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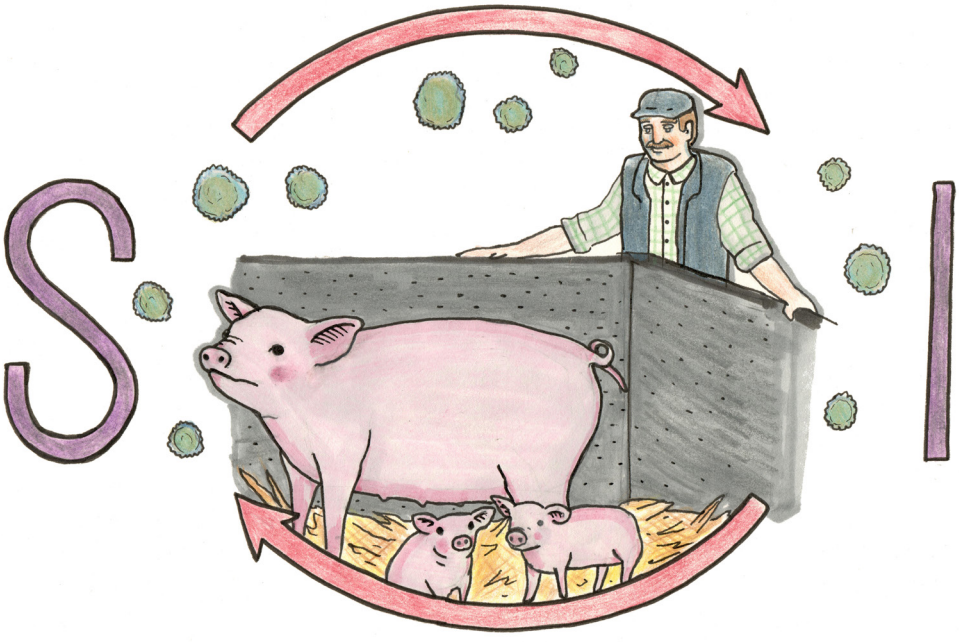
Control strategies for LA-MRSA in Swedish pig production: A disease modelling approach

Krista Tuominen



Control strategies for LA-MRSA in Swedish pig production: A disease modelling approach

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Abstract

Antimicrobial resistance is a threat to global health. Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is a category of multiresistant bacteria that primarily colonises livestock animals. While pigs are considered its main reservoir, LA-MRSA is zoonotic and causes an occupational risk to those working with livestock. However, LA-MRSA is also capable of spreading to humans without livestock contact. Although LA-MRSA carriers are usually asymptomatic, LA-MRSA can cause a wide range of infections in humans.

The aim of this thesis was to use disease modelling to study the spread of LA-MRSA and assess possible control strategies in a Swedish farrow-to-finish pig herd—additionally, the thesis aimed to fill knowledge gaps regarding the survival of LA-MRSA in the farm environment.

The modelling studies concluded that eradicating LA-MRSA is challenging. Early detection and introduction of control measures were considerably more effective in lowering the within-herd prevalence than measures that were implemented when LA-MRSA had become established in the herd. The time to disease elimination was at least 300 days even with the most effective control measures that were introduced early at the outbreak phase of disease spread.

In an experimental study, the survival of LA-MRSA strains belonging to the clonal complex (CC) 398 varied on different surface materials (concrete, polypropylene plastic and stainless steel). This finding can be beneficial when planning efficient cleaning and disinfection routines in pig farms.

In conclusion, investing in early detection and intensive early control measures may be justified if a low LA-MRSA prevalence country aims to have LA-MRSA-free pig herds.

Keywords: antimicrobial resistance, biosecurity, disease control, LA-MRSA, modelling, One Health, pig, survival, zoonosis

Kontrollstrategier för LA-MRSA i svensk grisproduction: En sjukdomsmodelleringsmetod

Sammanfattning

Antimikrobiell resistens är ett hot mot den globala hälsan. Lantbruksdjurassocierad meticillinresistent *Staphylococcus aureus* (LA-MRSA) är en kategori av multiresistenta bakterier som främst koloniserar lantbruksdjur. Även om grisar anses vara dess huvudsakliga reservoar är LA-MRSA zoonotisk och utgör en yrkesrisk för dem som arbetar med boskap. LA-MRSA kan dock även spridas till människor utan kontakt med boskap. Även om bärare av LA-MRSA vanligtvis är symptomfria kan LA-MRSA orsaka ett brett spektrum av infektioner hos människor.

Syftet med denna avhandling var att använda sjukdomsmodellering för att studera spridningen av LA-MRSA och bedöma möjliga kontrollstrategier i svenska grisbesättningar—dessutom hade avhandlingen som mål att fylla kunskapsluckor angående överlevnaden av LA-MRSA i gårdsmiljön.

Modelleringsstudierna visade att utrota LA-MRSA är utmanande. Tidig upptäckt och införandet av kontrollåtgärder var betydligt effektivare för att minska prevalensen inom besättningen än åtgärder som genomfördes när LA-MRSA hade etablerats i besättningen. Tiden för att eliminera sjukdomen var minst 300 dagar även med de mest effektiva kontrollåtgärder som infördes tidigt under utbrottsfasen av sjukdomsspridningen.

I en experimentell studie var överlevnaden av LA-MRSA-stammar tillhörande klonalt komplex (CC) 398 varierande på olika ytmaterial (betong, polypropylenplast och rostfritt stål). Detta resultat kan vara till nytta vid planering av effektiva rengörings- och desinfektionsrutiner på grisgårdar.

Avslutningsvis kan det vara motiverat att investera i tidig upptäckt och intensiva tidiga kontrollåtgärder i ett land med låg förekomst av LA-MRSA, om målet är att uppnå LA-MRSA-fria grisbesättningar.

Nyckelord: antimikrobiell resistens, biosäkerhet, gris, LA-MRSA, modellering, One Health, sjukdomskontroll, zoonos, överlevnad,

LA-MRSA:n torjuntastrategiat ruotsalaisessa sikatuotannossa tautimallinnuksen näkökulmasta

Tiivistelmä

Mikrobilääkeresistenssi on uhka globaalille terveydelle. Tuotantoeläimiin liitetty metisilliini-resistentti *Staphylococcus aureus* (LA-MRSA) kuuluu ryhmään moniresistenttejä bakteereita, jotka kolonisoivat ensisijaisesti tuotantoeläimiä. Vaikka siat ovat LA-MRSA:n tavallisin reservoaari, zoonoottisena bakteerina se aiheutaa tautiriskin tuotantoeläinten parissa työskenteleville ja levitä ihmisiin joilla ei ole kontaktia tuotantoeläimiin. LA-MRSA-kantajat ovat useimmiten oireettomia, mutta LA-MRSA pystyy aiheuttamaan ihmissä laajan kirjon erilaisia infektioita.

Tämän väitöskirjan tarkoituksena oli tutkia LA-MRSA:n leviämistä ja mahdollisia torjuntatoimenpiteitä ruotsalaisessa yhdistelmäsikalassa käyttäen tautimallinnusta. Lisäksi väitöskirjan tavoitteena oli lisätä tietämystä LA-MRSA:n selviytymisestä sikalaympäristössä.

Tautimallinnukset osoittivat, että LA-MRSA:n hävittäminen sikatilalta on haasteellista. Bakteerin varhainen tunnistaminen ja torjuntatoimenpiteiden käyttöönotto olivat huomattavasti tehokkaampia alentamaan taudin esiintyvyyttä kuin toimenpiteet, jotka otettiin käyttöön vasta kun LA-MRSA oli jo vakiintunut tilalle. Varhaisesta tautihallinnasta huolimatta myös tehokkaimmilla torjuntatoimenpiteillä bakteerin hävittämiseen kului vähintään 300 päivää.

Kokeellisessa tutkimuksessa klonaalikompleksi (CC) 398:aan kuuluvien LA-MRSA-kantojen selviytyminen vaihteli eri pintamateriaaleilla (betoni, polypropeenimuovi ja ruostumaton teräs). Tämä havainto voi auttaa suunnittelemaan tehokkaampia puhdistus- ja desinfiointirutiineja sikatiloille.

Yhteenvetona voidaan todeta, että varhaiseen tunnistamiseen ja alkuvaiheen torjuntatoimenpiteisiin panostaminen voi olla perusteltua, jos LA-MRSA:n esiintyvyys maassa on matala ja tavoitteena on sikatuotanto ilman LA-MRSA:ta.

Asiasanat: bioturvallisuus, LA-MRSA, mallinnus, mikrobilääkeresistenssi, selvityminen, sika, tautivalvonta, One Health, zoonoosi

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Tuominen, K.S., Sternberg Lewerin, S., Jacobson, M., Rosendal, T. (2022). Modelling environmentally mediated spread of livestock-associated methicillin-resistant *Staphylococcus aureus* in a pig herd. Animal, 16, 100450.

II. Gröndal, H., Tuominen, K.S., Sternberg Lewerin, S. Perspectives of on-farm biosecurity and disease prevention among pig veterinarians and pig farmers in Sweden (accepted).

III. Tuominen, K.S., Sternberg Lewerin, S., Widgren, S., Rosendal, T. (2023). Assessment of control measures against livestock-associated methicillin-resistant *Staphylococcus aureus* in a farrow-to-finish pig herd using infectious disease modelling. Animal, 17, 100840.

IV. Tuominen, K., Frosth, S., Pedersen, K., Rosendal, T., Sternberg Lewerin, S. (2023). Survival of livestock-associated methicillin-resistant *Staphylococcus aureus* CC398 on different surface materials. Acta Veterinaria Scandinavica, 65, 13.

The contribution of Krista Tuominen to the papers included in this thesis was as follows:

I. Took major responsibility in model planning and building. Analysed and visualised the data. Drafted the manuscript and finalised it with input from the co-authors. Corresponded with the journal.

II. Actively participated in planning and observing the focus group discussions. Took part in writing the manuscript. Corresponded with the journal.

III. Took major responsibility in planning and model programming. Analysed and visualised the data. Drafted the manuscript and finalised it with input from the co-authors. Corresponded with the journal.

IV. Took major resbonsibility for setting up and performing the experiments. Analysed and visualised the data. Drafted the manuscript and finalised it with input from the co-authors. Corresponded with the journal.

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Abbreviations

ABC	Approximate Bayesian computation
AIAO	All-in all-out pens
AMR	Antimicrobial resistance
AMU	Antimicrobial use
BA	Bovine blood agar
BS+	Improved biosecurity
CA-MRSA	Community-associated methicillin-resistant *Staphylococcus aureus*
CC	Clonal complex
ccr	Cassette chromosome recombinase
CF	Continuous flow pens
CFU	Colony forming units
CrF	Cross-fostering (mixing practice)
CTMC	Continuous-time Markov chain
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EU	European Union
FM	Finishing pig mixing
G	Gilts

G+S	Gilts and sows
HA-MRSA	Healthcare-associated methicillin-resistant *Staphylococcus aureus*
IEC	Immune evasion cluster
LA-MRSA	Livestock-associated methicillin-resistant *Staphylococcus aureus*
M-	No mixing in finishing and farrowing units
MRSA	Methicillin-resistant *Staphylococcus aureus*
NLS	Non-linear least squares regression
PVL	Panton-Valentine leucocidin
qPCR	Quantitative polymerase chain reaction
S	Sows
SCC*mec*	Staphylococcal cassette chromosome *mec*

1. Introduction

Antimicrobial resistance (AMR) threatens the health of humans, animals and plants. Increased spread of AMR and the lack of effective antimicrobial drugs leads to more difficult-to-treat infections, reduced safety of many medical procedures and consequently to increased mortality (World Health Organization 2021). Antimicrobial resistance also has a negative impact on the global economy through, for example, increased healthcare costs and production losses (OECD 2019).

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) are a group of *Staphylococcus aureus* bacteria strains associated with livestock reservoirs. LA-MRSA are resistant to most β-lactam antibiotics as well as to tetracyclines. However, resistance to other antimicrobials has also been described (EFSA & ECDC 2022). While pigs are considered to be the primary reservoir of LA-MRSA, they are usually only asymptomatic carriers (Verkade & Kluytmans 2014). LA-MRSA is zoonotic and people working with livestock are at increased risk of becoming LA-MRSA carriers (Chen & Wu 2021). Additionally, spillover of LA-MRSA to people without livestock contact has been reported (Larsen *et al*. 2015). While LA-MRSA—like the other *S. aureus* bacteria—are considered commensals, they are also facultative pathogens that can cause various diseases, ranging from skin infections to potentially life-threatening conditions (Quinn *et al*. 2011). Therefore, LA-MRSA is considered a risk to public health (EFSA & ECDC 2022).

While LA-MRSA have been detected in pigs in many countries worldwide, the distribution of different LA-MRSA strains varies. In Europe, the majority of the LA-MRSA isolates belong to clonal complex (CC) 398 (EFSA & ECDC 2022). Even though LA-MRSA are widely spread globally, current knowledge about the spread of LA-MRSA, as well as efficient

control or eradication measures that do not require culling the whole herd, is limited. Therefore, further studies on control measures against LA-MRSA are needed to establish effective control plans.

2. Background

2.1 *Staphylococcus aureus* and MRSA

Staphylococcus aureus are commensal bacteria colonising numerous animal species, including humans (Quinn *et al*. 2011). They are robust and able to grow in various conditions, resistant to environmental stress (Clements & Foster 1999) and capable of forming biofilms (Flemming *et al*. 2016; Shen *et al*. 2021). *Staphylococcus aureus* are also opportunistic pathogens capable of causing a range of infections varying from minor local infections to life-threatening septicaemia (Quinn *et al*. 2011). In pigs, *S. aureus* carriage is common, but it only rarely causes clinical disease (Frana & Hau 2019). However, *S.aureus* has been associated with septicaemia, arthritis, osteomyelitis, mastitis, metritis, vaginitis and endocarditis (Aarestrup *et al*. 2008; Frana & Hau 2019).

The term methicillin-resistant is used for those *Staphylococcus aureus* strains that were originally found to be resistant to methicillin and other penicillins (Lee *et al*. 2018). Penicillins are a group of antimicrobial substances classified as β-lactam antibiotics. The methicillin-resistant *S. aureus* have developed from methicillin-susceptible *S. aureus* clones by acquiring the staphylococcal cassette chromosome mec (SCC*mec*) complex through horizontal gene transfer (Lee *et al.* 2018). The SCC*mec* is a mobile genetic element that can carry *mecA* and *mecC* genes responsible for encoding the resistance against most β-lactam antibiotics (Lee *et al*. 2018; EFSA & ECDC 2022). The SCC*mec* can be classified into different types based on the type of the cassette chromosome recombinase (*ccr*) and the class of the *mec* gene(s) in the mobile genetic element (Liu *et al*. 2016; Lee *et al*. 2018).

Different MRSA strains are usually classified into three categories based on their epidemiological origin: healthcare-associated (HA-), community-associated (CA-) and livestock-associated (LA-) MRSA (Lee *et al*. 2018; EFSA & ECDC 2022). The division of MRSA into these categories is based on genetic and phenotypic (including virulence) criteria, as well as on different epidemiological characteristics and clinical presentation. However, the differentiation between the categories is not always clear-cut and its relevance has been questioned (Bal *et al*. 2016; Lee *et al*. 2018; EFSA & ECDC 2022). Of the three categories, HA-MRSA and CA-MRSA are primarily associated with strains that affect humans, while LA-MRSA has been found in most livestock species (EFSA & ECDC 2022). However, as LA-MRSA is zoonotic, it also impacts public health. While there is no formal definition for LA-MRSA, it is usually associated with the CC398 and CC9 lineages (Bal *et al*. 2016).

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

In addition to pigs and humans, *Staphylococcus aureus* belonging to the LA-MRSA CC398 have been detected in several food-producing animals, such as dairy cattle, veal calves and poultry (Nemati *et al*. 2008; Hansen *et al*. 2019; Schnitt & Tenhagen 2020). These bacteria have also been found in horses, goats, minks, rodents and companion animals (Pletinckx *et al*. 2013; Islam *et al*. 2017; Fertner *et al*. 2019b). LA-MRSA contamination has also been reported in food for human consumption and animal feed (Hansen *et al*. 2017; Anjum *et al*. 2019).

2.2.1 Evolution of LA-MRSA CC398 and other MRSA CC398

LA-MRSA was first reported in pigs in the Netherlands in 2005 (Voss *et al*. 2005). The first LA-MRSA isolates belonged to multilocus sequence type clonal complex 398 (MRSA CC398; Huijsdens *et al*. 2006). Since then, several other sequence types belonging to multiple clonal complexes have been recognised in livestock (Fitzgerald 2012). It has been suggested that livestock-associated MRSA CC398 most likely originated from human-associated methicillin-susceptible CC398 strains and that LA-MRSA CC398 acquired the mobile genetic element SCC*mec* as well as methicillin and tetracycline resistance when it spread to livestock (Price *et al*. 2012). During

the switch from human hosts to livestock, *Staphylococcus aureus* CC398 also lost its immune evasion cluster (IEC; Price *et al*. 2012), a genetic element that protects the bacteria from the human immune system (Thammavongsa *et al*. 2015).

Based on phylogenetic studies, MRSA CC398 strains have been divided into separate livestock- and human-associated clades (Price *et al*. 2012; Stegger *et al*. 2013). The livestock-associated clade has been associated with the *tet(M)*-gene, which encodes tetracycline resistance. In contrast, the human clade has been associated with the staphylococcal complement inhibitor (*scn*) gene and other genes part of the IEC (Price *et al*. 2012; Stegger *et al*. 2013). Price *et al*. (2012) also reported that the *lukF-lukS* genes that encode the virulence factor Panton-Valentine leucocidin (PVL), were only found in the human-associated strains of CC398. Several studies worldwide have reported MRSA CC398 strains adapted to the human host, which have likely developed from the MSSA CC398 of human origin (Welinder-Olsson *et al*. 2008; Møller *et al*. 2019; Lu *et al*. 2021; Coombs *et al*. 2022). While some of the MRSA CC398 have been reported to be PVL-positive (Koyama *et al*. 2015; Becker *et al*. 2017; Møller *et al*. 2019; Coombs *et al*. 2022), PVL-negative strains with other virulence factors have also emerged (Lu *et al*. 2021; Coombs *et al*. 2022). In China, the PVL-positive human variant of MRSA CC398 has been reported to be fairly frequent in healthcare and community setting (Møller *et al*. 2019). The PVL-positive CC398 variant has also been encountered in Europe, for example in a hospital outbreak in Denmark, but known cases have mainly been associated with contacts to south-eastern Asia (Møller *et al*. 2019). In Australia, clinical cases of PVL-positive MRSA CC398 without a travel history have been reported (Coombs *et al*. 2022).

The MRSA CC398 strains of human origin have been considered to be more virulent than LA-MRSA CC398. However, some studies have demonstrated that IEC harbouring LA-MRSA CC398 can be present in animal isolates, and it has also been proposed that LA-MRSA CC398 is capable of re-adapting to the human host (Cuny *et al*. 2015a; Sieber *et al*. 2019; Sieber *et al*. 2020; Avberšek *et al*. 2021). However, virulence factors, such as enterotoxin, exotoxin and exfoliative toxin genes, have also been reported, and LA-MRSA CC383 can acquire foreign genetic material by horizontal gene transfer (Krüger-Haker *et al*. 2023). The capability of re-obtaining virulence factors and the recent evolution of MRSA CC398 of

human origin might pose a greater risk to human and animal health in the future. The acquisition of IEC has also been proposed to have caused increased LA-MRSA CC398 transmission among people living in the same household, but these isolates have not been found to be self-sustainable in the general human population (Sieber *et al*. 2020). In light of these studies, distinguishing separate human- and livestock-associated LA-MRSA CC398 clades is not straightforward. As livestock acts as a reservoir for LA-MRSA, decreasing the prevalence in animal herds and closely monitoring the development of LA-MRSA are needed.

2.2.2 Antimicrobial resistance in LA-MRSA CC398

Even though the abbreviation of MRSA refers to methicillin resistance, resistance against numerous other antimicrobial agents have been detected in *Staphylococcus aureus* bacteria. When MRSA adapted to the livestock hosts, it acquired the antimicrobial resistance genes *tet(M)* (tetracycline), *mecA* (β-lactams) as well as *czrC* gene that is responsible for zinc resistance (Kadlec *et al*. 2012; Price *et al*. 2012). Since then, several genes responsible for antimicrobial resistance have been detected in LA-MRSA CC398 isolates, some of which are considered novel or uncommon for staphylococci (Kadlec *et al*. 2012). Of these, the *cfr*-gene has been raised as a particular concern due to resistance properties against the so-called "last-resort" antimicrobials like oxazolidinones (Kadlec *et al*. 2012; EFSA & ECDC 2022). Resistance to linezolid—an antibiotic that belongs to oxazolidinones, which is used for treating highly resistant MRSA infections in humans—has recently been found in LA-MRSA CC398 from pigs in Europe (Ruiz-Ripa *et al*. 2021; EFSA & ECDC 2022; Leão *et al*. 2022). Additionally, phenotypic antimicrobial resistance or resistance genes against trimethoprim, macrolides, lincosamides, streptogramin A and B, phenicols, aminoglycosides and mupirocin have been detected in LA-MRSA CC398 (Kadlec *et al*. 2012; Butaye *et al*. 2016; Conceição *et al*. 2017; Leão *et al*. 2022). Studies have also highlighted that LA-MRSA CC398 can have a high diversity of different antimicrobial patterns and that it is able to acquire resistance from other bacteria (Kadlec *et al*. 2009; Kadlec *et al*. 2012; Leão *et al*. 2022).

2.3 Occurrence of LA-MRSA

2.3.1 Occurrence of LA-MRSA globally

When livestock-associated MRSA was discovered, it had transmitted between pigs and pig farmers and their household members (Voss *et al*. 2005). Currently, the distribution of different LA-MRSA strains found in animals, humans and food varies globally. While in Europe, the majority of the livestock-associated strains belong to the CC398 (EFSA & ECDC 2022), the strains found in the United States are more distributed between clonal complexes CC398, CC395 and CC8 (Smith 2015). On the other hand, strains belonging to CC9 predominate in many Asian countries (Chuang & Huang 2015), while CC398 is the major lineage in Korea (Back *et al*. 2020). In Australia, LA-MRSA CC398 and community-adapted CC93 have been found in pigs (Sahibzada *et al*. 2017), but recently a community-associated CC398 has become established in the Australian community (Coombs *et al*. 2022). Knowledge of the occurrence of LA-MRSA CC398 in Africa is limited. While MRSA CC398 has been detected from a dog in Zambia (Youn *et al*. 2014), it has not been reported in livestock (Lozano *et al.* 2016). However, MRSA isolates have been found in pigs, for example in South Africa and Nigeria (Van Lochem *et al*. 2018; Nwaogaraku 2019), but further genotyping analysis of the isolates was not reported.

