrise?	Overskydende ☐ De største ☐ De mindste ☐ Andet ☐ Hvis andet, beskriv:			
	78. Hvordan opstaldes ammesøerne?	Alle ammesøerne samles i en separat sektion ☐ Sammen med det hold, hvis afkom de ammer ☐ Andet ☐ , specificér venligst:			
79. Bruger du kuldudjævning?					
Hvis Ja:	**Hvornår?**	Kun indenfor 1-2 døgn efter faring ☐ Flere gange ☐ , typisk: _____			
80. Andet?				Beskriv:	

				Kommentar
81. Pattegrisenes alder ved fravænning?	Typisk: dg	Min: dg	Max: dg	
82. Pattegrisenes vægt ved fravænning	Typisk: kg	Min: kg	Max: kg	

E. Smågrise/klimagrise (fravænnede grise 7-30 kg)

E1. Opstaldning – Smågrise

83. Hvor gammel er din smågrisestald?	Antal år:		
84. Hvilken gulvtype anvendes til smågrisene?	Drænet gulv: ☐ Delvist spaltegulv: ☐ Fast gulv: ☐ Andet: ☐ Kommentar:		
85. Hvilken type strøelse anvendes der i smågrisestalden?	Ingen: ☐ Halm i begrænset mængde: ☐ Halm som dybstrøelse: ☐ Spåner: ☐ Andet: ☐		
86. Hvor mange m^2 er der pr. gris på INDENDØRS arealet?	<0,5: ☐	≥ 0,5: ☐	Kommentar

		Ja	Nej	Usikker	Kommentar
87. Er smågrisestalden sektioneret?		Nej: ☐ Fuldsektionering: ☐ Vaskemure: ☐			
Hvis Ja:	**88. Hvor mange smågrise er der pr. sektion?**	Typisk: Min: Max:			
89. Hvor mange smågrise er der pr. sti?		Typisk: Min: Max:			
90. Har smågrisene mulighed for trynekontakt mellem stierne?					
91. Bruger du konsekvent holddrift i smågrisestalden (rengøring til gødnings-frit niveau mellem alle hold)?					
Hvis Ja:	**92. Betyder dette at dyr aldrig flyttes ml. holdene?**	Altid : ☐ For det meste: ☐ Typisk et par flytninger pr. hold: ☐			
93. Bruger du desinfektionsmiddel efter rengøring?					
Hvis Ja:	**94. Hvordan gør du?** (her tænkes på form (gas/væske/pulver) og procedure)				
95. Tørrer du smågrisestalden ud inden indsætning af nye dyr (hvidtør)?					
Hvis Ja:	**96. Hvor lang tomperiode/tid til udtørring har du ml. holdene (i dg.)?**	Typisk: Min: Max:			
97. Har du syge-/aflastningsstier inde i selve smågrisestalden?					
98. Størrelsessorteres smågrisene i tidsrummet ml. placering i sti ved ankomst til smågrisestalden og salg/afgang til slagtesvinestalden?					
Hvis Ja:	**99. Hvor mange gange?**	Typisk: Min: Max:			
100. Passerer smågrisene igennem afsnit med yngre svin, når de sælges/overflyttes til ung-/slagtesvinestalden?		Ja:	Nej:	Af og til:	Kommentar:
101. Hvordan håndterer du restgrise, der ikke er klar til at blive solgt eller overflyttet?		Bruger bufferstald: ☐ % Sendes til videre uanset størrelse: ☐ % Flyttes til efterfølgende hold: ☐ % Andet: specificér: ☐ %			

E2. Fodring – Smågrise

	Ja	Andel af svin	Nej	Usikker	Kommentar
102. Bruger du pelleteret færdigfoder til smågrisene?		%			
103. Bruger du hjemmeblandet foder til smågrisene?		%			
Hvis Ja: **104. Bruger du færdigt tilskudsfoder?**		%			
105. Bruger du tør- eller vådfodring til smågrisene?	Tørfodring: ☐ Vådfodring: ☐				
106. Deles der krybbe med nabostien?					

	Ja	Andel af grise	Nej	Usikker	Kommentar
107. Har smågrise adgang til halm andet end som stibunds- eller rodemateriale?		%			

F. Ungsvin (ca. 30-45 kg)

108. Går ungsvinene separat?	Ja: ☐ Nej: ☐ Usikker: ☐	Kommentar

Hvis Nej, gå til spørgsmål G.