In the EU, the most recent baseline study on MRSA in pigs was conducted in 2008 in holdings with breeding pigs. The study concluded that the MRSA prevalence varied between the member states: while 26.9% of the herds were MRSA-positive at the EU level, the prevalence in individual member states varied between 0–51.2% (European Food Safety Authority 2009). The proportion of MRSA CC398 was 92.5% of all detected MRSA isolates (European Food Safety Authority 2009). However, knowledge of the current occurrence of LA-MRSA is largely incomplete as the monitoring and molecular characterisation practices vary between countries. In Europe, human MRSA data are summarised in the European Centre for Disease Prevention and Control's (ECDC) Antimicrobial resistance surveillance in Europe report-series. The European Food Safety Authority (EFSA) and ECDC also publish a joint report on antimicrobial resistance in humans, animals and food. However, these reports rely on voluntary MRSA reporting, which does not always include molecular characterisation of the bacteria.

Of the countries that participated in the EFSA's and ECDC's 2022 report, Norway is the only country with a systematic MRSA surveillance and eradication programme in animals (Grøntvedt *et al*. 2016; EFSA & ECDC 2022). This policy was introduced after several LA-MRSA introductions were detected in Norwegian pig herds between 2013–2014, and it has been successful in keeping Norwegian pig herds free from LA-MRSA. Contrary to Norway, Denmark is an example of a country with high LA-MRSA prevalence in pig herds, where 88% of finishing herds were reported as positive in 2016 (DANMAP 2018). Denmark is also characterised by having more intensive pig farming than other Nordic countries (Petersen *et al*. 2021). In humans, Denmark also has a high proportion of LA-MRSA CC398 of all MRSA cases (35% in 2021; DANMAP 2022). However, the high number of human LA-MRSA cases is partially explained by the Danish screening guidelines, which include testing persons with pig contact and their household members for MRSA when hospitalised (The Danish Health Authority 2016; Petersen *et al*. 2021).

Updated information about the MRSA situation in European pigs should be available in the upcoming years, as the EU is preparing a new baseline survey for an EU-wide monitoring program on the prevalence of LA-MRSA in fattening pigs at slaughter (EURL-Antimicrobial Resistance 2022). This survey is currently scheduled for 2025. Some countries have previously studied the prevalence of LA-MRSA in pigs at slaughter: for example, in England the prevalence varied between abattoirs, and LA-MRSA was found in 43.8% of the pig batches (Smith *et al*. 2021). In the Netherlands, all batches were LA-MRSA positive, and the prevalence in pigs was as high as 83–99% (Dierikx *et al*. 2016).

The knowledge of the occurrence of LA-MRSA in animals other than pigs varies. Moreover, surveillance in other species is often targeted to MRSA in general. Within the EU, some countries have performed surveillance or monitoring of non-clinical cases of MRSA (EFSA & ECDC 2022). Between 2019–2020, this voluntary monitoring included turkeys, laying hens, broilers, wild boars, freshwater fish and fur animals; LA-MRSA CC398 was detected in Belgian fattening turkeys flocks (11.1%) and broiler flocks (3.3%; EFSA & ECDC 2022). Additionally, LA-MRSA was detected in 9.6% of Danish veal calf herds in 2019 (EFSA & ECDC 2022).

In previous studies, LA-MRSA occurrence in veal calves has been high: in the Netherlands, Germany and Italy, the prevalence has varied between

27.3–82% (Bos *et al*. 2012; Tenhagen *et al*. 2014; Zoppi *et al*. 2021). In dairy herds, strains belonging to LA-MRSA CC398 are the most common MRSA in Europe, but CC398 has also been found in Brazil, China and Israel (Schnitt & Tenhagen 2020). However, the prevalence of LA-MRSA in dairy cattle varies between countries and regions (Tenhagen *et al*. 2018; Schnitt & Tenhagen 2020).

A recent Danish study found that the prevalence of LA-MRSA CC398 in horses and horse farms was 3.5% and 6.8%, respectively (Islam *et al*. 2017). In older studies, 0.53–10.9% of the horses were CC398 carriers (Van den Eede *et al*. 2009; Van den Eede *et al*. 2012). The data on LA-MRSA in horses is otherwise scarce and mostly limited to reports on individual infection cases.

LA-MRSA contamination has been reported in meat products worldwide and in ready-to-eat products (DANMAP 2017; Bernier-Lachance *et al*. 2020; Gelbíčová *et al*. 2022). Various studies have also reported isolates belonging to CC398 in bulk tank milk, bovine quarter milk samples, milk products and sheep and goat milk (Cortimiglia *et al*. 2015; Caruso *et al*. 2016; Basanisi *et al*. 2017; Kadlec *et al*. 2019; Tegegne *et al*. 2019). Similar to animals, LA-MRSA in food and feed is not monitored routinely within the EU, and the detection methods have not been harmonised (EFSA & ECDC 2022). In 2019-2020, some member states reported LA-MRSA CC398 in pork, beef, broiler and sheep meat (EFSA & ECDC 2022).

2.3.2 Occurrence of LA-MRSA in Sweden

In Sweden, antimicrobial usage and resistance levels in humans and animals are generally lower than in most other countries (Swedres-Svarm 2021; Swedish Medical Products Agency 2022). The Swedish national legislation requires notification of all detected MRSA cases in animals (SJVFS 2021:10). The suspected MRSA isolates must also be confirmed with molecular typing methods. However, Sweden does not currently have active surveillance of LA-MRSA in pigs. The latest MRSA screening in pig herds was done in nucleus and multiplying herds in 2014, where all samples were found negative (Swedres-Svarm 2019). LA-MRSA was also not detected in Swedish herds in the 2008 EU baseline study (European Food Safety Authority 2009). As LA-MRSA is usually asymptomatic in pigs, the passive surveillance of MRSA is insufficient for monitoring the LA-MRSA carriage status in pig herds. Combined with the relatively old active surveillance data,

it is reasonable to conclude that the current status of LA-MRSA carriage in Swedish pigs is largely unknown.

Similar to animal cases, human MRSA infections are notifiable, according to the Swedish Communicable Diseases Act (SFS 2004:168). In humans, patients hospitalized in another country for at least 24 hours are routinely screened for MRSA (Swedish Medical Products Agency 2022), but LA-MRSA screening is not routinely done for people with possible occupational exposure to LA-MRSA. Between 1997 and 2016, only two human cases of LA-MRSA CC398 were detected in Sweden (Petersen *et al*. 2021). In 2021, ten cases of MRSA belonging to spa-types t011 and t034 were detected (Swedres-Swarm 2021). These spa-types are considered to be livestock-associated (Hetem *et al*. 2013).

Livestock-associated CC398 strains are among the most common MRSA isolates in Swedish horses (Swedres-Svarm 2020; Swedres-Svarm 2021). In 2021, a CC398 outbreak was detected in an equine hospital with a total of eight reported cases (Swedres-Svarm 2021). Otherwise, LA-MRSA CC398 has not been recently detected in MRSA samples from Swedish animals.

2.4 LA-MRSA in pigs

2.4.1 Factors affecting the spread of LA-MRSA in pigs

The acquisition of antimicrobial resistance due to antimicrobial exposure is a natural evolutionary response for microorganisms (Holmes *et al*. 2016). The spread of MRSA strains of human origin has been shown to be related to antimicrobial use (AMU; Guardabassi *et al*. 2013). Similarly, the spread of LA-MRSA has likely been promoted by the selective pressure of antimicrobials used in veterinary medicine (Broens *et al*. 2012a; Guardabassi *et al*. 2013). Studies have also shown that the use of medical in-feed zinc oxide promotes the selection of MRSA CC398 and increases its prevalence (Cavaco *et al*. 2011; Moodley *et al*. 2011; Slifierz *et al*. 2015). Zinc oxide has been used in pig feed to prevent post-weaning diarrhoea caused by enterotoxigenic *Escherichia coli* (Fairbrother *et al*. 2005). Zinc-resistant MRSA CC398 isolates have also been found in hospital patients in pig-farming dense areas, and concern has been raised that these strains may promote the co-selection of resistance genes in humans (van Alen *et al*. 2018). The use of zinc oxide as a veterinary medicinal product was banned

in the EU in 2022 (European Medicines Agency [EMA] 2017), but its use is allowed as a low-dose feed additive.

However, other factors—such as environmental contamination, the transmission of the bacteria through animal movement and the genetic traits of the bacteria—can also contribute to the spread of resistant bacteria. For LA-MRSA, the trade of pigs has been recognised as an important part of the between-herd spread (Broens *et al*. 2011c; Espinosa-Gongora *et al*. 2012; Grøntvedt *et al*. 2016; Sieber *et al*. 2018; Sørensen *et al*. 2018a; Pirolo *et al*. 2020). Human carriers and fomites have also been recognised as a possible route to introduce LA-MRSA to a herd (Broens *et al*. 2011b; Grøntvedt *et al*. 2016). It has been suggested that house- and stable flies might spread LA-MRSA between farms (Stelder *et al*. 2021). The spread may be further accelerated if LA-MRSA is introduced into a breeding herd which supplies pigs to many other herds (European Food Safety Authority 2010; Sieber *et al*. 2018). These findings are supported by a modelling study, which concluded that outbreaks in farms with a high outbound flow of pigs resulted in a significantly higher proportion of contaminated farms than outbreaks in farms with low outdegree of pigs (Bastard *et al*. 2020). Similarly, outbreaks in breeding farms lead to a higher proportion of contaminated farms than outbreaks in farms with only farrowing, post-weaning and finisher production (Bastard *et al*. 2020).

At the within-herd level, larger herd size has been associated with a higher prevalence of LA-MRSA (Alt *et al*. 2011; Broens *et al*. 2011a; Broens *et al*. 2011c; Fromm *et al*. 2014; Sørensen *et al*. 2018a; Golob *et al*. 2022). However, it has been suggested that the size of the herd may not be the primary reason for high LA-MRSA prevalence, but rather a combination of management practices or other risk factors that also are related to herd size that may, in turn, affect the prevalence (Broens *et al*. 2011a; Sørensen *et al*. 2018a). The production type of the herd has also been identified as a risk factor for LA-MRSA, where several studies have implicated that herds with sows have a lower risk of being positive (Broens *et al*. 2011a; Sørensen *et al*. 2018a). However, this conflicts with the European MRSA baseline study, which concluded that the risk of MRSA contamination increased when the number of breeding pigs increased in both breeding and production farms (European Food Safety Authority 2010).

The type of pig production (e.g. conventional, organic) may also impact the LA-MRSA status of pigs (van de Vijver *et al*. 2014; Kobusch *et al*. 2022).

In the study by van de Vijver *et al*. (2014), the proportion of LA-MRSA-positive herds was lower in organic production than in conventional production (21% and 70%, respectively). Similarly, Kobusch *et al*. (2022) reported that the within-herd prevalence was lower in organic herds than in conventional or alternative farms. The alternative farms referred to farms with straw bedding or outdoor climate, or both.

The LA-MRSA prevalence within pig herds has also been reported to vary during the growth of the pigs and different production phases (Weese *et al*. 2011; Broens *et al*. 2012a; Schmithausen *et al*. 2015b; Bangerter *et al*. 2016; Golob *et al*. 2022; Kobusch *et al*. 2022). The presence of an LA-MRSA-positive sow seems to be associated with a higher probability of colonisation in piglets (Weese *et al*. 2011; Golob *et al*. 2022), and the prevalence in sows increases during the time spent in the farrowing unit (Broens *et al*. 2012a). Studies have also reported that the LA-MRSA prevalence in piglets increases at weaning and decreases in the late weaning or finishing period (Weese *et al*. 2011; Broens *et al*. 2012a). The reasons behind these variations are largely unknown. It has been proposed that the piglets' immature immune system might affect their susceptibility to LA-MRSA (Broens *et al*. 2012a). However, a recent study could not find a connection between piglets' immune status and LA-MRSA colonisation (Rosen *et al*. 2020). The LA-MRSA colonisation dynamics in sows and gilts during breeding and gestation are also poorly understood. These mature pigs may be kept in groups that do not necessarily follow the all-in all-out principle and might act as a reservoir of LA-MRSA for piglet production.

2.4.2 Transmission within a pig farm

LA-MRSA has been recognised to spread through direct transmission between animals or humans (Broens *et al*. 2012a; Broens *et al*. 2012b) but also by indirect transmission through air or contaminated surfaces (Friese *et al*. 2012; Schmithausen *et al*. 2015b; Rosen *et al*. 2018). However, the relative importance of direct and indirect transmission on the colonisation of pigs is widely unknown. Still, a recent study found that LA-MRSA carriage on the skin of the pigs was more common than nasal carriage, which could indicate that the environment is important in maintaining LA-MRSA in the herd (Verkola *et al*. 2022). Moreover, LA-MRSA seems to contaminate the farm environment efficiently. In the study by Kobusch *et al*. (2020), weaned pigs and the surfaces within their reach were sampled over a 7-week

observation period. During this period, the LA-MRSA prevalence in the surface samples increased from 1.7% to 83.7%, while the prevalence in pigs increased from 71.7% to 100%.

Dust in the pig farm environment has been recognised as one of the potential sources of indirect LA-MRSA transmission among pigs (Feld *et al*. 2018; Golob *et al*. 2022). Rodents like rats and mice can harbour and spread pathogens (Firth *et al*. 2014). Therefore, it has also been suggested that they may transmit LA-MRSA in the pig farm environment (Pletinckx *et al*. 2013; Rothenburger *et al*. 2018). Additionally, there is some evidence that cats, dogs and goats may contribute to the spread of LA-MRSA in a farm environment (Pletinckx *et al*. 2013).

2.4.3 Survival of LA-MRSA in the pig farm environment

The survival of LA-MRSA has been studied by Feld *et al*. (2018), who collected dust from Danish pig farms. They found that the half-life in dust for LA-MRSA strains belonging to CC398 and CC30 lineages was approximately five days, with a 99.9% die-off rate of 66–72 days. According to the study, the survival of LA-MRSA has similar patterns as what was observed in the total *S. aureus* populations, which could indicate that LA-MRSA resembles other *S. aureus* in terms of survival. However, the study also found that LA-MRSA survival was longer in a farm where the isolates belonged to spa-type t571. This result could indicate differences in survival even between different LA-MRSA isolates, but the study could not exclude the possibility of other factors (e.g. the composition of the dust) affecting the results. As the surrounding conditions affect the survival of bacteria, this thesis includes a study of the survival of LA-MRSA CC398 on different surface materials commonly found in the pig farm environment.

2.4.4 LA-MRSA control in pig herds

While LA-MRSA control and eradication measures have been previously explored in several studies, there is still a lack of effective control and eradication measures that would not require culling the herd. Field studies have been used to assess the effectiveness of cleaning in LA-MRSA prevalence in pig herds. Based on these studies, cleaning and disinfection of pig farms where no animals are present can remove all viable LA-MRSA from the environment (Schmithausen *et al*. 2015a; Elstrøm *et al*. 2019). This finding is also supported by the study by Grøntvedt *et al*. (2016), which

concluded that using an MRSA-negative pig supplier who follows an all-in all-out routine, combined with good cleaning and disinfection procedures, may be effective in preventing MRSA establishment to finishing farms.

However, in the study by Kobusch *et al*. (2020), routine cleaning and disinfection measures were insufficient to fully remove LA-MRSA from the environment when the prevalence in pigs and the farm environment was high. According to the study, cleaning and disinfection substantially reduced the proportion of positive LA-MRSA samples in both easy- and difficult-to-clean areas (from 73.9% to 3.3% and 70.0% to 2.1%, respectively). However, the persons responsible for cleaning were unaware that the cleaning was being measured. Therefore it may be possible that the cleaning results could be improved. Kobusch *et al*. (2020) also argued that areas within the animals' reach are generally more contaminated than areas out of reach. However, it is noteworthy that the number of positive samples outside the pigs' reach was high even before cleaning and disinfection (65.7%), although it was less than what was observed within reach (79.6%). These results indicate that LA-MRSA spreads easily to areas without direct contact with pigs.

Other approaches to remove LA-MRSA from the farm environment and pigs have also been explored in studies. In one study, spraying the environment twice a week with a biocide (BioVir) during the suckling period could not reduce the MRSA occurrence in the environment or the animals (Bækbo *et al*. 2018). Similarly, washing sows with water and a cleaning product in the gestation or farrowing barns had no significant effect on the MRSA status of the sows' skins or nasal cavities (Verhegghe *et al*. 2013).

Several modelling studies have also explored the effectiveness of various control measures (Sørensen *et al*. 2018b; Schulz *et al*. 2019a; Schulz *et al*. 2019b; Bastard *et al*. 2020). In summary, Sørensen *et al*. (2018b) concluded that improving biosecurity only marginally reduced the LA-MRSA in the model herd. The same applied to reducing the number of pigs per section and mixing of pigs.

Sørensen *et al*. (2018b) and Schulz *et al*. (2019a) have also modelled the effect of antimicrobial use by changing the disease transmission rates in the models. At the within-herd level, reducing the LA-MRSA transmission rates to 30% or less from the original eradicated the bacteria from the herd (Sørensen *et al*. 2018b). According to Schulz *et al*. (2019a), reducing the proportion of pig herds using high-risk antimicrobials reduced the between-

herd spread of LA-MRSA. However, while the reduced AMU seems to impact the LA-MRSA transmission rates (Broens *et al*. 2012a), little is known about the magnitude of this change. Therefore, it is challenging to evaluate which level of effect of reduced AMU would be closest to reality and how big the reduction would be in the Swedish context. It can also be speculated that reducing the use of antimicrobials is likely more effective in countries or farms where the usage is high. In addition to abandoning the prophylactic use of antimicrobials, reducing the AMU in a farm requires minimizing the overall incidence of diseases, which requires changes in herd management practices (Speksnijder & Wagenaar 2018). This change of management could on its own also impact the LA-MRSA prevalence. However, reducing the AMU does not seem to be efficient in short-time reduction of LA-MRSA prevalence in pig herds where the prevalence is high: a Dutch study found that reducing AMU in pig farms by 50% did not lower the prevalence in pigs at slaughter in five years (Dierikx *et al*. 2016), and the LA-MRSA prevalence remains high in Danish herds regardless of the national antimicrobial reduction programmes (DANMAP 2021).

At the between-herd level, Schulz *et al*. (2019a); Schulz *et al*. (2019b) concluded that reducing indirect transmission (e.g. transmission through human movements), restricting the movement of LA-MRSA positive pigs and eradicating some of the LA-MRSA positive herds caused small reductions in the between-herd spread when the interventions were used as a single control measure. In their model, the herd eradications were performed risk-based or by randomly culling 5–7.5% of the positive herds. Combining several measures led to larger reductions but could not fully eradicate LA-MRSA (Schulz *et al*. 2019a). The modelling study by Bastard *et al*. (2020) concluded that if LA-MRSA was newly introduced to a farm network, targeting surveillance to the herds with the largest number of incoming pigs led to the lowest proportion of contaminated herds at the time of LA-MRSA detection. Additionally, if LA-MRSA has become established in the network and control measures can be introduced only to a limited number of herds, targeting herds with the highest outbound pig movements has the biggest impact on the between-herd LA-MRSA prevalence (Bastard *et al*. 2020).

2.5 LA-MRSA in humans

While LA-MRSA CC398 colonisation is usually asymptomatic, it is also considered to be able to cause similar infections as other MRSA strains (Cuny *et al*. 2015b; Becker *et al*. 2017; EFSA & ECDC 2022). The range of possible human infections is wide, and even fatal cases have been reported (Berning *et al*. 2015; Koyama *et al*. 2015). These infections include, for example, various skin, soft-tissue, wound and urinary tract infections, conjunctivitis, bone and joint infections, mastitis, pneumonia and blood-stream related infections (e.g. bacteraemia, septicaemia; Verkade & Kluytmans 2014; Becker *et al*. 2017; Slott Jensen *et al*. 2020; Coombs *et al*. 2022). In Denmark, where the LA-MRSA prevalence in pig herds is high, LA-MRSA CC398 caused 16% of human MRSA infections in 2016 (Sieber *et al*. 2019). In 2021, six of the 40 Danish human MRSA bacteraemia cases (=15%) were caused by LA-MRSA (DANMAP 2022).

Meta-analyses have confirmed that people with occupational exposure to livestock (especially pigs) have a significantly increased risk of becoming colonised with LA-MRSA (Chen & Wu 2021; Dong *et al*. 2021). While LA-MRSA colonisation is more common in people with livestock contact, spillover to the rest of the community occurs, especially in areas with a high density of pig farming (Feingold *et al*. 2012; Larsen *et al*. 2015; Sieber *et al*. 2019). In Denmark, the majority of healthcare-associated CC398 infections (64.2% in 2007–2016) were associated with livestock origin, and the transmission had likely occurred through between-human or environmental transmission chains (Sieber *et al*. 2019).

2.5.1 Transmission between pigs and humans

According to the study by Grøntvedt *et al*. (2016), humans are a possible route of introducing LA-MRSA to pig herds, as the bacteria were detected in Norwegian herds even in the absence of imported pigs. However, detailed information on the LA-MRSA transmission from humans to pigs and humans' role in spreading the bacteria between the pigs is largely unknown.

For transmission from pigs to humans, the concentration of LA-MRSA in pig farm air correlates with the risk of LA-MRSA nasal carriage (Feld *et al*. 2018). Several studies have explored possible routes for the spread of LA-MRSA from farms to people without livestock. While *S. aureus* can transmit outside the farm through dust from farm ventilation (Gibbs *et al*. 2004), the MRSA concentrations are reduced quickly by distance (Angen *et al*. 2021).

LA-MRSA spread outside the farm environment also depends on the wind direction and weather conditions (Schulz *et al*. 2012; Angen *et al*. 2021). Therefore, airborne transmission from farms has not been considered an important route for human MRSA exposure (Angen *et al*. 2021). LA-MRSA survival in liquid manure varies based on the outside temperature (Astrup *et al*. 2021). While the 90% decimation time (T90) was the longest in low temperatures (at least 32 days at 5°C), the decimation time was reduced to 15 days at 15°C (Astrup *et al*. 2021). Therefore, spreading manure to fields can predispose to LA-MRSA but is likely affected by external conditions.

2.5.2 Occupational exposure

Studies have shown a strong association between LA-MRSA carriage and LA-MRSA concentration in the farm air (Bos *et al*. 2016; Angen *et al*. 2017). However, nasal LA-MRSA carriage seems to be dependent on the frequency and time spent in the pig farm (Graveland *et al*. 2011; Bos *et al*. 2016), and it has been suggested that people with long-term occupational exposure can be persistently colonised by LA-MRSA (Köck *et al*. 2012; van Cleef *et al*. 2015). While it can be debated if the suggested persistent colonisation is rather a repeated re-colonisation, the study by Köck *et al*. (2012) shows that LA-MRSA colonisation can persist in pig farmers for at least two weeks after taking a leave from the farm.

A short-time exposure to LA-MRSA-positive pig farms is often sufficient to make visitors LA-MRSA carriers, but this carrier state usually resolves within a few days (van Cleef *et al*. 2011; Angen *et al*. 2017). On the contrary, the study by Bosch *et al*. (2015) reported that veterinarians and their household members carried LA-MRSA for prolonged periods of up to 14 months. However, it has been proposed that persistent LA-MRSA colonisation in pig veterinarians depends on individual host factors (Sun *et al*. 2017). As with other occupations working with pigs, veterinarians visiting pig farms also have an increased risk of LA-MRSA colonisation (Cuny *et al*. 2009; Garcia-Graells *et al*. 2012; Walter *et al*. 2016). Similarly, pig truck drivers have been shown to acquire LA-MRSA during their workday (Ingham *et al*. 2021). Exposure to LA-MRSA-positive pigs at slaughter may pose an occupational risk to abattoir personnel, especially if the prevalence in pigs is high (Dierikx *et al*. 2016).

While the carriage or colonisation with LA-MRSA seems common for those working with livestock, the incidence of occupational LA-MRSA

infections is unknown (Goerge *et al*. 2017). For pig-farm visitors and farmers' household members, using facemasks significantly reduced the risk of nasal MRSA contamination, but masks did not fully protect from MRSA (van Cleef *et al*. 2015; Angen *et al*. 2018). However, pig farmers are usually in long-term contact with pigs and at higher risk for LA-MRSA colonisation; it is currently unknown how well masks protect against long-term exposure.

2.5.3 Transmission to people without livestock contact

Household members of pig farmers can become LA-MRSA carriers (Cuny *et al*. 2009; Bosch *et al*. 2015; van Cleef *et al*. 2015), and the carriage status is associated with the presence of an LA-MRSA-positive pig farmer (van Cleef *et al*. 2015). In the Dutch study by van Cleef *et al*. (2015), LA-MRSA was present in 26% of the household members that worked less than 20 hours a week on the farm. This proportion was higher than what was observed in a German study, where 4.3% of family members were LA-MRSA carriers (Cuny *et al*. 2009). However, this value only included people without exposure to pigs. Van Cleef *et al*. (2011) also identified that 4% of the household members carried LA-MRSA persistently.

LA-MRSA has also been transmitted to the rest of the community without livestock contact (Wulf *et al*. 2008; Verkade *et al*. 2012; Bosch *et al*. 2016). Studies have identified living in rural and livestock-dense areas as risk factors for LA-MRSA carriage (Feingold *et al*. 2012; van Rijen *et al*. 2014). However, the results on the effect of distance to farms as a risk factor are conflicting. In the Dutch study by Zomer *et al*. (2017), living near a livestock farm increased the risk of LA-MRSA carriage, while a Danish study suggested that the distance to pig farms in pig-farming-dense areas does not increase the risk of MRSA CC398 infections in humans without livestock contact (Anker *et al*. 2018).

It has been estimated that the risk of between-human transmission is 4.4 times lower for LA-MRSA than other MRSA (Hetem *et al*. 2013). Regardless, an overall increasing trend in human LA-MRSA CC398 cases was observed in Denmark between 2007-2016 (DANMAP 2017). The number of LA-MRSA human infections decreased during the COVID pandemic (DANMAP 2021; DANMAP 2022), which aligns with what has been observed as a consequence of social distancing with other infectious diseases (Yeoh *et al*. 2020; Komori *et al*. 2022). Before the pandemic, Danish officials suspected that the LA-MRSA spread from pig farms to the

community was mainly due to humans carrying MRSA from the farms (DANMAP 2017). The statistics of LA-MRSA infection during the COVID years support this view.

While most human LA-MRSA cases identified in healthcare are spillover from pig farms, LA-MRSA has caused nosocomial outbreaks in hospitals and nursing homes in Netherlands and Denmark (Verkade *et al*. 2012; Nielsen *et al*. 2016; Reynaga *et al*. 2018; Sieber *et al*. 2019; Slott Jensen *et al*. 2020). A few of these cases have resulted in fatal septicaemia (Nielsen *et al*. 2016).

2.6 LA-MRSA in food

While LA-MRSA has been found in meat products, LA-MRSA-contaminated meat has not been considered a source of human infections (Statens Serum Institut 2014; Bal *et al*. 2016). Similarly, occupational exposure to handling meat or raw meat products has not been associated with LA-MRSA colonisation (de Jonge *et al*. 2010; Cuny *et al*. 2019). However, an experimental study has demonstrated that LA-MRSA CC398 can transmit to farmed minks through contaminated feed (Fertner *et al*. 2019a).

So far, LA-MRSA CC398 strains isolated from meat have not been linked to enterotoxins or the presence of genes that code for enterotoxins and are, therefore, unlikely to cause food poisoning (Sergelidis & Angelidis 2017). According to Bal *et al*. (2016), handling meat might pose a low risk for LA-MRSA colonisation in humans, but they did not consider meat as an important source of LA-MRSA. However, a Danish study has found a hybrid LA-MRSA CC9/CC398 in urban living people (Larsen *et al*. 2016). As similar isolates have been previously found in livestock and retail food in Europe, it was hypothesised that the origin of this strain was in contaminated poultry meat or between-human transmission (Larsen *et al*. 2016).

2.7 LA-MRSA in other livestock and horses

As described in the sections ”Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA)” and ”Occurrence of LA-MRSA”, LA-MRSA has been detected in several livestock species and horses. This section focuses on veal calves, dairy cattle, poultry and horses, as they are most commonly mentioned in the literature.

Pigs have been suggested to be a potential LA-MRSA reservoir for veal and dairy farms (Hansen *et al*. 2019). The prevalence of LA-MRSA in veal calves is high in some countries (see section ”Occurrence of LA-MRSA”). In the Netherlands, veal calves are gathered to fattening farms from several countries (Bos *et al*. 2012), which poses a risk of disease spread. In addition to the risk of between-herd spread, group treatment with antimicrobials, farm hygiene, farm size and the age of the calves have been identified as risk factors for LA-MRSA carriage in veal calves (Graveland *et al*. 2010; Bos *et al*. 2012; Gravel *et al*. 2012). However, differences in management practices at different age phases might impact the age-related differences in LA-MRSA carriage (Bos *et al*. 2012).

In dairy farms, LA-MRSA has been found in bulk and mastitis milk samples as well as in nasal and udder swabs (Schnitt & Tenhagen 2020). Studies have found that LA-MRSA CC398 may cause bovine mastitis (Silva *et al*. 2014), and it can spread widely between animals or with milking equipment (Locatelli *et al*. 2016; Lienen *et al*. 2021). In Germany, the detected MRSA isolates in dairy cattle presented a broad range of virulence factors that might be connected to mastitis, but virulence factors associated with human infections were not found (Lienen *et al*. 2021). Based on the review by Schnitt and Tenhagen (2020), LA-MRSA CC398 seems to transmit between pigs and cows. Young calves have also been proposed as a potential LA-MRSA reservoir in dairy cattle (Schnitt *et al*. 2020).

Transmission and risk factors for poultry are widely unknown. The LA-MRSA status of broilers seems to vary between batches (Pletinckx *et al*. 2011; Friese *et al*. 2013). However, Pletinckx *et al*. (2011) have suggested that broilers might be less susceptible to LA-MRSA CC398. Their study found major differences in the prevalence of LA-MRSA between broiler flocks and pigs on the same farm (0–28% and 82–92%, respectively). However, these differences could have been caused by other factors, such as the differences in production time (Pletinckx *et al*. 2011). According to Friese *et al*. (2013), LA-MRSA can spread to the surroundings outside broiler and turkey farms.

Isolates belonging to LA-MRSA CC398 are among the most prevalent MRSA strains in horses (Van den Eede *et al*. 2009; Islam *et al*. 2017; Swedres-Svarm 2021). Notably, horses often carry the so-called horse-adapted clone of MRSA CC398, which are IEC-positive (Islam *et al*. 2017). However, the pig-adapted LA-MRSA CC398 and other MRSA such as

CC130 have been detected in horses (Islam *et al*. 2017). Horses seem capable of transmitting the bacteria to humans, and the Danish study found isolates from equine veterinarians that were closely related to the horse-adapted strains (Islam *et al*. 2017).

2.8 Disease modelling

Disease models are a type of mathematical model that can be used to gain information on infectious disease dynamics and predict outcomes of different control strategies. Disease models can also support decision-making when experimental studies are not possible for practical, ethical or economic reasons. They have also been used for advising on disease preparedness (Kirkeby *et al*. 2021). While all models are approximations of reality and cannot perfectly represent the modelled phenomenon, they can be valuable tools for understanding complex systems and events.

The usefulness of a model depends on its purpose—a good model should have the appropriate balance of accuracy, transparency and flexibility for the particular problem while being as simple as possible (Keeling & Rohani 2008). For predicting the effect of interventions, it is necessary to emphasise the model's accuracy to reflect the real-world phenomenon accurately (Keeling & Rohani 2008; Mancy *et al*. 2017). When models are used to guide decision-making, they should have a biologically sound basis and include known complexities to be applicable in practice (Keeling & Rohani 2008; Thrusfield 2018). The other side of the coin is that with increased accuracy, the detailed model can be harder to understand (transparency), more difficult to adapt to new situations and require a lot of computational power (flexibility; Keeling & Rohani 2008). However, the number of approximations and simplifications needed in the model is often driven by data availability: lack of data requires more approximations, which lowers the accuracy of the model output (Kirkeby *et al*. 2021).

2.8.1 Model types

Disease models can be divided into several different types. However, the differentiation of the model types can be unclear (Vynnycky & White 2010), and the terms used vary between authors. This subchapter is not exhaustive of all different model types but is limited to those most relevant in the scope of the thesis.

This thesis presents a stochastic continuous-time compartment model to study the spread of LA-MRSA and possible control measures at a herd level. Stochastic models use various methods for approximating the probability or randomness that occurs in real-world situations (Keeling & Rohani 2008). In contrast, in deterministic models the input parameters are fixed, and these models do not consider random variation in the parameters (Vynnycky & White 2010). Because of this, every time the model is run, it results in the same output (Keeling & Rohani 2008; Vynnycky & White 2010). Unlike deterministic models, stochastic models require running a large number of trajectories[1] to enable a more precise estimation of the results (e.g. prevalence or confidence intervals). It also reduces the impact of random fluctuations as the number of simulations increases (Vynnycky & White 2010).

Models can also be compartment or individual-based models. In compartment models, the population is divided into subgroups where the individuals are tracked as a collective group (Vynnycky & White 2010). Individuals in these subgroups move between different states, such as susceptible, exposed, infected and recovered, and only the number of individuals in each state is recorded. In contrast, individual-based models track the state of each animal (e.g. an individual pig) separately (Keeling & Rohani 2008). The advantage of individual-based models is that each animal can be tracked throughout the system and assigned individual properties (Kirkeby *et al*. 2021). However, due to the complexity of individual-based models, they tend to be computationally demanding (Kirkeby *et al*. 2021).

Compartment models can be further divided into discrete or continuous time models. The former refers to models where chance determines the number of secondary cases from the infected individuals from the previous generation (Vynnycky & White 2010). In discrete-time compartment models, the length of the time steps between the transitions is fixed (Vynnycky & White 2010). This approach is also used in individual-based models (Vynnycky & White 2010). In continuous time compartment models, chance determines when the next event (e.g. susceptible individual becomes infected) occurs (Vynnycky & White 2010).

[1] A trajectory referes to a single random realisation of the simulated model output.

2.8.2 Stages of disease model building

Building a model is a multiple-step and iterative process; a diagram of the process is presented in Figure 1. Stakeholders, such as experts of the disease in question and end-users (e.g., policymakers and farmer representatives), should be included in the process throughout the stages of model development (Reeves *et al*. 2011; Kirkeby *et al*. 2021). This is important for keeping the model and its results relevant in practice.

Good planning is the essential foundation for a model. It should begin by formulating the study question and defining the level of detail of information the model should produce (Taylor 2003; Mancy *et al*. 2017; Kirkeby *et al*. 2021). For example, the study question could be: "Which herds should be targeted by disease surveillance and subsequent actions to get the biggest reduction in herd prevalence?" After the initial phase, one should determine the unit of interest, such as a single animal or a single herd (Kirkeby *et al*. 2021). Then existing data and knowledge relevant to the disease are gathered, analysed and translated into a model framework (Taylor 2003; Thrusfield 2018; Kirkeby *et al*. 2021). The availability of this information determines the level of accuracy the model can produce and what kind of features can be included in the model (Kirkeby *et al*. 2021). There are several methods for the estimation of the model parameters (parameterisation) from existing data, such as maximum likelihood estimation and approximate Bayesian computation (ABC), each with advantages and disadvantages (Toni *et al*. 2009; Heesterbeek *et al*. 2015; Roosa & Chowell 2019). In this thesis we have used the ABC method, which allows model parameterisation without deriving the analytical likelihood function, which may be difficult to compute for complex models (Sunnåker *et al*. 2013; Lintusaari *et al*. 2017).

The background information is transferred into a model by first choosing the appropriate programming language and framework (Taylor 2003; Kirkeby *et al*. 2021). The knowledge of the disease and desired unit of interest are then transformed into a model of the population structure and consequently modelling the chosen disease states (e.g. susceptible, exposed, infected and recovered) and disease transmission (Mancy *et al*. 2017; Kirkeby *et al*. 2021). Depending on the complexity of the model, this state is often the most time-consuming part of the model building. The model functionality and reliability should be verified throughout the programming process by generating appropriate checks (Taylor 2003; Reeves *et al*. 2011; Kirkeby *et al*. 2021).

A model should also go through validation, which involves evaluating if the model is representative of the biological system it aims to model (Taylor 2003; Reeves *et al*. 2011; Thrusfield 2018). Valid models should make sense biologically and fit for the use they are designed for (Taylor 2003; Reeves *et al*. 2011; Thrusfield 2018). Several model validation techniques exist and different model types may require different validation techniques (Kopec *et al*. 2010). However, model validation techniques are usually more or less subjective evaluations of the model and there is no universal test for model validity (Taylor 2003; Reeves *et al*. 2011).

In addition to the steps above, the modelling process should include a sensitivity analysis (Thrusfield 2018; Kirkeby *et al*. 2021). Sensitivity analyses assess how the model output is affected by the model input parameters (Taylor *et al*. 2011; Thrusfield 2018; Kirkeby *et al*. 2021). There are several different methods for performing sensitivity analysis, such as one-at-at-time perturbations and algebraic "no box" sensitivity analysis (Kopec *et al*. 2010; Norton 2015).

When the model is deemed appropriate, the model simulations are run and the outputs are analysed and presented (Kirkeby *et al*. 2021). The number of trajectories needed for the simulations and the produced model output depends on the model type. For stochastic models, it is necessary to ensure that enough trajectories are run to stabilise the model output variance (Kirkeby *et al*. 2021). Because of the variation caused by stochasticity, reporting output from stochastic models also requires presenting the output distributions (Kirkeby *et al*. 2021). Finally, if the final model and its results are appropriate for its purpose, it can be combined with expert knowledge and used in decision-making (Taylor 2003; Thrusfield 2018).

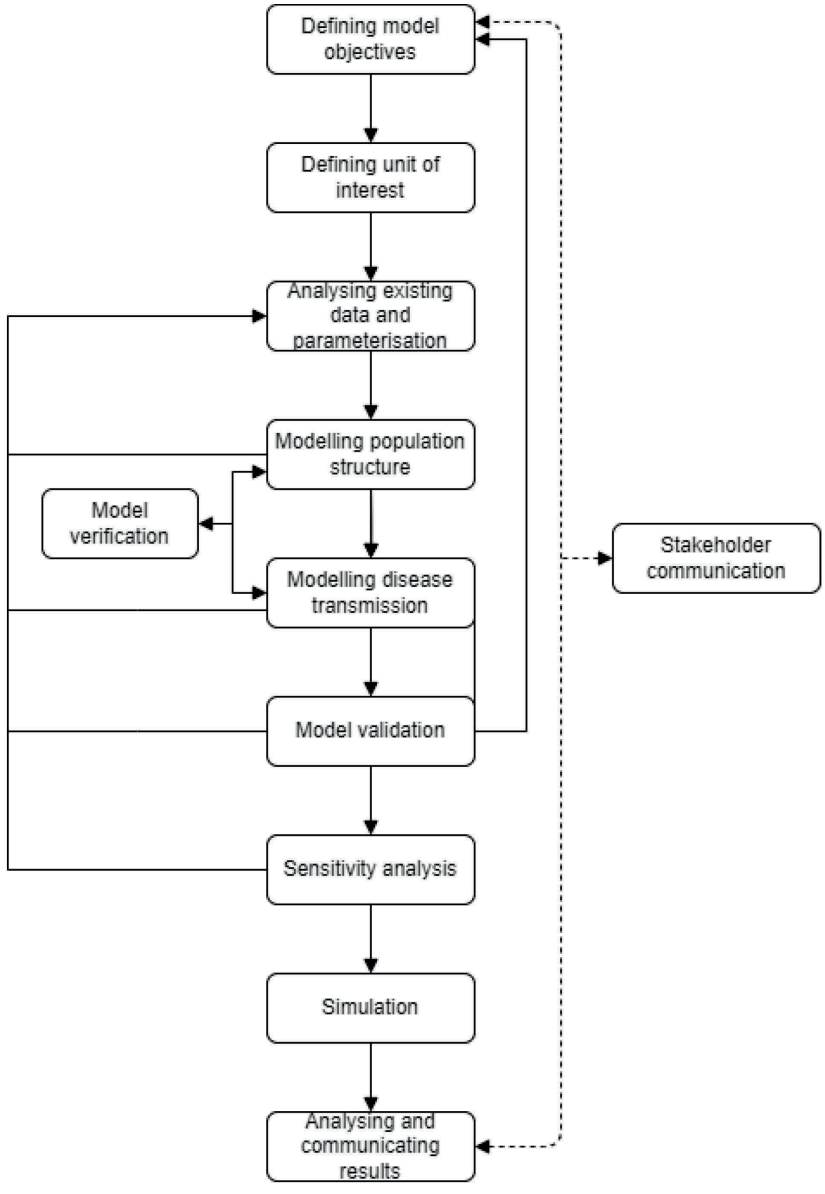

Figure 1. Different stages of building a disease model. Adapted from Taylor (2003), Reeves *et al.* (2011) and Kirkeby *et al.* (2021).

3. Aims of the thesis

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is a risk to human and animal health. The general aim of this thesis was to study the spread of LA-MRSA and assess possible control strategies which would help or limit the spread of the bacteria in a pig farm environment. The studies were aimed to provide reliable scientific input to decision-making when control strategies against LA-MRSA are planned in Sweden and highlight the current knowledge gaps.

The specific aims for studies I-IV were:

I. To develop a robust pig herd model to study the spread of LA-MRSA in a pig herd. The model was aimed to be representative of a Swedish farrow-to-finish farm.
II. To assess pig farmers' and veterinarians' views on feasible biosecurity and disease control measures in the pig farm environment.
III. To study the effect of different control measures on the within-herd LA-MRSA prevalence by utilising the pig herd model developed in study I and stakeholder opinions obtained from study II.
IV. To improve the knowledge of LA-MRSA persistence in the pig farm environment by studying the survival of LA-MRSA on different surface materials. The results of the study were also aimed at improving the input parameters of future LA-MRSA modelling studies.

4. Materials and methods

This chapter describes an overview of the material and methods used in this thesis. The in-depth descriptions are presented in the respective papers.

4.1 Data analysis and visualisation

Apart from study II, all data analysis was performed using the R programming environment (R Core Team 2022). The result graphs were produced using the ggplot2 package for R (Wickham 2016). In the LA-MRSA survival study (study IV), the bacterial decay rates were computed using non-linear least squares regression (NLS).

In the modelling studies (study I and III), the SimInf package (Widgren *et al.* 2019) was used to build the disease spread models, run the model simulations and compute the disease prevalence. SimInf is a framework for spatio-temporal disease spread modelling, where the disease spread between metapopulations is achieved with an event-based approach (Widgren *et al.* 2019). The events—such as births, deaths and disease transmission—are implemented with continuous-time Markov chains (CTMC) using the Gillespie stochastic simulation algorithm (Gillespie 1977; Widgren *et al.* 2019).

4.2 Modelling the spread of LA-MRSA in a pig herd (study I)

4.2.1 Pig herd model

In study I, a model of a farrow-to-finish herd with 500 sows in production was designed and implemented. The model structure was built to be

representative of a Swedish pig herd: the pig movements within the model (e.g. time spent in different production phases and pig mixing practices) were based on production statistics provided by the Swedish Farm and Animal Health Organisation (Farm and Animal Health 2019; Farm and Animal Health 2020b; Farm and Animal Health 2020a) and on expert opinion about common herd management practices in Swedish pig herds. A conceptual presentation of the pig herd structure and animal flow is presented in Figure 2.

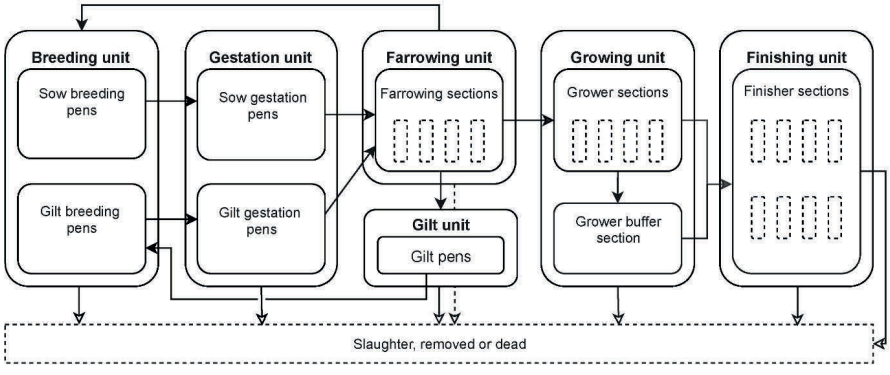

Figure 2. Conceptual presentation of the pig herd structure and movements between different units in the model.

The smallest unit in the model was a pen and each pen was its own metapopulation. The model was structured as an SIS_E compartment model, where the pigs moved between susceptible (S) and infected (I) categories. The infected category represented pigs that were carriers/colonised by LA-MRSA without making assumptions about the possible clinical disease status of the pig. If exposed, the pigs could be recolonised immediately after returning to the susceptible category. The disease spread in the model was environmentally mediated (E), meaning that LA-MRSA was transmitted through the contaminated farm environment. In this environmentally mediated disease spread approach, each infected pig was shedding to the environment, which increased the contamination of the farm environment, while the decay of the bacteria simultaneously reduced the contamination. This approach was necessary to allow LA-MRSA to persist in the herd and the pens between batches of pigs, which is consistent with previous studies. During the trial, model simulations using only direct transmission without

environmental accumulation caused LA-MRSA to die out from the herd (Appendix I).

In study I, the pig herd model was also used to simulate the spread of LA-MRSA within the herd and assess how the mixing of pigs in the farrowing unit (cross-fostering) and finishing unit affected the LA-MRSA prevalence in these units. Mixing of finishing pigs when the pigs enter the finishing unit and cross-fostering young piglets to even the litters are common practices in Swedish pig herds. The proportions of animals to mix were based on discussions with Swedish pig experts. The different mixing practices were:

- Baseline mixing where 10% of the piglets in each farrowing pen were randomly mixed one day after birth and all finishing pigs (100%) were mixed when moved from the growing to the finishing unit.
- Cross-fostering 100% of the piglets one day after birth.
- Cross-fostering 100% of the piglets two days after birth
- Reducing the mixing of finishing pigs to 0% when the pigs were moved from growing to finishing unit.

All pigs in the herd were infected at the same time point after the model burn-in period. This approach was taken to find a steady state of infection in the herd.