F1. Opstaldning – Ungsvin

109. Hvor gammel er din ungsvinestald?	Antal år:			
110. Hvilken gulvtype anvendes til ungsvinene?	Drænet gulv:☐ Delvist spaltegulv:☐ Fast gulv:☐ Andet:☐ Kommentar:			
111. Hvilken type strøelse anvendes der i ungsvinestalden?	Ingen:☐ Halm i begrænset mængde:☐ Halm som dybstrøelse:☐ Spåner: ☐ Andet: ☐			
112. Hvor mange m^2 er der pr. ungsvin på INDENDØRS arealet?	<0,5:☐	0,5-0,75:☐	>0,75:☐	Kommentar

		Ja	Nej	Usikker	Kommentar
113. Er ungsvinestalden sektioneret?		Nej: ☐ Vaskemure: ☐ Fuldsektionering: ☐			
Hvis Ja:	**114. Hvor mange ungsvin er der pr. sektion?**	Typisk: Min: Max:			
115. Hvor mange ungsvin er der pr. sti?		Typisk: Min: Max:			
116. Har ungsvinene mulighed for trynekontakt mellem stierne?					
117. Bruger du konsekvent holddrift i ungsvinestalden (rengøring til gødnings-frit niveau mellem alle hold)?					
Hvis Ja:	**118. Betyder dette at dyr aldrig flyttes ml. holdene?**	Altid : ☐ For det meste: ☐ Typisk et par flytninger pr. hold: ☐			
119. Bruger du desinfektionsmiddel efter rengøring?					
Hvis Ja:	**120. Hvordan gør du?** (her tænkes på form (gas/væske/pulver) og procedure)				
121. Tørrer du ungsvinestalden ud inden indsætning af nye dyr (hvidtør)?					
Hvis Ja:	**122. Hvor lang tomperiode/tid til udtørring har du ml. holdene (i dg.)?**	Typisk: Min: Max:			
123. Har du syge-/aflastningsstier inde i selve ungsvinestalden?					
124. Størrelsessorteres ungsvinene i tidsrummet ml. placering i sti ved ankomst til ungsvinestalden og flytning til slagtesvinestalden?					
Hvis Ja:	**125. Hvor mange gange?**	Typisk: Min: Max:			
126. Passerer ungsvinene igennem afsnit med yngre svin, når de overføres til slagtesvinestalden?		Ja:	Nej:	Af og til:	Kommentar:
127. Hvordan håndterer du restgrise, der ikke er klar til at blive solgt eller overflyttet?		Bruger bufferstald: ☐ % Sendes til videre uanset størrelse: ☐ % Flyttes til efterfølgende hold: ☐ % Andet: specificér: ☐ %			

G. Slagtesvin (evt. + ungsvin >30 kg)

G1. Opstaldning – Slagtesvin (evt. + ungsvin)

128. Hvor gammel er din slagtesvinestald?	Antal år:			
129. Hvilken gulvtype anvendes til slagtesvin?	Drænet gulv:☐ Delvist spaltegulv:☐ Fast gulv:☐ Andet:☐ Kommentar:			
130. Hvilken type strøelse anvendes der i slagtesvinestalden?	Ingen:☐ Halm i begrænset mængde:☐ Halm som dybstrøelse:☐ Spåner:☐ Andet:☐			
131. Hvor mange m^2 er der pr. slagtesvin på INDENDØRS arealet?	<0,75:☐	0,75-1:☐	≥1:☐	Kommentar

		Ja	Nej	Usikker	Kommentar
132. Er slagtesvinestalden sektioneret?		Nej: ☐ Vaskemure: ☐ Fuldsektionering: ☐			
Hvis Ja:	**133. Hvor mange slagtesvin er der pr. sektion?**	Typisk: Min: Max:			
134. Hvor mange slagtesvin er der pr. sti?		Typisk: Min: Max:			
135. Har slagtesvinene mulighed for trynekontakt mellem stierne?					
136. Bruger du konsekvent holddrift i slagtesvinestalden (rengøring til gødnings-frit niveau mellem alle hold)?					
Hvis Ja:	**137. Betyder dette at dyr aldrig flyttes ml. holdene?**	Altid : ☐ For det meste: ☐ Typisk et par flytninger pr. hold: ☐			
138. Bruger du desinfektionsmiddel i forbindelse med rengøring?					
Hvis Ja:	**139. Hvordan gør du?** (her tænkes på form (gas/væske/pulver) og procedure)				
140. Tørrer du slagtesvinestalden ud inden indsætning af nye dyr (hvidtør)?					
Hvis Ja:	**141. Hvor lang tomperiode/tid til udtørring har du ml. holdene (i dg.)?**	Typisk: Min: Max:			
142. Har du syge-/aflastningsstier inde i selve slagtesvinestalden?					
143. Størrelsessorteres slagtesvinene i tidsrummet ml. placering i sti ved ankomst til slagtesvinestalden og afsendelse til slagtning?					
Hvis Ja:	**144. Hvor mange gange?**	Typisk: Min: Max:			
14x. Passerer slagtesvinene igennem afsnit med yngre svin, når de afsendes til slagtning?		Ja: ☐ Nej: ☐ Af og til: ☐			

145. Hvad gør du med restgrise? (ca. andele)	Bruger bufferstald:☐ %	Sendes til slagtning uanset størrelse: ☐ %
	Flyttes til efterfølgende hold:☐ %	Andet: ☐ %, specificér:

Fodring af ungsvin og slagtesvin

	Ja	Nej	Usikker	Kommentar
146. Får ungsvin og slagtesvin det samme foder?				