4.2.2 Parameterisation of transmission rates

To obtain transmission parameters for LA-MRSA, in studies I and III, Approximate Bayesian Computation (ABC; Toni *et al.* 2009) was used to parameterise the model against previously published prevalences (Broens *et al.* 2012a). As the name implies, approximate Bayesian computation is based on Bayesian methods. According to Toni *et al.* (2009) and Sunnåker (2013), in ABC, particles (candidate parameters) that are sampled from the prior distribution are simulated within the model to obtain a simulated dataset. This simulated dataset is compared to the observed data to calculate the distance of the simulated dataset from what is expected. The particle is accepted as part of the posterior distribution if this distance is within a pre-defined tolerance value. The tolerance gradually evolves between consequent data generation steps towards the target posterior, and the

outcome of the process is parameters approximately distributed according to the desired posterior distribution.

In study I, the target transmission rates obtained from Broens *et al.* (2012a) were divided into three different target prevalence sets based on their magnitude (low, medium and high parameter set). This was done to address the high variation in LA-MRSA prevalence between the study herds that might have been caused by, for example, different phases of disease spread or management practices. The parameterisation with ABC was run for all three target prevalence sets. In study III, only the medium target prevalence set was used. This limitation was necessary to decrease the number of models to a manageable level for one study.

4.2.3 Model validation

The disease spread models used in studies I and III were validated by evaluating the model structure and animal flow using face and trace validity (Sørensen 1990). In practice, this was done by assessing the model animal flow and comparing the model output to the target Swedish pig production statistics (mean number of sows and gilts in production, mean number of produced finishing pigs, proportion of gilts of all breeding pigs). In study I, the goodness of fit of the parameterised transmission rates were evaluated by visually comparing the model-predicted prevalences to the target prevalences obtained from the literature. The model fit indicators obtained from parameterising the transmission rates with approximate Bayesian computation were reported in both studies I and III. The operational validity of the models was also assessed by including unit tests of the model code.

The model sensitivity to the input transmission rates was assessed alongside the parameterisation progress, where the posterior densities of the parameterised transmission rates for the different target parameter sets were compared and visualised.

4.3 Pig farmers' and veterinarians' views on disease control measures (study II)

4.3.1 Participant selection

In study II, two focus group discussions were organised to assess pig farmers' and pig veterinarians' views on practically feasible farm-level

control measures against LA-MRSA. The discussions were organised before study III to obtain suggestions for practically possible control measures for the modelling study. The focus groups were organised separately for the pig farmers and the veterinarians. The veterinary participants worked in the Farm and Animal Health advisory services and the pig farmers were board members of the Swedish Pig Farmers Association. Invitations were sent to both groups and the final participant selection was based on the availability of participants.

4.3.2 Group discussion protocol

Due to pandemic restrictions, the focus group discussions were organised digitally by using Zoom software program (Zoom Video Communications, Inc. San José, USA). Focus groups are guided discussions that allow participants to elaborate freely on a chosen topic. The discussions were led according to a predetermined discussion guide by the study's main author (sociologist, PhD). After the discussions, the recordings of the occasions were transcribed and analysed.

4.4 Modelling control measures against LA-MRSA (study III)

Study III used the model from study I to investigate the effect of various herd-level control measures on the within-herd LA-MRSA prevalence. This model was extended to include a between-pen spread of LA-MRSA and the distribution of recovery time was changed from exponential to Erlang distributed. The former was implemented to allow studying the effects of improved biosecurity and the latter to obtain a distribution of recovery times that is biologically more plausible. Due to the changes in the model structure, the transmission rates were re-parameterised using the approximate Bayesian method described in study I.

The control measures used in this study were chosen based on the focus group discussions conducted in study II and on the authors' evaluation of practically feasible methods to implement within the limits of the model framework. These control measures were modelled as the sole control measure or in combination during either the outbreak or endemic phase of the disease spread.

The modelled control measures were:

- Improved herd biosecurity
- Disease surveillance (testing sows or gilts, or both, for LA-MRSA and replacing the test-positive pigs with susceptible pigs)
- Cleaning the environment
- Reducing the mixing of pigs in farrowing and finishing unit
- Extending the period that pens were kept empty before a new batch of animals

After the model burn-in period, the disease was seeded into the herd by infecting 20% of the growing gilts in the gilt unit. The number of infected gilts corresponded to approximately 0.4% of all pigs in the herd. In the simulations, 10 000 trajectories were run for each control measure or control measure combination and the within-herd prevalence was recorded. The probability of LA-MRSA elimination and the mean time to elimination were calculated based on the data.

4.5 The survival of LA-MRSA CC398 on surface materials (study IV)

In study IV, the survival of two LA-MRSA CC398 strains was studied on four surface materials: polypropylene plastic, stainless steel and two different types of concrete. These strains originated from two Danish field studies: the SPACE 95 project (Ministry of Food 2022) and the BioVir project (Bækbo *et al*. 2018). Both of the LA-MRSA CC398 strains belonged to the spa-type t034.

The survival of the LA-MRSA strains was assessed by extracting the bacteria from the samples and using the viable count method. The viable counts were calculated as triplicates on each surface material. The counts were repeated over a period of time, which was weekly for a total of 14 weeks for the plastic and steel samples, and weekly or every other week for a total of 5 or 11 weeks for the different concrete materials. The serial dilutions for the viable counts were plated on selective Oxoid Brilliance MRSA 2 agar (PO5310A, Thermo Fisher Scientific Inc.) and 5% bovine blood agar (B341960; National Veterinary Institute, Uppsala, Sweden). The selective plates were used to reduce the risk of contamination and blood agar plates were used to monitor the effect of the selective plates on the LA-MRSA.

At the end of the study, suspected MRSA colonies from the beginning and end of the study for each strain and material combination were confirmed to be MRSA by using a qPCR assay that detects the *mecA*, *mecC*, *nuc* and *lukS*-PV genes (Pichon *et al*. 2012).

Figure 3. Running the survival study in the laboratory. Left picture: agar plates being prepared for viable counts. Right picture: LA-MRSA CC398 bacteria are loosened from samples by shaking with sterile glass beads.

5. Results

This chapter describes the general findings of studies I-IV. For more detailed information, please refer to the corresponding papers.

5.1 Modelling the spread of LA-MRSA (study I)

Based on the results of study I, the environmentally-mediated transmission allowed LA-MRSA to persist in the herd without making assumptions about persistent shedders.

5.1.1 Model validation

The model presented was considered a reasonable approximation of a Swedish farrow-to-finish pig farm based on the comparison of the model production data and target production values. In the model, the proportion of gilts of all breeding pigs was 23% and the corresponding value in production statistics was on average 24.2% (Farm and Animal Health 2020b). The target number of sows and gilts in production was 500, which on average should produce 133 50 pigs annually (Farm and Animal Health 2020a). The corresponding model-predicted mean number of sows and gilts in production and produced finishing pigs per year are presented in Figure 4.

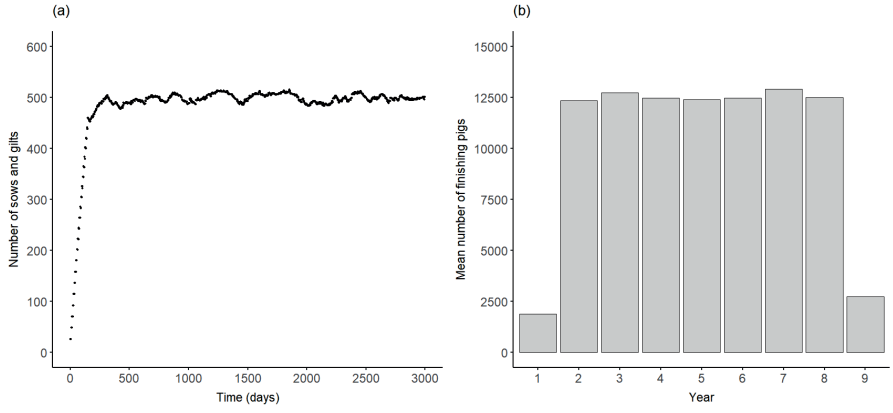

Figure 4. The model-predicted mean number of sows and gilts in the pig herd over time (a) and the mean number of finishing pigs slaughtered per year (b). The figure includes the model burn-in period where the pig population grows towards the target values. The year-9 observation of the number of finishing pigs slaughtered had only 80 days.

5.1.2 Pig mixing practices

Reducing the mixing of finishing pigs from 100% to 0% lowered LA-MRSA prevalence in the unit when low transmission parameters were used (Figure 5). The median difference in prevalence between these two models was 8.8%. In medium and high transmission parameter sets, no difference in prevalence was observed when the mixing proportion was changed. Changing the cross-fostering practices in the farrowing unit did not affect the prevalence in the farrowing unit in any of the models (Figure 6). Therefore, the results indicate that changing pig mixing practices in farrowing and finishing units is insufficient to eradicate LA-MRSA from these units. However, in this study, LA-MRSA was introduced by infecting all pigs in the model herd. With a smaller disease introduction the different mixing practices may have had a bigger impact on the prevalence.

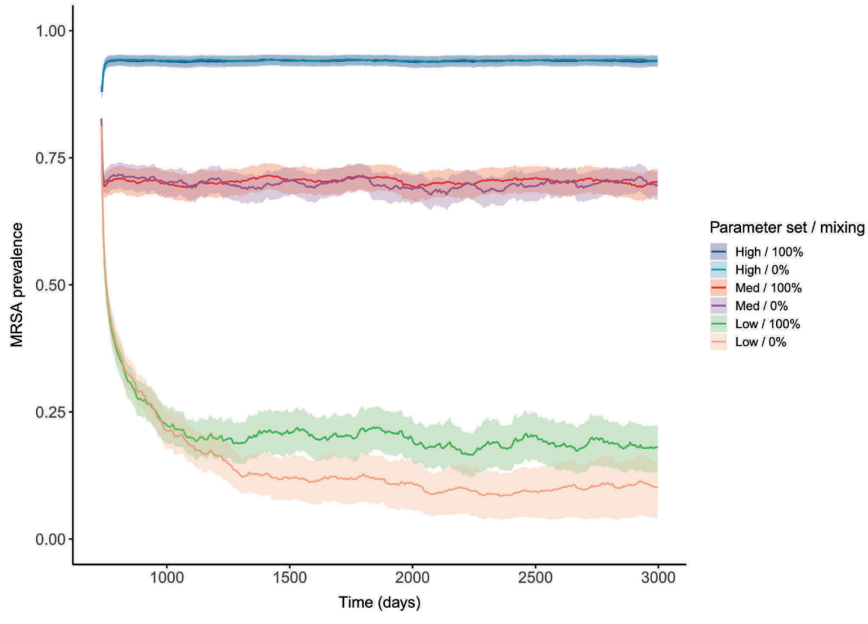

Figure 5. Model-predicted mean LA-MRSA prevalence and associated 95% credible intervals in the finishing unit when 0 or 100% of pigs were mixed one day after arrival to the unit. Prevalence was simulated for three transmission parameter sets (Low, medium [Med] and High). The model was run over 1 000 trajectories. All animals in the herd were infected at day 730 when the herd had reached its steady state.

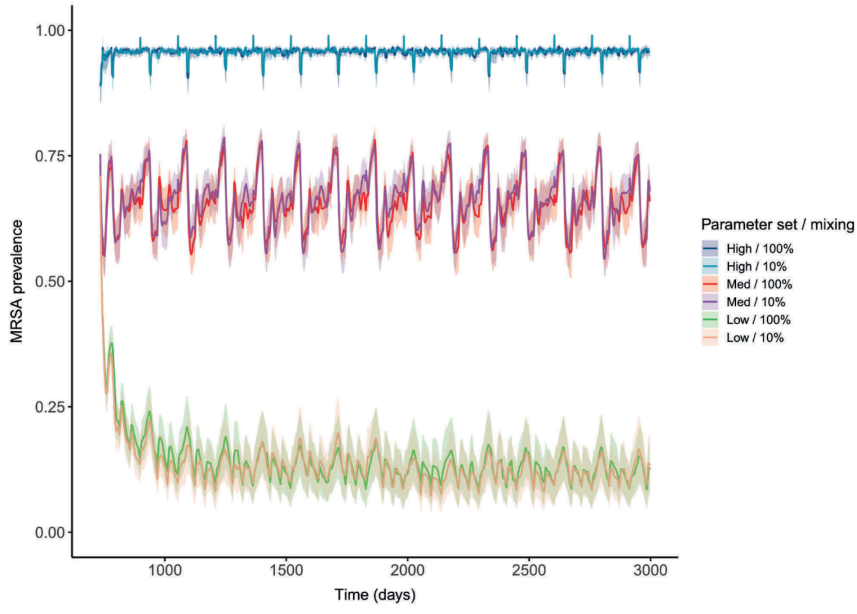

Figure 6. Model-predicted mean LA-MRSA prevalence in the piglets in the farrowing unit and the associated 95% credible intervals when 10 or 100% of the piglets were mixed one day after birth. Prevalence was simulated for three transmission parameter sets (Low, Medium [Med] and High). The model was run over 1 000 trajectories. All animals in the herd were infected at day 730 when the herd had reached its steady state.

5.2 Pig farmers' and veterinarians' views on disease control measures (study II)

While the discussions with the pig farmers and veterinarians were initially started with questions related to possible control measures against LA-MRSA in pig herds, both discussions evolved to focus on basic biosecurity routines. The study's main finding was that the pig farmers and veterinarians had diverging views on the current status of pig farm biosecurity. While the veterinarians described that farms often have inadequate biosecurity, farmers viewed the general level of biosecurity in Swedish pig farms as good. The veterinarians also felt that communicating about biosecurity to the farmers is challenging.

Contrary to the veterinarians, the pig farmers considered the flexibility of the farming system as important, and that biosecurity is only one piece of the puzzle. The veterinarians emphasised that the pig production system should be strict and focus on limiting possible disease spread. These results support the findings of previous studies, which have concluded that producers tend to accept and adapt to biosecurity threats, which may not follow the official recommendations (Higgins *et al.* 2018; Enticott & Little 2022). Although the veterinarians were not fully satisfied with the biosecurity practices in farms, they recognised that growing healthy, happy pigs was important to the farmers and appreciated their desire for a "good farmer life". In good farmer life, the farmers can enjoy their work. This can include many different aspects, such as being able to interact with animals and people rather than doing only hard labour within strictly set barriers.

Both veterinarians and farmers also shared similar views on the biosecurity challenges in pig production. The farmers implied that they would implement any disease control measures that were proven effective.

5.3 Modelling control measures against LA-MRSA study III)

The results suggest that eradicating LA-MRSA from a pig herd can be difficult, which is consistent with previous modelling studies (Sørensen *et al.* 2018b; Schulz *et al.* 2019a). Achieving disease eradication with the tested control measures was generally more likely if the control measures were introduced early in the outbreak phase of disease spread and when several control measures were combined. When simulating individual control

measures, cleaning all pens to remove the environmental infectious pressure was most effective in reducing the within-herd LA-MRSA prevalence (Figures 7 and 8).

When the control measures were combined, using a larger number of control measures led to larger reductions of within-herd prevalence and increased the probability of disease elimination (Figures 9 and 10). For example, combining all the modelled control measures resulted in 100% disease elimination with a mean time to disease elimination of 365 days (Table 1). However, the results indicate that the relative impact of improving biosecurity and removing pig mixing in farrowing and finishing units is small compared to the other control measures. In contrast, cleaning all-in all-out pens weekly combined with disease surveillance (testing gilt and sows before moving them to the breeding unit) had the largest impact on reducing the within-herd prevalence and the highest probability of causing disease elimination.

As presented in Table 1, the mean time to disease elimination was long in all of the control measures that were capable of causing disease elimination in the herd. The shortest mean time to disease elimination with a single control measure was observed when new gilts were tested for LA-MRSA before they were moved to breeding from the growing gilt unit (300 days). However, the probability of elimination was low (2.96% of all trajectories). In general, the effectiveness of testing gilts is likely affected by the route of disease introduction in the simulations. As LA-MRSA was introduced to the herd through the growing gilts, disease surveillance by testing these animals probably caused the LA-MRSA to be caught before it had spread to the rest of the herd.

Combining all the tested control measures achieved a 100% probability of disease elimination, but even in this case, the mean time to elimination was 365 days. Testing gilt and sows had a good elimination probability (94.33%), but the mean time to elimination (920 days) increased substantially, which might reduce the attractiveness of using only these control measures in disease eradication.

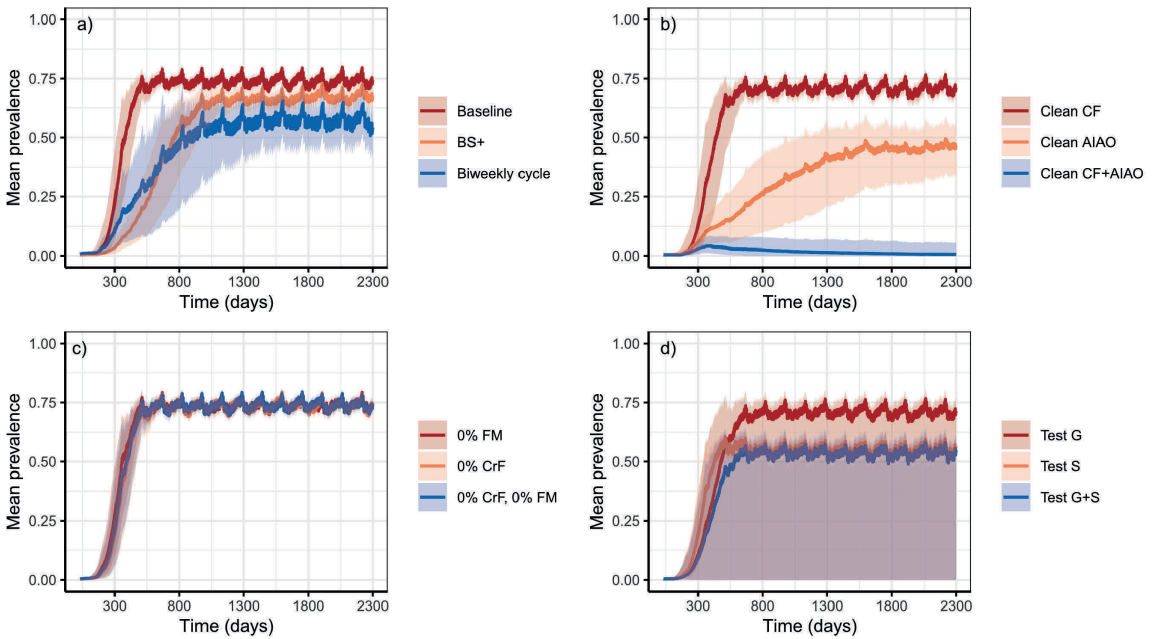

Figure 7. The model predicted mean LA-MRSA prevalence and the corresponding 95% credible intervals when single control measures were introduced in the disease outbreak phase. a) Prevalence without control measures (Baseline), with improved biosecurity (BS+) and when animals were moved between units only every other week (Biweekly). b) Prevalence when the environmental infectious pressure was removed by the weekly cleaning routine either in continuous flow (CF) pens, all-in all-out pens (AIAO) or simultaneously in both pen types. c) Prevalence when either mixing of finisher pigs (FM) or cross-fostering (CrF) or both were reduced to 0% 1 day after birth. d) Prevalence when new gilts (G), sows (S) or both new (G+S) were tested for LA-MRSA and positive pigs were replaced with susceptibles (diagnostic sensitivity 70%).

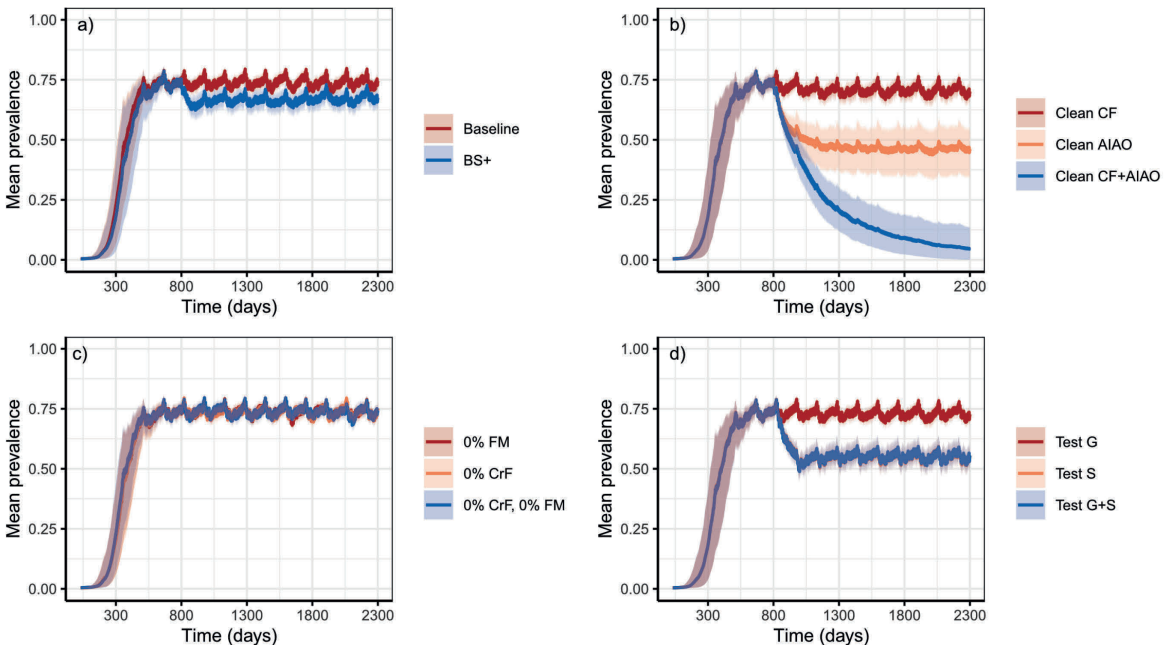

Figure 8. The model predicted mean LA-MRSA prevalence and the corresponding 95% credible intervals when single control measures were introduced in the endemic phase of disease spread. a) Prevalence without control measures (Baseline), with improved biosecurity (BS+) and when animals were moved between units only every other week (Biweekly).b) Prevalence when the environmental infectious pressure was removed by the weekly cleaning routine either in continuous flow (CF) pens, all-in all-out pens (AIAO) or simultaneously in both pen types. c) Prevalence when either mixing of finisher pigs (FM) or cross-fostering (CrF) or both were reduced to 0% 1 day after birth.d) Prevalence when new gilts (G), sows (S) or both new (G+S) were tested for LA-MRSA and positive pigs were replaced with susceptibles (diagnostic sensitivity 70%).

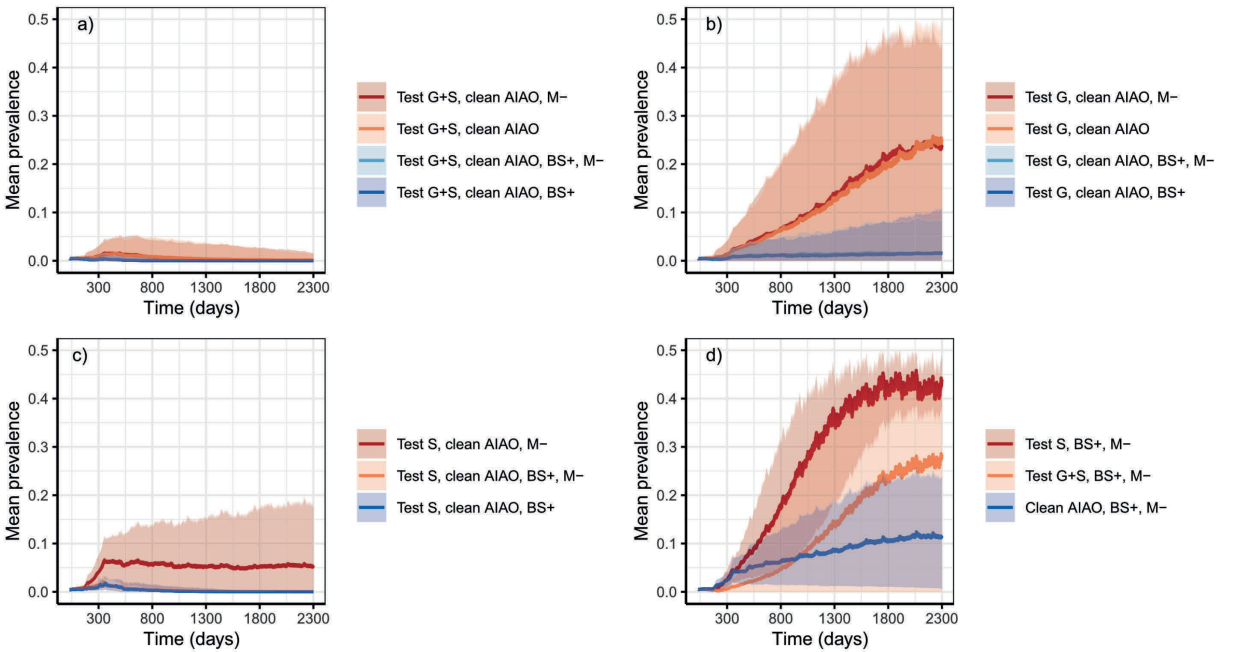

Figure 9. The model predicted mean LA-MRSA prevalence and the corresponding 95% credible intervals when combined control measures were introduced in the disease outbreak phase. The possible control measures used in different combinations were: testing gilts (test G) or sows (test S) or testing both gilts and sows (test G+S), cleaning all-in all-out (AIAO) pens when the pens were empty, improving biosecurity by removing between-pen disease transmission (BS+) and reducing cross-fostering piglets and mixing of finishing pigs to 0% (M-).

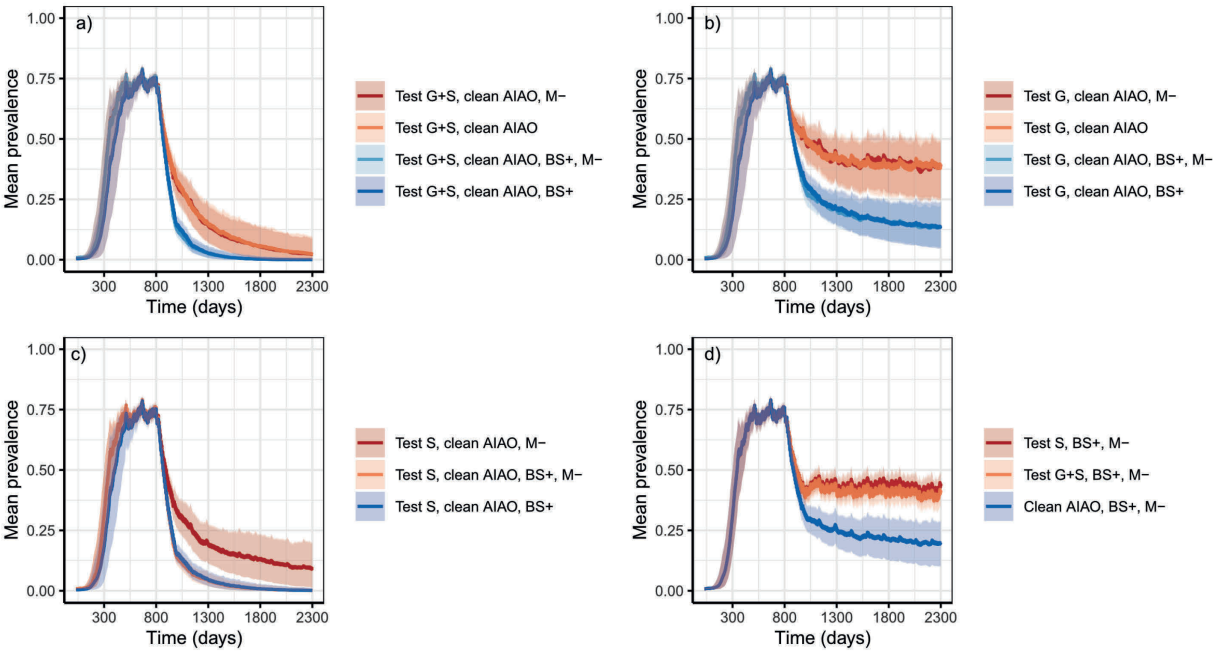

Figure 10. The model predicted mean LA-MRSA prevalence and the corresponding 95% credible intervals when combined control measures were introduced in the endemic phase of disease spread. The possible control measures used in different combinations were: testing gilts (test G) or sows (test S) or testing both gilts and sows (test G+S), cleaning all-in all-out (AIAO) pens when the pens were empty, improving biosecurity by removing between-pen disease transmission (BS+) and reducing cross-fostering piglets and mixing of finishing pigs to 0% (M-).

Table 1.The probability of LA-MRSA elimination and the mean time to elimination when different control measures were applied at the outbreak phase of disease spread. Only control measures that had >0% probability of elimination are included in the table.

Control measure	Mean time (days) to elimination	Probability of elimination (%)
Single control measures		
BS+	559	0.01
Biweekly[1]	587	0.07
Test[2] gilts	300	2.96
Clean AIAO	1158	0.02
Combined control measures		
Test G+S, clean CF and AIAO, BS+, M-	365	100.00
Test G+S, clean CF and AIAO, M-	536	100.00
Test G+S, clean AIAO, BS+, M-	533	99.99
Test G+S, clean AIAO, BS+	492	99.98
Test G+S, clean AIAO, M-	946	94.04
Test G+S, clean AIAO	920	94.33
Test G+S, BS+, M-	565	23.7
Test G+S	291	3.26
Test gilts, clean AIAO, BS+, M-	868	54.39
Test gilts, clean AIAO, BS+	780	63.31
Test gilts, clean AIAO, M-	660	18.92
Test gilts, clean AIAO	648	23.63
Test sows, clean AIAO, BS+, M-	977	99.1
Test sows, clean AIAO, BS+	931	99.39
Test sows, clean AIAO, M-	1600	18.67
Test sows, BS+, M-	1109	0.02
Clean AIAO, BS+, M-	1510	1.46
Clean CF and AIAO	1370	73.02

5.4 The survival of LA-MRSA CC398 on surface materials (study IV)

The study results indicate that different surface materials may affect the survival of LA-MRSA CC398. Figure 11 presents the viable counts (CFU/mL) of LA-MRSA on steel and plastic over time. The half-life of the bacteria was longer on polypropylene plastic ($t_{½}$ = 11.08–15.78 days) than on stainless steel ($t_{½}$ = 2.45–7.83 days). Figure 12 presents the viable counts of LA-MRSA on the two concrete materials over time. The bacteria diminished rapidly from these surfaces and became undetectable after 3 to 9 weeks. Therefore, it was not possible to determine half-life values for concrete. While concrete is likely an unfavourable surface for many bacteria phyla (Maresca *et al.* 2016), its porous structure might have limited the number of viable bacteria that were recoverable with the employed method. Determining the half-life for concrete would at least require more frequent sampling intervals.

The survival of LA-MRSA on barn surfaces is likely affected by several factors, such as temperature, the presence of organic matter and the condition of the surfaces. Therefore it is not possible to draw conclusions about the risk of transmission of an individual material. However, it may be worthwhile to consider the properties of the surface materials when planning on-farm cleaning and disinfection routines.

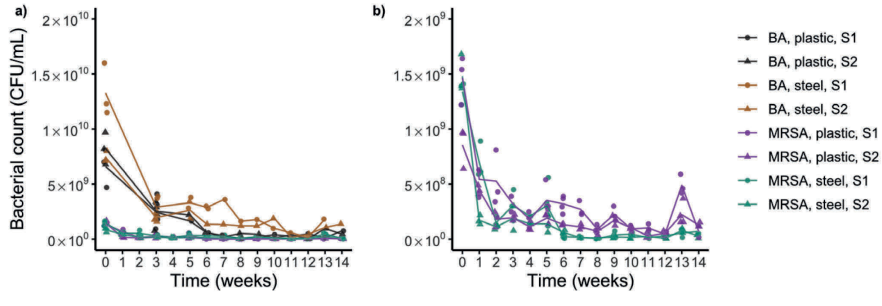

Figure 11. The LA-MRSA counts (CFU/mL) on steel and plastic surfaces over time. The points represent the viable counts within the quantification range (30–300 CFU) that were obtained from the samples; the lines represent the mean viable count of each material, strain and plate combination.
a) 5% bovine blood agar (BA) and selective Oxoid Brilliance MRSA 2 agar (MRSA).
b) selective MRSA plates from the same data.

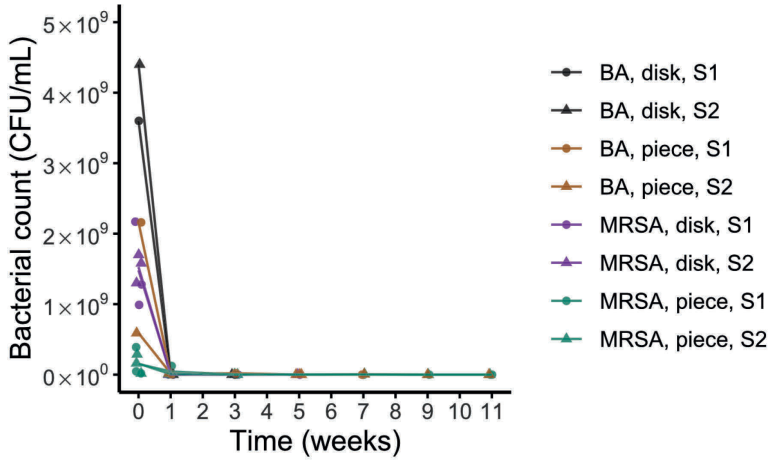

Figure 12. The LA-MRSA counts (CFU/mL) on the concrete surfaces over time. Two different concrete surfaces were used (concrete disks and concrete pieces). Concrete disks were observed for 4 weeks and concrete pieces for 11 weeks. All strain and material combinations were plated in triplicates on Oxoid Brilliance MRSA 2 agar (MRSA) and as a single sample on 5% bovine blood agar (BA). The points represent the observed viable counts; counts with less than 30 CFU are also included in the figure. If a sample had a count below the quantification limit (<30) on multiple dilution plates, the count from lowest dilution is presented. The lines represent the mean viable count of each concrete type, strain and plate combination.

6. Discussion

The World Health Organisation has declared antimicrobial resistance as one of the top 10 global public health threats facing humanity (World Health Organization 2021). In this thesis, I have taken a multifaceted approach to explore ways to control the spread of LA-MRSA in pigs, with an emphasis on the Swedish context. However, as LA-MRSA is often asymptomatic in animals and humans, motivating and implementing control programmes can be challenging.

The virulence of LA-MRSA CC398 has traditionally been considered to be low (see section "2.2.1 Evolution of LA-MRSA CC398 and other MRSA CC398"), but the recent findings of more virulent strains raise concerns about the future development of the bacteria. Due to this, several studies have called for establishing continuous monitoring of LA-MRSA (Sieber *et al.* 2020; Avberšek *et al.* 2021; Leão *et al.* 2022). However, only a few countries currently have an active LA-MRSA monitoring programme (EFSA & ECDC 2022). While monitoring programmes depend on necessary resources, the lack of efficient control measures might impact the willingness to establish these programmes. Providing input on the control measures was one of the key motivations for conducting the studies in this thesis.

6.1 LA-MRSA control in pig herds

6.1.1 Prevention is better than the cure

This thesis supports previous modelling studies' findings that eradicating LA-MRSA from a herd is challenging (Sørensen *et al.* 2018b; Schulz *et al.* 2019a; Bastard *et al.* 2020). The results also highlight that introducing the control measures early in the outbreak phase is crucial to improve the

likelihood of eradicating LA-MRSA. While it may seem self-evident that early interventions are usually more effective than late ones, in the case of LA-MRSA, it is worthwhile to emphasise the need to act early if the goal is to eradicate the disease without culling the entire herd. However, detecting LA-MRSA early would require establishing monitoring programmes, which are currently not in use in Sweden. Previous studies have shown that pig movements have had a significant role in the between-herd spread of LA-MRSA (Broens *et al.* 2011c; Espinosa-Gongora *et al.* 2012; Grøntvedt *et al.* 2016; Sieber *et al.* 2018; Sørensen *et al.* 2018a; Pirolo *et al.* 2020). These published findings and the present modelling results indicate that quarantining and testing new animals are likely important in preventing LA-MRSA introduction to herds.

6.1.2 Eradicating LA-MRSA in high-prevalence herds

Based on the current knowledge from disease models, in high-prevalence herds culling might be the only option for fast disease eradication. However, in countries where the number of LA-MRSA-positive herds is high, eradication by culling may not be seen as a reasonable goal. A Danish modelling study has concluded that depopulating the LA-MRSA-positive farms would not be cost-effective under these conditions compared to the estimated cost reduction in healthcare (Olsen *et al.* 2018). However, from an individual's perspective, just a single fatal case due to an untreatable infection is unwanted.

Another Danish cost-benefit study estimated that the societal costs of culling all the positive herds exceed the costs of using a "containment strategy" (Jensen *et al.* 2020). In the study, "containment strategy" referred to measures where hygiene requirements are implemented to prevent farm personnel from spreading the bacteria outside the farm. The strategy included changing clothes and showering at the end of the working day (Jensen *et al.* 2020). Whether this strategy would succeed in containing LA-MRSA within the farms is debatable, as the bacteria can be carried in the human nasal cavity. Furthermore, as highlighted in our focus-group study and previous studies, motivating farmers to maintain a high biosecurity level is difficult unless it has an apparent production benefit. Additionally, the cost estimations do not consider the possibility of more virulent LA-MRSA strains emerging over time, which could cause more significant consequences and costs to public health.

6.1.3 Modelled control measures

In our modelling study, combining cleaning of all-in all-out pens and continuous flow pens was effective in eradicating LA-MRSA in the herd. However, the cleaning interval (every seven days) for continuous flow pens is extreme and not feasible in real-life farms. Modelling hard-to-implement control measures was still considered justified, as observing the effect of these measures can help understand the within-herd disease dynamics. Based on the model results, continuous-flow pens might serve as a disease reservoir and should be considered when planning LA-MRSA control measures.

Cleaning only the all-in all-out pens after every batch of pigs also had a considerable impact on the herd LA-MRSA prevalence. Cleaning and disinfection between batches is common practice in farms, but LA-MRSA seems to persist in these herds regardless. This difference between the model output and real-world experience could result from several reasons. Firstly, the cleaning and disinfection practices in farms might not have fully removed the infectious pressure from the environment. Secondly, the model output represents the modelled herd structure, whereas in the real world, the herd structures and management practices vary. Thirdly, the disease model assumed all disease transmission to occur through the environment, which might overestimate the effect of cleaning. This assumption was necessary due to the limited information on the relative importance of direct and indirect transmission.

Combining the cleaning of all-in all-out pens with disease testing practice similar to a test-and-removal strategy was also capable of removing LA-MRSA from the model herd. The latter measure included testing either sows or new gilts before they were moved to the breeding unit or a combination of both. The combination of cleaning and testing could be a feasible control measure to test in practice. It should be considered, however, that differences in diagnostic sensitivity and the chosen sampling protocol can change the obtained results in practice.

The disease testing control measure used in the model was implemented either simultaneously with the disease introduction or when LA-MRSA had reached an endemic state in the herd. The limitation of the employed scenarios was that neither represented the case where LA-MRSA is detected later during the outbreak phase but before the endemic state of disease spread, e.g. after a positive case has been found in a clinically sick pig or from farm workers. While modelling this type of scenario would be

interesting, it would require either making assumptions on the possible herd prevalence when the first LA-MRSA case is detected or modelling control measures applied at several different herd prevalences. The latter approach was considered during the construction of the model simulations, but it was not implemented because interpreting and summarising the large amount of result data for all control measures was not possible within the limits of a single study.

In study III, the spread of LA-MRSA was initiated through gilts, as farms can purchase them to replace sows. However, in different herd structures, e.g. in fattening farms, LA-MRSA could be introduced to another pig group, which may impact the spread dynamics. In closed pig herds, the risk of introduction might be lower, but still possible, e.g. through humans. However, the relative risk of LA-MRSA introduction from humans compared to new animals is unknown. Depending on the risk, it could be worthwhile to target disease testing to farm workers who have visited countries with high LA-MRSA prevalence.

6.1.4 Survival of LA-MRSA in the farm environment

This thesis included a study on the survival of LA-MRSA CC398 on different surface materials commonly found in the pig farm environment. One of the study aims was to provide more input parameters for our disease spread model, but due to delays in executing the laboratory study, the obtained decay values were not included in the modelling work. Instead, a previously published value for LA-MRSA half-life in dust was used ($t_{1/2}$ = 5 days; Feld *et al*. 2018).

Our survival study showed a substantial difference in survival on different surface materials. While LA-MRSA CC398 survived the longest on plastic and the shortest on concrete, the survival on stainless steel was between the two ($t_{1/2}$ = 2.45–7.83 days). As the survival in the dust is within this interval, it can be speculated that using the value for dust was a reasonable approximation of the mean LA-MRSA survival in the pig farm environment. However, as we do not know the relative importance of the materials, this assumption has its drawbacks. For the same reason, incorporating the different survival times separately into a disease model would be challenging.

Based on the results of the survival study, considering the properties of different surfaces might help to improve the outcome of cleaning and

disinfection measures against LA-MRSA. However, the length of survival can be affected by several factors, such as the age and physical condition of the surfaces.

6.1.5 Farm biosecurity and motivations for disease control

Based on previous studies and the veterinarians who participated in our focus group discussions, on-farm biosecurity often has room for improvement (Nöremark & Stenberg-Lewerin 2014; Filippitzi *et al*. 2018; Verkola *et al*. 2021). While our focus group study did not result in specific suggestions for controlling LA-MRSA in the pig herds, the pig farmers implied that they were willing to implement any disease control measures as long as they were proven effective. The challenge is that scientific evidence on efficient disease-specific control measures is often lacking. Moreover, gathering such evidence is difficult, as it would require showing that the absence of the disease was a consequence of a control measure. The dynamics also vary between diseases and the spread can be affected by numerous factors, such as herd structure and management practices.

In the case of LA-MRSA, pigs are usually only asymptomatic carriers of the bacteria, and implementing control measures does not have an immediate impact on the pigs' health or the cost-effectiveness of the production. Therefore, motivating farmers to engage in additional control measures to limit the spread of LA-MRSA may be challenging. Based on our modelling, the time to disease elimination is also long, which can further decrease the willingness to engage with additional control measures. Therefore, introducing LA-MRSA control measures on farms may require legal obligation and decisions regarding how the costs would be covered.

6.2 Model validity

Based on conceptual and face validation, the model was seen as a good representation of a Swedish farrow-to-finish pig herd. However, the model also has some limitations.

It is often stated that a model's output is as good as its input. One of the main challenges in our modelling studies was the limited knowledge of the input parameters. The transmission parameters used in our study were extracted from a ten-year-old study done in Dutch and Danish pig herds (Broens *et al*. 2012a). This study included only prevalence data from

farrowing to finishing, and therefore LA-MRSA prevalence in adult pigs in breeding and gestation was largely unknown. However, better data on LA-MRSA prevalence and transmission is still lacking. These uncertainties related to the input data also impact the model outputs' accuracy. The data presented by Broens *et al.* (2012a) also only represents the herd structure and management practices of study herds, which may differ from what is seen in other countries and farms.

Regardless of the uncertainties and approximations, disease spread modelling can be useful in studying the dynamics of disease spread and control measures because large-scale experimental studies are not feasible. However, the limitations should be considered when the model results are taken into practice and part of decision-making.

6.3 Implications of the results

In summary, this thesis suggests that in low-prevalence countries, preventing the introduction of LA-MRSA to pig herds by disease monitoring is indicated. In high-prevalence herds, eradicating the bacteria without culling the entire herd is difficult. The probability of disease eradication by other measures is more likely the earlier LA-MRSA has been detected. The upcoming EU baseline study in pigs at slaughter will provide information on the LA-MRSA status of Swedish pigs and comparable information between member states. When the results are available, comparing factors that might impact the possible differences between countries could provide insight into factors affecting the spread of LA-MRSA. However, as the baseline study is limited to slaughter pigs, further studies on the LA-MRSA prevalence and dynamics are needed, especially for adult pigs in piglet production, as they could be a potential LA-MRSA reservoir to the offspring. As the current knowledge of LA-MRSA in Swedish pigs is unknown, an action plan for handling possible LA-MRSA-positive pig cases is needed before surveillance is launched and the results of this thesis will support such a plan.

7. Concluding remarks

We have used disease modelling to bring new insights into the possible effective control measures against LA-MRSA CC398 in pig production, with an emphasis on Swedish farm characteristics. To obtain suggestions for viable control measures to be modelled, we organised focus group discussions with stakeholders (pig farmers and veterinarians). We also studied the survival of LA-MRSA CC398 on different surface materials to support decision-making regarding cleaning and sanitation practices and to improve input parameters in future modelling studies. The main conclusions are:

- Eradicating LA-MRSA CC398 from the pig herd can be difficult, but more likely if control measures are introduced early in the outbreak phase of disease spread.
- Pig farmers are willing to implement any necessary disease control measures if they can be proven effective.
- Pig farmers and pig veterinarians have different views on the level of biosecurity in Swedish farms. For farmers, the flexibility of the production system was important, which presents challenges for the farm's biosecurity.
- The disease modelling suggested that combining several control measures causes larger reductions in the within-herd LA-MRSA prevalence than individual control measures.
- Removing the environmental infectious pressure by cleaning the pen environment and testing both gilts and sows before moving them to the breeding unit were the most effective control measures in reducing the prevalence.

- Even with the most effective control measures, the time to disease elimination was at least 300 days.
- The survival of LA-MRSA CC398 varies on different surface materials. The bacteria became rapidly undetectable on concrete surfaces, while on stainless steel and polypropylene plastic, the half-life ($t_{1/2}$) was 2.45–7.83 days and 11.08–15.78 days, respectively. These differences may impact the survival of LA-MRSA in the farm environment.