<u>Hvis Ja</u>: gå til spørgsmål G3, side 11, <u>Hvis Nej</u>: fortsæt med spørgsmål G2

G2. Fodring – Ungsvin (30-45 kg)

	Ja	Andel af svin	Nej	Usikker	Kommentar
147. Bruger du pelleteret færdigfoder til ungsvinene?		%			
148. Bruger du hjemmeblandet foder til ungsvinene?		%			
Hvis Ja: **149. Bruger du færdigt tilskudsfoder?**		%			
150. Bruger du tør- eller vådfodring til ungsvinene?	Tørfodring: ☐ Vådfodring: ☐				
151. Deles der krybbe med nabostien?					

	Ja	Andel af grise	Nej	Usikker	Kommentar
152. Har ungsvin adgang til halm andet end som stibunds- eller rodemateriale?		%			

Fodring af slagtesvin

G3. Alm. Fodring – Slagtesvin (over 45 kg)

	Ja	Andel af svin	Nej	Usikker	Kommentar
153. Bruger du pelleteret færdigfoder til slagtesvinene?		%			
154. Bruger du hjemmeblandet foder til slagtesvinene?		%			
Hvis Ja: **155. Bruger du færdigt tilskudsfoder?**		%			
156. Bruger du tør- eller vådfodring til slagtesvinene?	Tørfodring: ☐ Vådfodring: ☐				
157. Deles der krybbe med nabostien?		%			

	Ja	Andel af grise	Nej	Usikker	Kommentar
158. Har slagtesvin adgang til halm andet end som stibunds- eller rodemateriale?		%			

H. Fælles arealer og udstyr

		Ja	Nej	Ej relevant	Kommentar
159. Har du en karantænestald til nye grise?					
Hvis Ja:	**160. Hvor længe opholder de nyindkøbte grise sig der (i uger)?**	Typisk:	Min:	Max:	
161. Har du dedikeret udstyr til hver stald ? (fodtøj, skovl etc)					
Hvis Ja:	**162. Er tilhørsforhold tydeligt markeret?**				
163. Rengøres gangene efter flytning af svin?					
Hvis Ja:	**164. Hvordan fortages dette?**	Der… fejes ☐	støvsuges ☐	vaskes af ☐	andet ☐
165. Genbruges kanyler/medicinsk udstyr etc?					
Hvis Ja:	**166. Til alle i samme kuld/boks?**				
	167. Til alle i samme sektion?				
	168. Til alle i samme aldersgruppe?				
169. Er bad påkrævet før adgang til besætningen?					
170. Er bad påkrævet når besætningen forlades?					

		Ja	Nej	Nogle gange	Kommentar
171. Køres svinene til slagtning i egen vogn?					
Hvis Nej:	**172. Har chaufføren af vognen adgang til stalden?**				
	173. Står der typisk svin i vognen i forvejen, når den ankommer?				
	174. Hvilke krav stiller du til bilen?	Tom: ☐	Vasket og desinficeret: ☐	Andet: ☐	
175. Har i udleveringsrum?					
Hvis Ja:	**176. Holdes udleveringsrummet helt lukket til ind mod stalden indtil det er rengjort?**				

I. Ventilation

		Ja	Nej	Ej relevant	Kommentar
177. Har du samme type ventilation i alle stalde?					
Hvis Nej:	**178. De følgende spørgsmål besvares for den stald, hvor MRSA prøverne blev udtaget. Angiv om dette var i:**	Slagtesvinestalden ☐ Smågrisestalden ☐ Andet ☐ , specificér:			
179. Er alle ventilations ind- og udtag lukket tæt til under eventuelle tomperioder efter at rengøring er afsluttet?					
180. Hvilke(n) type(r) luftindtag er der i stalden?		Undertryksanlæg med diffust luftindtag ☐ Undertryksanlæg med ventiler ☐ Ligetryksanlæg ☐ Overtryksanlæg ☐ Naturlig ☐ Andet ☐ Hvis andet, specificer:			
181. Hvilke(n) type(r) luftudtag er der i stalden?		Luftudtag i loft ☐ Luftudtag i gulv ☐ Naturlig ☐ Andet, specificer: ☐			
182. Har du overbrusningsanlæg?					
Hvis Ja:	**183. Hvilken type?**	Højtryksanlæg ☐ Lavtryksanlæg ☐			
184. Er der iværksat støvreducerende tiltag i stalden?					
Hvis Ja:	**185. Hvad er der installeret?**	Olieudsprøjtningsanlæg ☐ Ioniseringsanlæg ☐ Elektrostatisk filter ☐ Afskærmning ved foderudløb/fodervogn ☐ Støv separator påsat halmsnitter ☐ Andet, beskriv: ☐			