8. Future perspectives

This thesis has increased the knowledge of the efficacy of control measures against LA-MRSA. However, it has also identified several knowledge gaps, which should be addressed to improve our knowledge of how to protect humans and animals from infections caused by LA-MRSA. Areas for further studies and actions include:

- **Improving the knowledge of LA-MRSA transmission between pigs.** Better input parameters enhance the output of disease models. To achieve this, experimental studies that determine transmission parameters among pigs in different production phases are needed.
- **Estimating the rates for between-pen transmission of LA-MRSA.** Experimental studies on between-pen transmission would allow us to predict the impact of biosecurity-related control measures more accurately.
- **Studying the relative impact of environmental LA-MRSA transmission and direct transmission to the colonisation of pigs.** Evaluating the importance of different transmission routes could also improve modelling studies and increase understanding of potentially more effective control measures.
- **Studying the effect of control measures used in study III in a between-herd model.** Combining animal movement data with a multiherd model could be used to assess the impact of the control measures presented on a national level. Such a model could also be used to study which herds should be targeted to gain the biggest impact in prevalence reduction.

- **Assessing the importance of humans in introducing LA-MRSA to pig farms.** If humans are a significant route for LA-MRSA introduction, targeted screening to farm workers may be justifiable.
- **Finding ways to motivate farmers to protect their farms from LA-MRSA**. A better understanding of the drivers and priorities for implementing biosecurity measures is needed. Assessing the effectiveness of general biosecurity measures against LA-MRSA would provide input parameters for disease modelling. The results from modelling could be used as a basis for exploring feasible biosecurity options among farmer-vet groups.
- **Studying the impact of the condition of surface materials on the survival of LA-MRSA in pig farms.** While this thesis investigated LA-MRSA survival on different materials, these materials become worn out over time in the farm environment. This could make the materials harder to clean and promote the survival of LA-MRSA in the farm environment.
- **Surveying the prevalence of LA-MRSA in Swedish pig herds.** As highlighted in this thesis, LA-MRSA that is caught early is easier to eradicate from the herd. Additionally, knowledge of the current LA-MRSA status in Swedish herds could aid in studying and planning efficient control programmes for the particular situation. However, this forms a catch-22 situation as ideally we would want to have an efficient control programme available before the bacteria are detected.

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Popular science summary

Multiresistant bacteria, also known as superbugs, are bacteria resistant to several types of antibiotics. One group of these resistant bacteria is called methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA is a growing concern in humans and animals. Some MRSA bacteria are adapted to animals, which is why they are called livestock-associated MRSA (LA-MRSA). LA-MRSA was originally found in pigs, but it is also capable of causing infections in humans. LA-MRSA transmits to humans primarily through direct contact with animals, causing people working with livestock to be at higher risk of becoming LA-MRSA carriers. However, spread between people can also occur.

By controlling the spread of LA-MRSA in pigs, we can reduce human exposure to LA-MRSA. This thesis used infectious disease modelling to explore possible control measures to eliminate or reduce the spread of LA-MRSA in a Swedish pig herd. Additionally, this thesis studied the pig farmers' and veterinarians' views of farm biosecurity and disease control and the survival of LA-MRSA on surface materials commonly seen in pig farms.

The results of the thesis conclude that eliminating LA-MRSA is difficult if it has become widespread in the pig herd. The probability of disease elimination is more likely if the bacteria are detected early and multiple control measures are applied at an early phase of the disease spread. However, motivating farmers to implement control measures might be challenging as LA-MRSA does not usually cause production losses or infections in pigs.

The discussions between selected pig farmers and veterinarians suggested that these two groups had different views of the general level of biosecurity in pig farms. The farmers thought that biosecurity in pig farms was good, while the veterinarians saw room for improvement. The pig farmers also

implied that they are willing to implement any disease control measures if they can be proven effective.

The bacterial survival study showed that LA-MRSA survives longer on plastic than on stainless steel or concrete. The bacteria became undetectable the fastest on concrete. Therefore, it was concluded that the materials used in farms could affect the survival of LA-MRSA. This knowledge will be useful in planning more effective cleaning and disinfection practices.

This thesis has expanded the knowledge of the survival of LA-MRSA and possible control measures for battling against the bacteria in the pig farm environment. However, further studies are needed to fill the remaining knowledge gaps regarding its transmission and elimination.

Populärvetenskaplig sammanfattning

Multiresistenta bakterier, ibland kallade superbugs, är bakterier som är resistenta mot flera typer av antibiotika. En variant av sådana bakterier kallas meticillinresistent *Staphylococcus aureus* (förkortat MRSA) som har blivit ett allt större problem hos djur och människor. En särskild typ kallas Livestock-Associated (LA-MRSA), eftersom den tycks anpassad till djur. Den hittades ursprungligen hos grisar, men kan även orsaka infektioner hos människor. LA-MRSA överförs till människor främst genom direktkontakt med djur, vilket är anledningen till att personer som arbetar med produktionsdjur löper större risk att bli bärare av LA-MRSA. Spridning mellan människor kan dock också förekomma.

Genom att kontrollera spridningen av LA-MRSA bland grisar kan vi minska överföringen till människor. I denna avhandling användes sjukdomsmodellering för att undersöka vilka tänkbara kontrollåtgärder som skulle kunna eliminera eller minska spridningen av LA-MRSA i en svensk grisbesättning. Dessutom undersöktes grisproducenters och veterinärers syn på smittskydd och sjukdomsbekämpning samt överlevnaden av LA-MRSA på olika typer av material som är vanligt förekommande i grisstallar.

Resultaten av avhandlingen visar att det är svårt att utrota LA-MRSA när den väl har blivit utbredd i en grisbesättning. Sannolikheten att kunna utrota smittan är större om bakterierna upptäcks tidigt och flera kontrollåtgärder tillämpas i ett tidigt skede av sjukdomsspridningen. Det kan dock vara svårt att motivera lantbrukare att vidta frivilliga kontrollåtgärder eftersom LA-MRSA vanligtvis inte orsakar produktionsförluster eller sjukdom hos grisarna.

Diskussionerna mellan ett fåtal grisproducenter och inom en grupp av veterinärer tydde på att dessa två grupper hade olika syn på den allmänna smittskyddsnivån i svenska grisbesättningar. Grisuppfödarna ansåg att

smittskyddet i besättningarna är gott, medan veterinärerna såg utrymme för förbättringar. Grisuppfödarna antydde också att de är villiga att genomföra alla tänkbara åtgärder för sjukdomsbekämpning om de bara är bevisat effektiva.

Studien av bakteriernas överlevnad visade att LA-MRSA överlever längre på plast än på stål eller betong. På betong avklingade bakterierna snabbast. Därför drogs slutsatsen att de olika byggmaterial som används på gårdarna kan påverka överlevnaden av LA-MRSA i miljön. Denna kunskap kan vara användbar vid planering av effektivare rengörings- och desinfektionsmetoder.

Denna avhandling har bidragit med kunskap om hur LA-MRSA överlever och hur möjliga kontrollåtgärder kan användas för att bekämpa bakterierna i miljön i en grisbesättning. Ytterligare studier behövs dock för att fylla de återstående kunskapsluckorna när det gäller smitta till och mellan grisar, och utrotning av bakterien.

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Appendix I

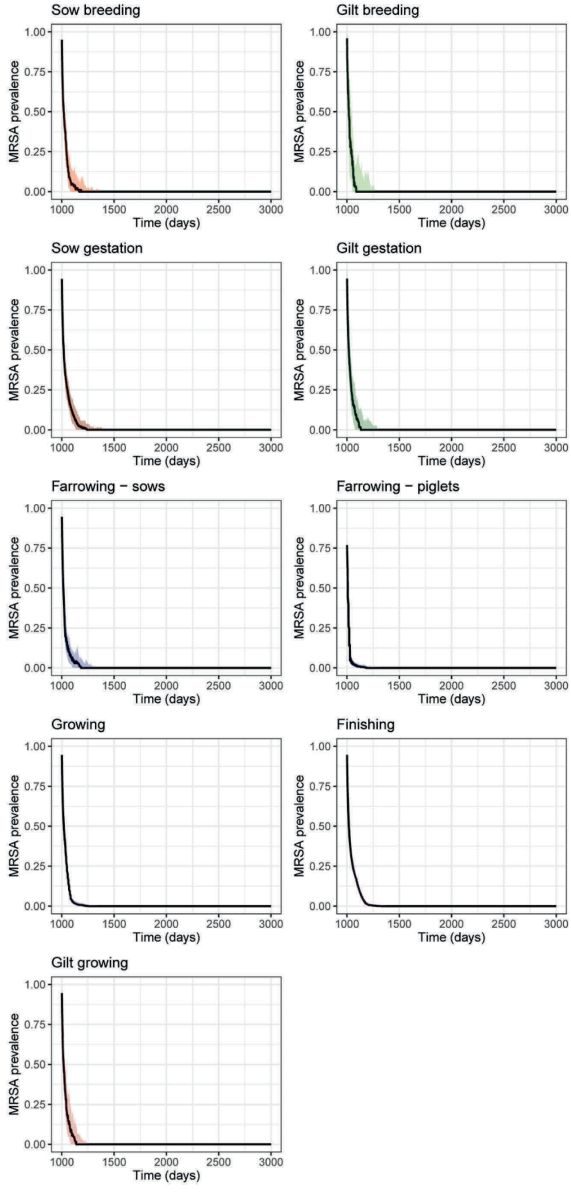
Sow breeding
Gilt breeding
Sow gestation
Gilt gestation
Farrowing – sows
Farrowing – piglets
Growing
Finishing
Gilt growing
MRSA prevalence
Time (days)
1.00
0.75
0.50
0.25
0.00
1000
1500
2000
2500
3000

Figure I. Model-predicted LA-MRSA prevalence over time for different pig production groups when LA-MRSA was transmitting only through direct transmission (no environmental accumulation). All pigs in the herd were infected at day-1000 of the model run. The model used transmission rates that were parameterised from the study by Broens et al. (2012a)

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Modelling environmentally mediated spread of livestock-associated methicillin-resistant *Staphylococcus aureus* in a pig herd

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ABSTRACT

Infectious disease models are a useful tool to support within-herd disease control strategies. This study presents a stochastic compartment model with environmentally mediated transmission to represent the spread of livestock-associated methicillin-resistant *Staphylococcus aureus* (**LA-MRSA**) in a farrow-to-finish pig herd. The aims of the study were to (1) construct a model of the spread of LA-MRSA that included spread of LA-MRSA through the environment; (2) parameterise the model to fit previously published observational data in order to obtain realistic LA-MRSA transmission rates; (3) and to investigate how changes in the mixing of animals in the farrowing and finishing units may affect the prevalence of LA-MRSA in a herd. The results showed that indirect transmission allowed LA-MRSA to persist in the herd without the assumption of persistently shedding individuals. Reducing the mixing of pigs upon entry to the finishing unit was also shown to lower the LA-MRSA prevalence in the unit if the initial LA-MRSA level in the unit was low, but at high prevalence, no effect of mixing was identified. In the farrowing unit, changing the proportion of piglets that were cross-fostered did not affect the within-herd LA-MRSA prevalence. The study demonstrates that there are several important knowledge gaps regarding the shedding and transmission of LA-MRSA in different animal age groups and further experimental studies are needed. This work also provides a new, robust and flexible model framework for the investigation of control and mitigation strategies for LA-MRSA and other infections in a pig herd.

Implications

Livestock-associated methicillin-resistant *Staphylococcus aureus* bacteria are capable of transmitting between animals and humans. This poses a health risk, especially to those working with pigs and other livestock, but also to the wider community. The study shows that reducing mixing of pigs may reduce the number of livestock-associated methicillin-resistant *Staphylococcus aureus* carriers in the herd. The model presented in this study will be useful for investigating the spread patterns and control strategies of the bacteria in a pig herd, aiming to provide tools to combat the spread. The model is also adaptable for studying other diseases in pig herds.

Introduction

The spread of infectious animal diseases is complex, which poses a challenge when evaluating possible outbreak scenarios and control strategies. This applies to individual herds where investments in internal biosecurity may need to be adapted to herd-specific risks; but it also affects choices made in regional or national responses to disease outbreaks or control programmes for endemic diseases. Mathematical models provide a useful tool to explore outbreak scenarios as well as possible control strategies, when experimental studies are not possible (Heesterbeek et al., 2015). Modelling has been used successfully to gain an understanding of many infectious diseases in animals (Keeling et al., 2001; Ivanek et al., 2004; Halasa et al., 2019).

Staphylococcus aureus is a commensal bacterium and opportunistic pathogen in both humans and animals. Since the introduction of antimicrobial therapies, *S. aureus* has gained resistance against antimicrobial agents, of which the resistance against β-lactams in methicillin-resistant *S. aureus* (**MRSA**) is the most notable (Crombe et al., 2013). The livestock-associated MRSA

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(**LA-MRSA**) strains belonging to clonal complex CC398 are frequently found in Europe and North America, both in pigs and people in contact with pigs as well as in other livestock species (Vanderhaeghen et al., 2010; Crombe et al., 2013; Hansen et al., 2017). While the zoonotic impact of LA-MRSA in pig herds is a concern in many countries, effective strategies for its control are still lacking. In Sweden, domestic pigs are assumed to be free of LA-MRSA or the prevalence is very low. It has also been deemed worthwhile to attempt to keep LA-MRSA out of the Swedish pig population (Höjgård et al., 2015).

Previous studies have shown that direct transmission (Broens et al., 2012a; Broens et al., 2012b) and indirect transmission via exposure to airborne LA-MRSA within the barn (Rosen et al., 2018) are both important routes of transmission between pigs as well as between humans and pigs (Bos et al., 2016; Feld et al., 2018). However, current knowledge on the effectiveness of environmental interventions is conflicting. A study by Kobusch et al. (2020) suggests that cleaning and disinfection of the barn can be worthwhile to decrease the infectious pressure of LA-MRSA in pig herds, whereas in a Danish study, different disinfection techniques were unsuccessful in reducing the environmental infectious pressure (Bækbo et al., 2019). To properly assess different interventions for reducing the environmental load of LA-MRSA, and consequently the risk of transmission and re-colonisation of the pigs, it is important to incorporate environmental transmission into a disease spread model.

The spread of LA-MRSA within a pig herd has been previously studied in an individual-based model, where the LA-MRSA carriers could be either intermittent or persistent shedders (Sørensen et al., 2017). Individual-based approach has also been used in a between-herd model (Schulz et al., 2018). In another study, within and between-herd dynamics were studied in a stochastic metapopulation model, where the within-herd transmission was modelled at the farm-section level (Bastard et al., 2020). In addition, different intervention and control strategies have been investigated in individual-based models (Sørensen et al., 2018; Schulz et al., 2019) and the spread from pigs to humans in a metapopulation model (Porphyre et al., 2012). The transmission of LA-MRSA through barn air has been previously modelled by Sørensen et al. (2020) to assess the potential hazard to humans, but to the best of our knowledge, a model of LA-MRSA spread that includes the spread among pigs via the environment has not previously been described.

The current study presents a stochastic compartment model of LA-MRSA spread in a pig herd, which incorporates environmentally mediated spread. The model provides a framework for testing the efficacy of potential LA-MRSA surveillance, prevention and control strategies to reduce the prevalence in the herd or to mitigate the spread after an introduction of the disease. It also allows future investigation of environmental interventions, such as cleaning and disinfection of pens, changes to downtime between groups of pigs and other factors that could affect the burden of LA-MRSA in the barn.

The aims of this study were to (1) build and (2) parameterise an efficient and flexible model of the animal movements within a pig herd, which would allow modelling the environmental infectious pressure of LA-MRSA within the herd and the spread of LA-MRSA in a Swedish context. The final aim was to (3) investigate the effects of different animal mixing practices on the LA-MRSA prevalence in the model herd. This study will serve as a basis for further study of the spread of LA-MRSA and intervention strategies to reduce the prevalence and probability of introduction to a herd.

Material and methods

The simulation model was built in the R programming language version 4.0.3 – "Bunny-Wunnies Freak Out" (R Core Team, 2020) with the SimInf package version 8.2.0.9000 (Widgren et al., 2019). SimInf is a framework for discrete event-based epidemiological simulations, where transitions between compartments are modelled as a continuous-time discrete-state Markov chain with the Gillespie stochastic simulation algorithm. The framework incorporates both a stochastic simulation in continuous time and the ability to add scheduled events that can move individuals between compartments in the model at the end of each unit of time. This allows for the precise simulation of movement, birth, ageing and death of animals within a herd, as well as testing the effects of changing pig flows on the within-herd spread.

Disease spread model

In this model, the term 'infected' is used to describe animals that are colonised by LA-MRSA, even though LA-MRSA rarely causes clinical disease in pigs. Therefore, 'infected' should be interpreted as a way of clearly communicating the infectious disease model results rather than as an indication of the state of disease of the pig. The disease spread model is an SIS_E compartment model, where animals move between susceptible (S) and infected (I) states and E represents the LA-MRSA-contaminated environment and farm air (Fig. 1). Animals in the model were also divided into metapopulations (nodes) which were interpreted as pens. Environment, in this context, should be interpreted as every surface and the air in each pen. The infected state was assumed to be transient; the pigs could return to the susceptible state and subsequently become recolonised. Based on previous studies, LA-MRSA prevalence varies by age of the animal (Broens et al., 2011; Broens et al., 2012a; Bangerter et al., 2016), but the underlying reasons for this variation have not been fully clarified. Therefore, the susceptible and infected compartments were further divided into age categories including mature sows and gilts in the reproductive cycle, suckling piglets, growing pigs (from weaning and up to 13 weeks of age) and finishing pigs (from 13 weeks up to slaughter).

LA-MRSA may be transmitted through both direct and indirect transmission, but in the model, these routes of transmission were not separated, since the observations available in the literature were not sufficient to allow for this distinction to be made. Thus, the transmission parameters can be interpreted to represent their combined effect. The approach to include indirect spread via the environment is an improvement over direct-spread models, as it allows LA-MRSA to persist in the environment even when animals are removed from their pens.

The transitions from the susceptible to the infected states were dependent on the environmental infectious pressure in the node (pen), age group specific transmission rates and the number of susceptible individuals in the node as described in Supplementary Material S1. Recovery from infected to susceptible states was driven by the number of infected individuals in the node and the average duration of carriage (17.4 days). The duration of carriage was based on the study by Broens et al. (2012b). The environmental infectious pressure in the transitions was specific for each individual node in the herd, and the transmission rates were specified for the four animal age groups.

To incorporate the indirect transmission in the model, a continuous value representing environmental infectious pressure $\varphi_i(t)$ was stored for each node during the simulation. The level of $\varphi_i(t)$ was updated when the simulated time had progressed by one unit (day). Each infected animal in a node contributed to $\varphi_i(t)$ by shedding one unit of contamination per day, which was assumed to be the same for all age categories. The environmental infectious pressure decayed over time by the daily decay rate ($N_{env} = 0.871$), based

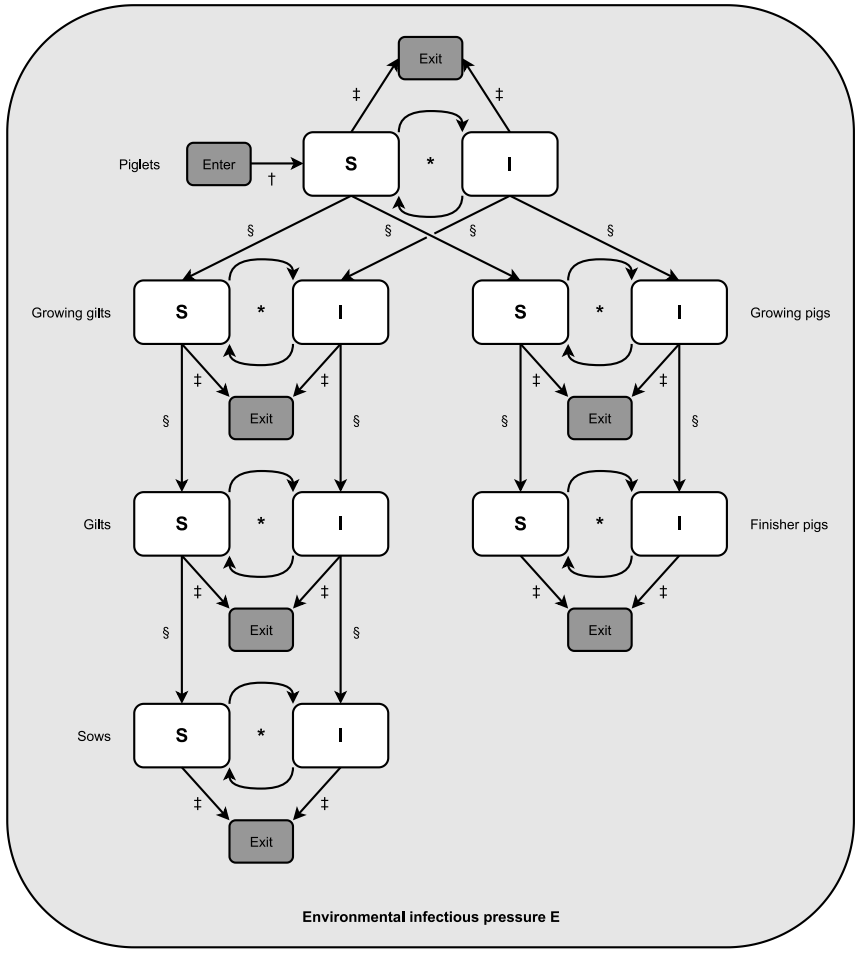

Fig. 1. Conceptual disease spread model for livestock-associated methicillin-resistant *Staphylococcus aureus* in a pig herd. Model has susceptible (S) and infected (I) disease states. Transitions from S to I are dependent on environmental infectious pressure (E). The environmental infectious pressure is specific for each individual node (pen) in the model. The herd consists of six different animal categories which are divided into twelve compartments depending on the animal production phase and disease status. A detailed description of the disease spread model, descriptions of transmission functions and model compartments are presented in Supplementary Figure S1, Supplementary Material S1 and Supplementary Table S1, respectively. *State transitions between S and I states. †Enter events. The birth of piglets into the model is controlled by a Poisson process after sows enter the farrowing room. ‡Exit events. Deaths and culling of animals from the model. §Predetermined scheduled ageing events.

on the reported half-life of LA-MRSA in dust of 5 days (Feld et al., 2018). The level of contamination was determined daily by the amount of existing contamination, the daily decay rate and the shedding from infected animals in the node. This change in the environmental infectious pressure (accumulation and decay) can be expressed as:

$$\frac{d\varphi_i}{dt} = \alpha \sum_j I_{i,j}(t) - \beta(t)\varphi_i(t) \quad (1)$$

where α is the shedding rate per day per infected individual and $I_{i,j}(t)$ is the number of infected individuals in node i and age group j at time t. The parameter $\varphi_i(t)$ is the environmental infectious pressure in node i at time t and $\beta(t)$ is the decay of $\varphi_i(t)$ over one day.

Parameterisation of transmission rates

Transmission rates based on large-scale sampling in Danish and Dutch herds have been presented in a previous study by Broens et al. (2012a). As this study included a transmission rate only for preweaned piglets and a total rate for all pigs in each herd, parameterisation was used to estimate the transmission rates for each different age group in the present model. Having separate animal group transmission rates was seen as justified, as it would help fit the possible age-dependent susceptibility.

The parameterisation was performed using approximate Bayesian computation (**ABC**; Sunnåker et al., 2013) included in the SimInf package. In this method, the simulated LA-MRSA prevalence in the model was compared against expected target values to produce best fitting transmission rates (see Supplementary Material S2). The simulated values were collected from the last year of the

model timespan, when LA-MRSA was at a steady state. The target values were obtained from the within-herd prevalences presented by Broens et al. (2012a) and used to obtain separate transmission rates for mature pigs (sows and gilts), suckling piglets, growing pigs and finishing pigs. The within-herd prevalences presented by Broens et al. (2012a) were assumed to be obtained when LA-MRSA was at steady state in the herds. Because the variation of within-herd prevalence in this data was high, i.e. originating from farms with very different prevalence levels, parameterisation was performed against three different target prevalence sets (low, medium and high), which are presented in Table 1.

To find suitable priors for the parameterisation, a preliminary evaluation was done by observing the effect of different transmission rates on LA-MRSA prevalence. Based on these observations, a prior range from 0.1×10^{-3} to 0.3 was used as a starting point for all three target prevalence sets (Table 1). For each generation of the ABC, two hundred accepted particles were acquired. An accepted particle refers to a set of four transmission rates for the respective animal age categories that are considered to produce suitable age-specific prevalences. Particles were accepted if the distance of the model output data was less than the tolerance of the ABC rejection function (Supplementary Material S2), which was reduced stepwise for each generation.

The model output distance was calculated from the sum of the squared differences of the prevalence at each of the time points presented in Table 1 over the last year of each model trajectory, where the model was at a steady state of prevalence. One model trajectory is a single random realisation of the simulated model output, in this case prevalence, over time. The parameterisation process for each target parameter set was halted when the latest produced generation took at least two days to process, or the number of proposed particles exceeded one million. The identified transmission rates obtained through parameterisation were used in the transmission functions, which, together with the recovery functions, are presented in Supplementary Material S1.

Within-herd animal flow

Production statistics for Swedish pig production

The presented model will be used to investigate interventions to control LA-MRSA in a Swedish context and therefore production statistics, such as the average number of piglets born per sow, return to oestrus rate and pig mortality, were obtained from the Winpig production monitoring programme's statistics provided by the Swedish Farm and Animal Health organisation (Farm and Animal Health, 2020a; 2020b). In 2017, the Winpig statistics covered 49% of the Swedish sow population and 14% of the total production of pigs grown for slaughter (Farm and Animal Health, 2019). Values such as group sizes and standard strategies for animal movement and mixing of pigs were obtained by interviewing three Swedish pig experts: one professor in pig medicine and two pig health practitioners.

Herd size and type

The conceptual model of the herd is based on a farrow-to-finish farm, which covers all the production phases in a herd. The herd was set to be closed (no animal influx from outside of the herd) as this is common practice in Sweden. The model was designed to be representative of a farrow-to-finish farm with approximately 500 sows in production.

Farm structure

The hierarchical structure of the farm—including units, sections and pens and the associated animal flow—is presented in Supplementary Figure S2. The herd was conceptually divided into six basic units: breeding, gestation, farrowing, growing, finishing and gilt units. The farrowing, growing and finishing units were further divided into several sections. 'Sections' can be interpreted as wall-separated rooms in a real farm. The growing unit had an additional buffer section, which represented the scenario where slow-growing pigs are moved to a separate room to grow for an extended period before they are moved to the finishing unit. Each section consisted of pens. These pens are nodes in the Siminf nomenclature, and they contained individuals in metapopulations from several model compartments (see section Infectious disease model). The breeding and gestation units consisted of separate pens for sows and gilts. The breeding unit also had separate buffer pens for both sows and gilts, which were a tool to manage the non-pregnant animals that were returning back to breeding.

The sections in the farrowing, growing and finishing units followed the all-in all-out principle, where each section was completely emptied before a new batch of animals entered. Farrowing occurred once a week, where one farrowing section was filled and another one emptied each week. Because of the all-in all-out system, pens that were emptied during a week stayed empty until the start of next production week (downtime period). Breeding, gestation and gilt units, as well as the grower buffer section, functioned as continuous flow, as only some of the pens within the same rooms were emptied and refilled each week. Individual pens in these units followed the all-in all-out principle. Further description of the housing in different production phases is presented in Supplementary Table S2.

Sow production cycle

The sow production cycle in the model was set to 155 days. The time spent in the breeding unit included the days from weaning to

Table 1
Target prevalence values in pigs for parameterisation of low, medium and high prevalence models.[1]

Sampling occasion[2]		Set[3]		
Name	Description	Low (%)	Medium (%)	High (%)
M1 sows	1 week before farrowing	8.0	40.9	84.3
M2 sows	3 days after farrowing	10.4	53.8	91.4
M2 piglets	3 days after birth	12.1	57.5	92.0
M3 sows	3 weeks after farrowing	13.7	67.1	94.1
M3 piglets	3 after birth	12.3	72.8	95.1
M4 growing pigs	6 weeks after birth	22.1	65.2	94.3
M5 growing pigs	10 weeks after birth	15.3	93.2	99.2
M6 finishing pigs	25 weeks after birth	19.0	70.3	94.1

[1] For each sampling occasion reported by Broens et al. (2012a), the 10th, 50th and 90th percentiles of prevalence were calculated and used to represent low, medium and high prevalence farms, respectively. These target values were used to fit age-specific transmission parameters in the disease spread model.
[2] Sampling occasion refers to the sampling moments as described by Broens et al. (2012a).
[3] Target prevalences were calculated from the within-herd prevalences reported by Broens et al. (2012a).

oestrus and the first 27 days of gestation. A pregnancy check was done on the 27th day in the breeding unit, where the probability of pregnancy failure (0.055) was equivalent to the average return-to-oestrus rate in the production statistics (Farm and Animal Health, 2020b). The non-pregnant animals were returned to the breeding buffer pens, and the pregnant animals were moved to the appropriate gestation section. Additionally, sows and gilts had a daily probability of reproductive failure during the first 28 days in gestation which was 0.0029 failures/sow per day, as described in Supplementary Material S3.

To mimic the routine of replacing part of the sow population in the herd, sows were removed from the herd at the time of weaning with a rate of 0.184 per weaned sow (Supplementary Material S3). Those sows and gilts that were found to be non-pregnant during gestation were randomly either returned to the breeding section or removed, where the probability of removal was 0.5. To maintain the target number of mature animals in the herd, the removed animals were replaced by new gilts from the gilt unit.

Pig growing cycle. At the time of farrowing, the sows and gilts were moved from the gestation to the farrowing unit. For simplicity, the farrowing events were set to occur one day later. The litter sizes were sampled from a Poisson distribution (λ = 14.8), based on the average reported number of live piglets born per litter (Farm and Animal Health, 2020b). Following weaning, 5% of the pigs in the section were transferred to the gilt unit and the rest were moved to a growing unit. After the growing period, pigs were moved either to a finisher section or to a grower buffer section. From the finishing unit, pigs were sent to slaughter on three occasions after spending 85, 92 or 99 days in the unit.

Mixing of pigs. As cross-fostering is routinely performed in many pig herds, this was included in the model. In the simulations, a baseline proportion of 10% of the piglets from each pen in the same farrowing section were randomly mixed one day after birth. When pigs in farms are moved from growing to the finishing unit, the slow-growing animals are often moved into a separate grower buffer section, or they might be left in the grower section and mixed with a new batch of growing pigs. In the model, 10% of animals from each grower pen were moved to a grower buffer section, while the rest of the pigs continued to the finishing unit. The pigs that were transferred straight from growing to finishing were mixed one day after the movement. This was implemented by randomly allocating the pigs into new pens. In the grower buffer section, pigs from the same grower section were placed together in the pens. After 23 days, they were merged together with the newest batch of finishing pigs, maintaining the original pen groups in the finishing unit.

Removal of pigs. Pigs can be removed from the herd in three ways: slaughter, euthanasia or death. Removal of animals by slaughter was simulated in the model as described in the Sow production cycle and Pig growing cycle sections. Euthanasia and death were assumed to be part of the mortality rates and were handled together using state transitions and scheduled events. The mortality rates for growing and finishing units were 0.0004 and 0.0002 mortalities per animal per day, respectively. This corresponded to the 2.0 and 1.7% mortalities reported in Swedish production statistics (Farm and Animal Health, 2020a; 2020b). The daily mortality rate for piglets was 0.006, which is based on the reported average total piglet mortality (17.7%) during the suckling period (Farm and Animal Health, 2020b). Calculations for the mortality rates are presented in Supplementary Material S4. Removal of sows and gilts was implemented as scheduled culling events as described in section Sow production cycle. Mortality in sows and gilts was not included in the model.

Model initialisation and run

For the baseline model, the model was run for 3 000 days over a total of 100 trajectories for the low, medium and high transmission parameter sets, where each trajectory is one random realisation of the model. The parameters were sampled from the accepted particles in the last generation of the ABC parameterisation for the corresponding model. In each trajectory, the herd was initiated by adding 22 susceptible gilts to the breeding unit on weekly intervals for a total of 21 weeks. The herd population had stabilised by model day 730. At this time point, the whole herd was infected by moving all animals from the susceptible to infected state from where the LA-MRSA prevalence settled to its steady state over time. The disease was initialised by infecting the entire herd to decrease the probability of disease die-out and to achieve a steady state of infection in all model trajectories.

Effect of mixing of pigs

In addition to the base model simulations, the impact of animal mixing on the LA-MRSA steady state prevalence in the farrowing and finishing units was investigated for all three transmission parameter sets (low, medium, and high). Simulations were completed by sampling parameters from the posterior of the final generation of each model presented in Table 2. In the finishing unit, the mixing of animals was turned off and the LA-MRSA prevalence in the unit was compared to the baseline model's full mixing practice. To investigate the impact of mixing in the farrowing unit (cross-fostering), the baseline LA-MRSA prevalence with 10% mixing of the piglets was compared with two other scenarios, where all piglets were mixed either one or two days after their birth.

Table 2
Parameterised median transmission rates in pigs with associated 95% credible intervals (in parentheses) and model fit values for the final generations of the approximate Bayesian computation (ABC) for the low, medium and high target parameter sets.

	Low set	Medium set	High set
Parameter estimates			
Mature	0.0010 (0.0009–0.0011)	0.0018 (0.0017–0.0020)	0.0071 (0.0065–0.0077)
Piglets	0.0020 (0.0019–0.0021)	0.0051 (0.0047–0.0056)	0.1337 (0.0833–0.2570)
Growing	0.0010 (0.0008–0.0012)	0.0035 (0.0030–0.0041)	0.0200 (0.0158–0.0249)
Finishing	0.0012 (0.0011 –0.0013)	0.0028 (0.0026–0.0030)	0.0140 (0.0124–0.0159)
Model fit			
Final generation tolerance	0.525	6.700	2.135
Proposed particles in final generation	37 586	233 563	1 197 796

Results

Parameterisation of transmission rates

The transmission rates obtained from parameterisation are presented in Fig. 2 and Table 2. The indicators of model fit are also presented in Table 2. The final generation tolerances are a measure of model fit and relate to how closely the model-predicted prevalence matched the targeted observations from the literature (Supplementary Material S2). A difference between the parameter estimates was defined as less than 5% overlap in the posterior density of the parameter distributions, corresponding to a lack of overlap in the 95% credible intervals (**CrI**). In all three parameter sets, the transmission rate for piglets differed from the other three animal groups, but in the high parameter set, the distribution of the identified values was very wide. In the low parameter set, there was no difference between the transmission rates for mature, growing and finishing pigs, whereas in the medium parameter set, the transmission rates differed for all animal groups. In the high set, the transmission rates for growing and finishing pigs overlapped, but the rate for mature pigs differed from the other transmission rates.

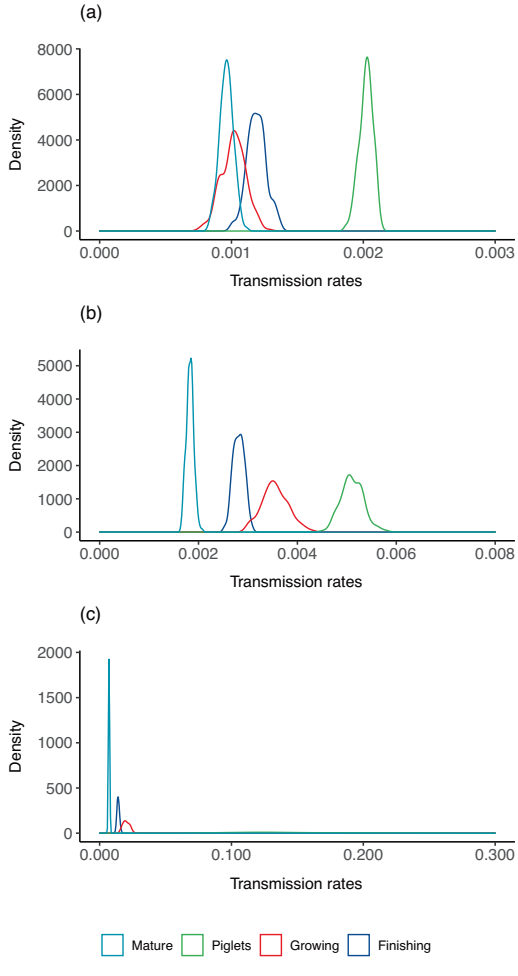

Fig. 2. The posterior densities of the parameterised transmission rates. Parameterisation was performed using approximate Bayesian computation (ABC) to estimate the four transmission rates for each pig age group (Mature, Piglets, Growing and Finishing pigs) against three different target prevalences: low (a), medium (b) and high (c).

Model within-herd prevalence

The model-predicted prevalences for the different animal groups, based on the transmission rates obtained through parameterisation, are presented in Fig. 3. The median prevalences in all three parameter sets for piglets, sows in the farrowing unit, growing pigs, and finishing pigs were similar to the target prevalence values presented in Table 1. The predicted prevalences of other mature animal groups could not be compared to the target prevalence values, as the available values for parameterisation included only sows from one week before farrowing to three weeks after farrowing, whereas Fig. 3 presents the within-herd prevalence for all different production phases of the mature animals.

Validation of the model animal flow

The model's production output was compared to the Swedish pig production statistics (Farm and Animal Health, 2020a; 2020b) to evaluate how well the animal flow reflected normal production. When run over 100 trajectories, the model farm produced on average 12 555 finishing pigs annually for slaughter, whereas an average Swedish farm with the same number of sows produces 13 350 pigs. The target total number of sows and gilts in the sow cycle was set to 500, and the result output is presented in Fig. 4a. The proportion of gilts relative to all breeding animals was 23%. In the production statistics, the proportion is presented as the proportion of gilt litters in the herd, which was on average 24.2%. The model reached a stable population structure within two years (Fig. 4); at this time point, the number of gilts and sows in each breeding cycle and the annual number of slaughtered finisher pigs both stabilised to the levels presented in the section Conceptual herd model.

Effect of mixing of pigs

Mixing of pigs in the finishing unit

When observing the difference in LA-MRSA prevalence between mixing and not mixing animals in the finishing unit, disabling mixing lowered the prevalence in the low transmission parameter set (Fig. 5) when LA-MRSA had reached steady state in the herd. For the low transmission parameter set, the median difference in prevalence between days 1 500 and 3 000 was 8.8% (95% CrI: [2.0–15.7%]). The prevalence was considered to differ because the credible interval did not include zero. This difference is also apparent in the lack of overlap in the 95% credible intervals illustrated in Fig. 5. Using the medium and high parameter sets, no difference in prevalence could be shown between the models with the different mixing scenarios (Supplementary Table S3).

Cross-fostering in the farrowing unit

When assessing the effect of cross-fostering on the transmission of LA-MRSA, no difference in the prevalence could be shown between different cross-fostering scenarios (Supplementary Table S4). This is illustrated by the overlapping credible intervals between different cross-fostering scenarios in Fig. 6. For clarity, as the results of mixing piglets one and two days after the birth were similar, mixing of the piglets two days after birth was excluded from Fig. 6. Complete results for all mixing scenarios are provided in Supplementary Table S5.

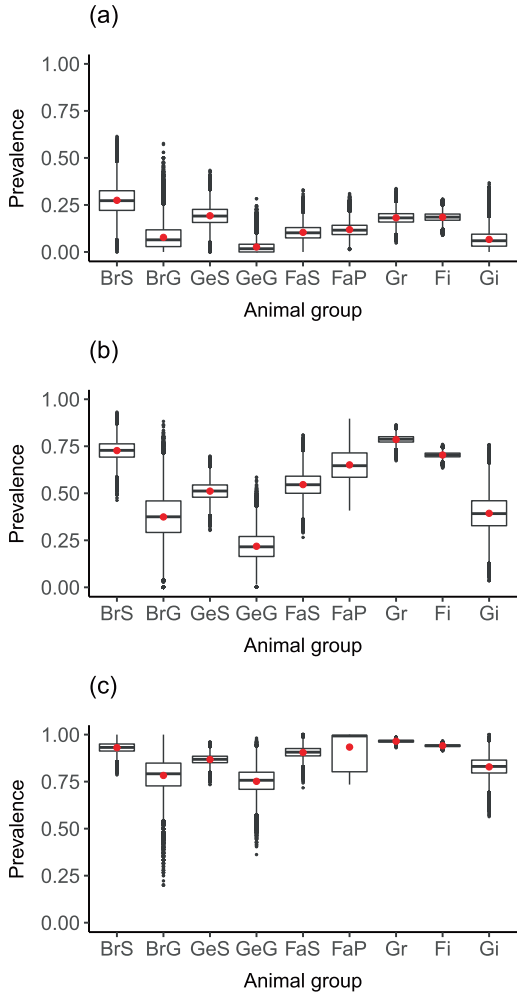

Fig. 3. Model-predicted within-herd livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) prevalence for different pig age groups when using the parameterised transmission rates. The transmission rates were classified into low (a), medium (b) and high (c) sets based on the target prevalences used in parameterisation. The prevalences were obtained over a period of 1 year (days 2 635–3 000) when LA-MRSA was in a steady state in the herd. The model was run over 1 000 trajectories. Mean prevalences are indicated with a red dot. LA-MRSA did not die out in the herd in any of the trajectories. Abbreviations: BrS = Breeding unit, sows; BrG = Breeding unit, gilts; GeS = Gestation unit, sows; GeG = Gestation unit, gilts; FaS = Farrowing unit, sows; FaP = Farrowing unit, piglets; Gr = Growing unit; Fi = Finishing unit; Gi = Gilt unit.

Discussion

Model structure and validation

This study presented a stochastic event-based model for simulating the environmentally mediated spread of LA-MRSA in pig herds in a Swedish context. In the study, the shedding of LA-MRSA was assumed to be intermittent. This differs from a previous modelling study where pigs could be either persistent or intermittent shedders (Sørensen et al., 2017). There is currently no scientific consensus on whether pigs can be persistent shedders of LA-MRSA or if they are being re-exposed to the bacteria either from the environment or by direct contact with other pigs. In humans, different *S. aureus* strains show varying degrees of persistence: persistent carriage of *S. aureus* has been described (Wertheim et al., 2005) but with LA-MRSA CC398, the possibility of re-colonisation with the same strain has not been ruled out (Goerge et al., 2017). Hence, without further experimental studies, it is not possible to ascertain whether the persistent carriage is a reality. As including environmentally mediated indirect transmission allowed LA-MRSA to persist in the herd, adding persistent shedders was not necessary in this modelling approach.

In this study, the entire herd was infected simultaneously for the purpose of finding a steady state of infection in the herd. This could impact the persistence of LA-MRSA as a smaller targeted introduction of the disease would result in a stochastic die-out of the disease from the herd in some cases. It was not known whether the prevalences used in parameterisation were from herds in a steady state with different disease dynamics, or if the sampled herds were from different phases of an epidemic of LA-MRSA. For the purpose of this study, it was assumed that the study herds were in a steady state which justifies the introduction of LA-MRSA into the entire herd simultaneously. Future work will investigate how the probability of LA-MRSA persistence in a pig herd is related to disease introduction intensity or introduction into specific age categories in the herd (e.g. purchased breeding stock).

In the current model, LA-MRSA was transmitted indirectly via the environment described by a single transmission term for each age category. These transmission parameters can be interpreted as the combined indirect and direct transmission that was required to achieve the LA-MRSA prevalences reported in the literature. This approach allowed the inclusion of environmental load in the model, reflecting how the infectious pressure can persist even when animals are not present in the pen. In a model with only direct transmission, the disease could not be perpetuated between animal groups subsequently housed in the same pens. Therefore, investigating environmental intervention strategies, such as cleaning or changing downtime between groups, would not be possible in a model with only direct transmission. One might argue that the inclusion of separate direct and indirect transmission in a spread model of LA-MRSA would be the best representation of the true disease dynamics. However, the reported observations of prevalence would not have allowed for separate parameters to be identified by the parameterisation method.

Based on the model output, the chosen values for animal housing and movements resulted in a realistic representation of a Swedish pig herd when comparing it to the Swedish pig production statistics. However, these statistics include only a portion of all Swedish herds, which limits their representativeness. On the other hand, the herds included in the statistics are mostly larger commercial herds, which are becoming more common while the total number of herds is decreasing in Sweden.

Parameterisation of transmission rates

Approximate Bayesian computation (ABC) was used to estimate the transmission rates for different animal age groups at three different prevalences. The ABC method is easy to implement, and it does not restrict the kind of model that can be fitted. However, ABC is not suitable for comparing models with different structures as increasing model complexity results in better fit without penalisation for the added complexity.

The transmission rates in the current study were estimated to fit previously published observational data by Broens et al. (2012a), which included six farrow-to-finish farms. The low

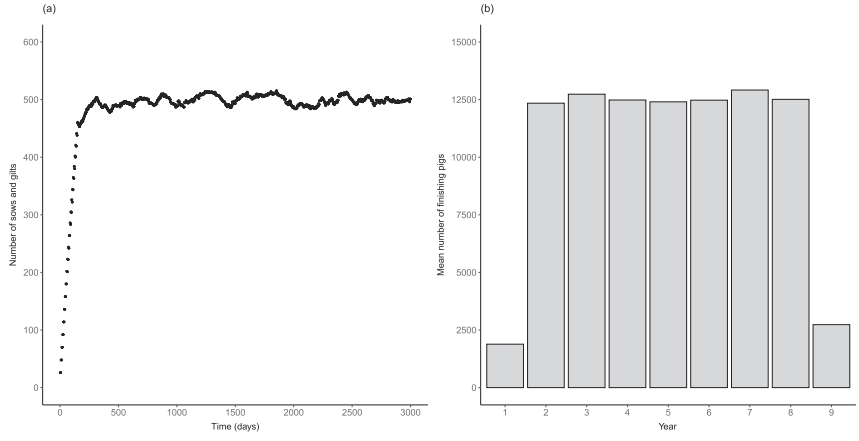

Fig. 4. Model-predicted mean number of sows and gilts in the pig herd over time (a) and the model-predicted mean number of finishing pigs slaughtered per year (b). The year-9 observation of the number of finishing pigs slaughtered included only 80 days. The model was run for 3 000 days and 100 trajectories.

number of farms in the study might limit the representativeness of the data. The within-herd prevalences reported by Broens et al. (2012a) are dependent on the diagnostic sensitivity of the used sampling methods, which was unknown in this case. However, the number of animals sampled in the study was high and several of the reported prevalences were approaching 100%. This would imply that the sensitivity of the sampling and testing methods were nearly perfect in these herds, assuming a test specificity of 100%.