J. Gylle og gødning

		Ja	Nej	Ej relevant	Kommentar
186. Omrøres der i besætningens gylletank?					
187. Behandles gyllen?					
Hvis Ja:	**188. Hvordan?**	Syretilsætning ☐ Andet ☐, specificer:			
189. Hvor stor er besætningens gylletank (m3)?		Ca. m3			
190. Hvor lang tid ligger gyllen i gennemsnit før udbringning?		Typisk: Min: Max:			

K.Medarbejdere og besøgende

191. Har du indgået sundhedsrådgivningsaftale?	Ja: ☐ Nej: ☐	
192. Hvor tit kommer dyrlægen i besætningen?	Hver . dag	
193. Navn på klinikken og dyrlægen:	Klinik: Veterinær(er):	
194. Er det den samme dyrlæge, der kommer hver gang	Ja: ☐ Nej: ☐	

195. Hvad er det gennemsnitlige antal besøgende pr. md, inkl. håndværkere?		Antal: _____	Kommentar
196. Hvor mange personer arbejder i besætningen?		Antal: _____	
197. Arbejder nogen af disse med andre dyr?		Ja: ☐ Nej: ☐	
Hvis Ja:	**198. Med hvilke dyrearter?**	Specificér:	
	199. På dette CHR nr?	Ja: ☐ Nej: ☐	
	200. På et tilknyttet CHR nr (multisite/grisering)?	Ja: ☐ Nej: ☐	
201. Benytter du udenlandsk arbejdskraft?		Ja: ☐ Nej: ☐	
202. Arbejder det samme personale med alle aldersgrupper af svin?		Ja: ☐ Nej: ☐	
203. Benyttes der en fast arbejdsgang i staldene? (de yngste svin og mest modtagelige håndteres først)		Ja: ☐ Nej: ☐	
204. (*Det er OK, hvis de ikke ønsker at svare på dette)* Er nogen af medarbejderne i besætningen testet for MRSA?		Ja: ☐ Nej: ☐	
Hvis Ja:	**205. Er nogen af disse testet positive?**	Ja: ☐ Nej: ☐	
	206. Har nogen af disse haft behandlingskrævende MRSA relateret sygdom (inkl. hudproblemer etc)?	Ja: ☐ Nej: ☐	

L. Behandlingsstrategi

Besætningsejeren gøres opmærksom på forholdene omkring anonymitet

		Ja	**Nej**	**Nogle gange**	**Kommentar**
207. Bruger du zink og kobber til dine dyr (udover indhold i færdigfoder)?		Zink ☐ Kobber ☐ Ingen af delene ☐			
Hvis Ja:	**208. På hvilken form?**	Dyrlægeordineret tilskud ☐ Tilsætning til hjemmeblandet foder ☐			
	209. Hvor ofte benyttes dette?	Fast brug til en aldersgruppe ☐ Ved behov ☐			
Hvis benyttes ved behov:	**210. Hvornår igangsætter du tilskud?**	Beskriv:			

Når du behandler med antibiotika:	**211. Hvilken aldersgruppe og vægtklasse er det så typisk?**	
	212. Behandles der typisk på sektion, sti eller individ niveau?	Sektion: ☐ Sti: ☐ Individ: ☐
	213. Ved flokmedicinering, gives behandlingen typisk via:	Foder: ☐ Vand: ☐

214. Er der anvendt probiotika (gavnlige bakterie-kulturer) eller andre alternative midler eller ikke-receptpligtig medicin til behandling/forebyggelse det sidste år?			Ja	Nej	Usikker	Kommentar
Hvis Ja:	215. Hvilket middel?	216. Mod hvilken lidelse?	217. Hos hvilke aldersgrupper (søer, pattegrise, smågrise, ungsvin, slagtesvin)?			
	1.	1.	1.			
	2.	2.	2.			
	3.	8.	3.			

218. Gør du noget for sundhed eller smitteforebyggelse i besætningen, som vi ikke har spurgt om?

M. Afsluttende spørgsmål

219. Må vi evt. kontakte dig igen på et senere tidspunkt?	Ja: ☐ Nej: ☐	Kommentar
220. Hvis ja: Hvad er det bedste tidspunkt og tlf.nr.? Tidspunkt: Tlf: Evt. Email:		

TAK FOR HJÆLPEN!