In the current study, the parameterisation was done for three different target prevalence levels, as the within-herd prevalences reported by Broens et al. (2012a) varied between the farms. This could have been caused by either variables that were not included in the data (eg. differences in management practices) or that the herds were in different phases of an LA-MRSA outbreak. The current modelling approach is suitable for the first type of variation. More detailed data, including observations over time from the same herds, would be required to build more accurate models of the dynamics of LA-MRSA.

Based on the transmission rates obtained by the parameterisation, the piglet transmission rate differed from the other three transmission rates in all three (low, medium and high) parameter sets. Therefore, it was evident that a separate rate for preweaned piglets was necessary when aiming to fit the model to the observational data. However, in the high parameter set, the distribution of the fitted transmission rates for piglets was considerably wider than in the other transmission rates, reflecting the difficulty in obtaining a precise value through parameterisation for this rate. This could be driven by the high overall transmission level, where the transmission rate for piglets becomes less influential when the high prevalence of infection in mature animals leaks to the piglets in the farrowing pens. When observing the medium parameter set,

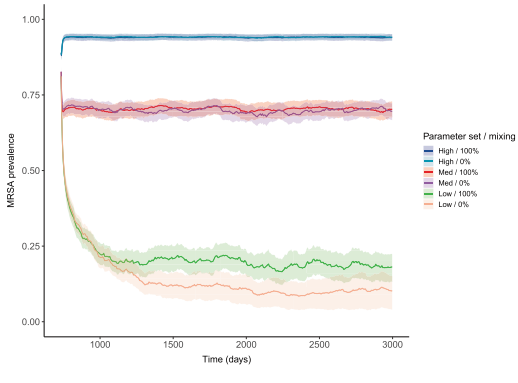

Fig. 5. Model-predicted mean livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) prevalence and associated 95% credible intervals in the finishing unit when 0 or 100% of pigs were mixed one day after arrival to the unit. Prevalence was simulated for three transmission parameter sets (low, medium [med], high). The model was run over 1 000 trajectories and all animals in the herd were infected at day 730 when the herd had reached its steady state. To assess the temporal variation of the production cycle, the mean and the credible intervals were plotted as the rolling mean over a 7-day period.

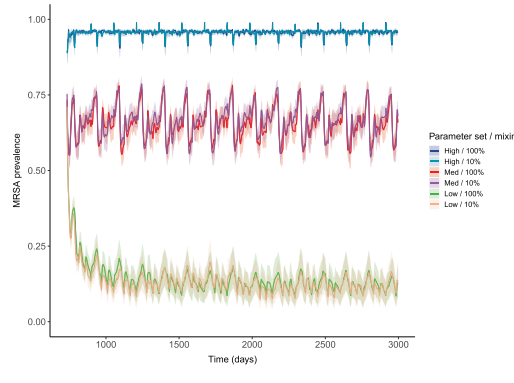

Fig. 6. Model-predicted mean livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) prevalence in the piglets in the farrowing unit and the associated 95% credible intervals when 10 or 100% of the piglets were mixed one day after birth. Prevalence was simulated for three transmission parameter sets (low, medium [med], high). The model was run over 1 000 trajectories and all animals in the herd were infected at day 730 when the herd had reached its steady state. To assess the temporal variation of the production cycle, the mean and the credible intervals were plotted as the rolling mean over a 7-day period.

using separate transmission rates for mature pigs, piglets, growing pigs and finishing pigs was justified, as in this set, the transmission rates for each animal group differed from each other. In the low parameter set, the lack of difference in transmission rates between age groups could be partially explained by the small numerical difference between the low target prevalences.

The model-predicted prevalence for piglets and sows in the farrowing unit and for pigs in growing and finishing units were comparable to the target values used for parameterisation. For other mature pigs (sows and gilts in the breeding, gestation, and gilt units), the model output varied among different animal groups. This could be explained by the limited number of sampling points in the parameterisation data which was focused only on farrowing sows. Interestingly, when model prevalence in sows was observed, all parameter sets indicated that LA-MRSA prevalence was higher in the breeding unit than in the farrowing unit, but the prevalence decreased again in the gestation unit. The higher prevalence in the sow breeding unit could be explained by the high prevalence in the farrowing unit—which is the origin of the animals in the sow breeding unit—and by the larger group sizes in the breeding pens. However, this phenomenon was absent when new gilts were moved from the gilt unit to breeding. This difference between gilts and sows in the breeding unit could be a consequence of the smaller group sizes of gilts, as well as the long gilt growing period prior the arrival to the breeding unit. During the growth period, the new gilts were housed in small fixed groups, which could have slowed down the spread of LA-MRSA. Similar to the sows, the prevalence among gilts decreased when the animals were moved to the gestation unit.

Effect of mixing of pigs

In addition to proposing a model and transmission rates of LA-MRSA in pigs, the effects of mixing pigs on the LA-MRSA prevalence in finishing pigs and cross-fostering piglets in the farrowing unit were investigated. In the finishing unit, removing the mixing of pigs at the time of entry to the unit had an effect on LA-MRSA prevalence when the low transmission parameter set was used. However, a similar effect was not observed with the medium and high parameter sets, and the difference between different mixing practices was smaller in the high than in the medium set. The lack of effect in the high parameter set could be explained by the high proportion of infected individuals entering the finishing unit, which overwhelmed the effect of reduced mixing. This finding indicates that, in circumstances of low disease spread, an intervention of reduced mixing in finishing pigs could be an effective reduction strategy.

In the cross-fostering scenarios, reducing or increasing the proportion of mixed animals did not have an effect on the LA-MRSA prevalence in any of the transmission parameter sets. Interestingly, performing the cross-fostering events one day later gave similar results, even though the piglets had more time to become infected. The lack of effect of both cross-fostering and mixing in finishing units can be linked to the way LA-MRSA is disseminated throughout the pens. If the infected individuals are spread uniformly over the pens in a section before the mixing events occur, mixing all the animals randomly will not substantially change the likelihood of an infected individual being added to pens that were previously free from infection. However, the difference in the effects of mixing between farrowing and finishing units could be explained by the different animal densities as well as different transmission rates due to the suspected higher susceptibility of piglets in the farrowing unit. It is also noteworthy that, unlike mixing in the finishing unit, cross-fostering was not completely turned off in the scenarios but only performed at the level commonly practised in Swedish herds.

Overall aspects

In case of an LA-MRSA outbreak, avoiding mixing in the finishing unit could be beneficial in reducing the prevalence when the LA-MRSA level is low in the herd. The practical importance of the observed reduction in prevalence (8.8%) would require a cost-benefit analysis also assessing the impact on human health. In other model scenarios, reducing the mixing as the only intervention strategy is not sufficient for reducing LA-MRSA prevalence. However, the study focused on the effect of reduced mixing when LA-MRSA had reached its steady state. The effect of the interventions could be different if performed earlier in an outbreak, perhaps even causing fade-out of LA-MRSA in the herd. Future work will investigate the effect of reduced mixing in different phases of an LA-MRSA outbreak or combining the reduced mixing with other interventions, for example, reduced environmental infectious load through thorough cleaning and disinfection.

Overall, further research on LA-MRSA transmission in different age groups and the relative role of indirect transmission are needed to fill the knowledge gaps and produce more accurate modelling results. With more observational data on indirect transmission, the model could be extended with between-pen transmission to simulate the animal contact between adjacent pens. In addition, the knowledge of LA-MRSA half-life in the environment is incomplete as previous knowledge is limited to analyses of dust collected from the barn air.

The advantage of this modelling approach was that including indirect transmission allowed the infection load to persist in the environment after the animals had been moved out from the pen. Using an event-based compartment model also provided a modelling framework that is faster and less resource-intensive than similar individual-based disease models. On the other hand, individual-based models make it possible to follow an individual animal and its status through the model, which is not possible in the current approach where the basic unit is the pen. From a control perspective, however, the status of individual animals is of less interest than the status at the group level or herd level.

This study presents a robust and flexible model with detailed herd representation and transmission through the environment. The model is a useful tool to investigate the effects of LA-MRSA and other infectious diseases in pig herds. The results show that using only transmission through the environment allows LA-MRSA to persist in the herd without assuming the presence of persistent shedders as has been previously suggested. The results also suggest that avoiding mixing of pigs in the finishing unit can reduce LA-MRSA prevalence in the herd when the within-herd prevalence is low. This study emphasises that there are still several substantial knowledge gaps regarding the transmission and shedding of LA-MRSA in pigs.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100450.

Ethics approval

Not applicable.

Data and model availability statement

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

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Declaration of interest

None.

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Modelling environmentally mediated spread of livestock-associated methicillin-resistant *Staphylococcus aureus* in a pig herd

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Animal journal

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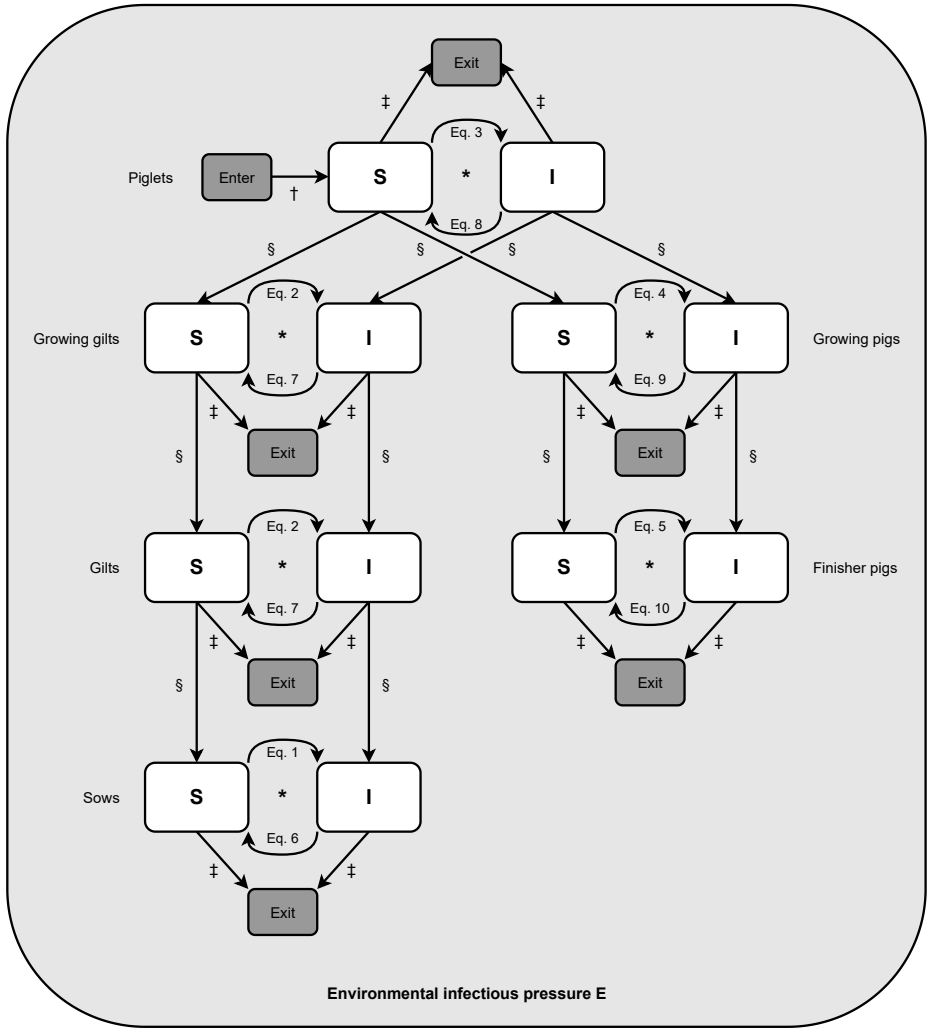
Supplementary Figure S1



Supplementary Figure S1: Detailed version of conceptual disease spread model of a pig herd as seen in section "Disease spread model". Model has susceptible (S) and infected (I) disease states. Transitions from S to I are dependent on environmental infectious pressure (E). The environmental infectious pressure is specific for each individual node (pen) in the model. The herd consists of six different animal categories which are divided into twelve compartments depending on their production phase and disease status. References to transmission functions (Eq) and model compartments are described in Supplementary Material S1 and Supplementary Table S1, respectively.

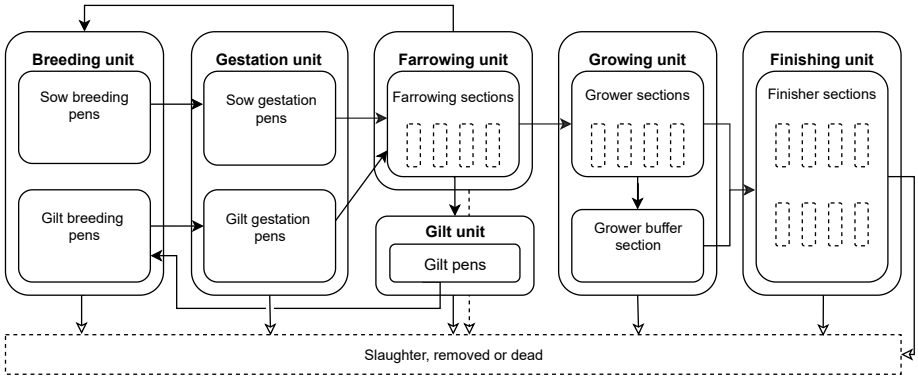
* State transitions between the S and I states.

† Enter events. The birth of piglets into the model is controlled by a Poisson process after sows enter the farrowing room.

‡ Exit events. Deaths and culling of animals from the model.

§ Pre-determined scheduled ageing events.

Supplementary Figure S2



Supplementary Figure S2: Conceptual representation of animal flows between units in the pig herd model. Boxes with dashed lines in farrowing, growing and finishing units indicate that the unit has several separate sections. The number of sections for each unit is specified in Supplementary Table S2. Individual nodes (pens) are not represented in this figure but are contained within each section or unit.

Supplementary Table S1

Supplementary Table S1: Description of the compartments in the pig herd model.

Compartment	Description
S_{sows}	Susceptible sows
I_{sows}	Infected sows
S_{gilts}	Susceptible gilts
I_{gilts}	Infected gilts
$S_{piglets}$	Susceptible piglets
$I_{piglets}$	Infected piglets
$S_{growers}$	Susceptible growing pigs
$I_{growers}$	Infected growing pigs
S_{finish}	Susceptible finishing pigs
I_{finish}	Infected finishing pigs

Sows: Adult animals which have farrowed at least once
Gilts: Pigs which are grown to be bred or have been bred but have not farrowed yet. Piglets that are grown to be new gilts get gilt status at the time of weaning.
Piglets: Small suckling pigs from the day of birth to weaning (0-35 days from birth)
Growing: Pigs from weaning to finishing (36-91 days from birth)
Finishing: Pigs from finishing to slaughter (92-177/184/191 days from birth).

Supplementary Table S2

Supplementary Table S2: Housing in different pig production phases in the model.

	Breeding	**Gestation**	**Farrowing**	**Growing**	**Growing buffer**	**Finishing**	**Gilts**
Time spent in unit (days)	32	88	35	56	22	85-99	Max. 216
Downtime period*	2	1	2	1	5	6	6
Animal type in pens	Sows, gilts	Sows, gilts	Sows, piglets	Growers	Growers	Finishers	Gilts
Housing type	Group	Group	1 sow with piglets	Group	Group	Group	Group
Number of sections	1	1	6	10	1	18	1
Pens per section	33†	65‡	26	26	26	30	25
Average number of animals in pen	17.4/5.7§	8.3/5.2§	1/13.4¶	11.6	9.8	10.0	6.4

The average number of animals in pen was calculated over 100 trajectories after the model reached steady state.
* Downtime period refers to the minimum time pens are kept empty before new animals are brought in.
† Section has 10 sow breeding pens, 8 gilt breeding pens, 5 sow breeding buffer pens, 10 gilt breeding buffer pens.
‡ Section has 35 sow gestation pens and 30 gilt gestation pens.
§ Values are separate for sows and gilts, respectively.
¶ Values are separate for sows and piglets, respectively.

Supplementary Table S3

The posterior distribution of prevalence from each mixing scenario was subtracted from the baseline scenario to generate a distribution of the differences in prevalence. If the 95% credible intervals of the difference did not include 0, this was interpreted as the scenario being different from the baseline (reference group).

Supplementary Table S3: The median difference in livestock-associated methicillin-resistant *Staphylococcus aureus* prevalence and 95% credible intervals between different pig mixing scenarios in finishing unit.

Parameter set	Mixing scenario	Reference group	Prevalence difference (%)		
			Median	2.5th percentile	97.5th percentile
Low	0%	100%	8.80	1.20	15.68
Medium	0%	100%	0.68	-3.48	4.88
High	0%	100%	-0.08	-1.63	1.46

The median difference and credible intervals were calculated from 1000 trajectories measured from days 1500-3000 in the model where the prevalence in the herd was in a steady state. The mixing scenario percentages refer to the percentage of pigs that are mixed on the day of entry to the finishing unit. In the reference group all pigs (100%) are mixed and in alternate, none are mixed (0%). Note that only the credible interval of the change in mixing in the low prevalence model does not include 0.

Supplementary Table S4

The posterior distribution of prevalence from each mixing scenario was subtracted from the baseline scenario to generate a distribution of the differences in prevalence. If the 95% credible intervals of the difference did not include 0, this was interpreted as the scenario being different from the baseline (reference group).

Supplementary Table S4: The median difference in livestock-associated methicillin-resistant *Staphylococcus aureus* prevalence and 95% credible intervals between different pig mixing scenarios in farrowing unit (cross-fostering).

Parameter set	Mixing scenario	Reference group	Prevalence difference (%)		
			Median	2.5th percentile	97.5th percentile
Low	100% / 1st day	10% / 1st day	0.23	-7.53	7.89
	100% / 2nd day		3.13	-4.65	11.46
Medium	100% / 1st day	10% / 1st day	-0.73	-7.66	5.96
	100% / 2nd day		-0.06	-5.55	5.45
High	100% / 1st day	10% / 1st day	0.00	-3.15	2.30
	100% / 2nd day		-0.03	-3.36	3.17

The median difference and credible intervals were calculated from 1000 trajectories measured from days 1500-3000 in the model where the prevalence in the herd was in a steady state. In the reference group 10% of the piglets are cross-fostered one day after farrowing while in the alternate scenarios are all the piglets (100%) are cross-fostered on either one or two days after farrowing. Note that all of the credible intervals of the difference in prevalence include 0.

Supplementary Table S5

Supplementary Table S5: Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) prevalence in cross-fostering of pigs.

Parameter set	Cross-fostering scenario	Prevalence (%)		
		Median	**2.5th percentile**	**97.5th percentile**
Low	10% / 1st day	12.21	7.83	17.14
	100% / 1st day	12.20	7.60	17.19
	100% / 2nd day	14.58	9.28	21.08
Medium	10% / 1st day	65.63	51.04	80.47
	100% / 1st day	64.98	50.85	80.36
	100% / 2nd day	65.61	50.90	80.51
High	10% / 1st day	95.64	77.17	99.08
	100% / 1st day	95.61	77.44	99.08
	100% / 2nd day	95.56	77.40	99.03

Median prevalence and credible intervals were calculated for farrowing unit over 1000 trajectories when LA-MRSA had reached steady state in the herd (days 2635-3000).

Supplementary Material S1

Transmission functions

Transmission through environment

$$S_{sows} \xrightarrow{\varphi_i(t)\cdot\beta_{mature}\cdot S_{sows}} I_{sows} \tag{1}$$

$$S_{gilts} \xrightarrow{\varphi_i(t)\cdot\beta_{mature}\cdot S_{gilts}} I_{gilts} \tag{2}$$

$$S_{piglets} \xrightarrow{\varphi_i(t)\cdot\beta_{piglet}\cdot S_{piglets}} I_{piglets} \tag{3}$$

$$S_{growers} \xrightarrow{\varphi_i(t)\cdot\beta_{growing}\cdot S_{growers}} I_{growers} \tag{4}$$

$$S_{finishers} \xrightarrow{\varphi_i(t)\cdot\beta_{finishing}\cdot S_{finishers}} I_{finishers} \tag{5}$$

Recovery

$$I_{sows} \xrightarrow{\frac{I_{sows}}{D}} S_{sows} \tag{6}$$

$$I_{gilts} \xrightarrow{\frac{I_{gilts}}{D}} S_{gilts} \tag{7}$$

$$I_{piglets} \xrightarrow{\frac{I_{piglets}}{D}} S_{piglets} \tag{8}$$

$$I_{growers} \xrightarrow{\frac{I_{growers}}{D}} S_{growers} \tag{9}$$

$$I_{finishers} \xrightarrow{\frac{I_{finishers}}{D}} S_{finishers} \tag{10}$$

Descriptions of the parameters used in the transmission functions

Parameter	Description
$\varphi_i(t)$	Environmental infectious pressure in node i at time t
β_{mature}	Indirect transmission rate for adult pigs
β_{piglet}	Indirect transmission rate for piglets
$\beta_{growing}$	Indirect transmission rate for growing pigs
$\beta_{finishing}$	Indirect transmission rate for finishing pigs
D	Duration of carriage
$\beta(t)$	Decay rate of environmental infectious pressure
S_{sows}	The number of susceptible sows in a node
I_{sows}	The number of infected sows in a node
S_{gilts}	The number of susceptible gilts in a node
I_{gilts}	The number of infected gilts in a node
$S_{piglets}$	The number of susceptible piglets in a node
$I_{piglets}$	The number of infected piglets in a node
$S_{growers}$	The number of susceptible grower aged pigs in a node
$I_{growers}$	The number of infected grower aged pigs in a node
$S_{finishers}$	The number of susceptible finisher aged pigs in a node
$I_{finishers}$	The number of infected finisher aged pigs in a node

Supplementary Material S2

Calculation of the acceptance function for the approximate Bayesian computation

For each generation of the Approximate Bayesian Computation (ABC), particles (sets of parameters) are tested against published data (the expectation). To determine if a particle was accepted or rejected, the simulated trajectory for that particle had to have a score in the acceptance function below a tolerance level. The tolerance was decreased for each generation. The score for a trajectory was calculated using the least squares method (Eq. 11). The sum of the squared differences of the observed prevalence was calculated at 508 points during the last year of the model trajectory. This was assumed to be at steady state of disease.

$$\sum_{t,j}(observed - expected)^2 \tag{11}$$

where t represented the time points for comparison and j the age categories for which the comparison was made. The scale of this score is dependant of the number of comparison points (508). A rough interpretation of the fit of the model to the expected data is:

$$fit = \sqrt{score/508} \tag{12}$$

The overall fit of the "low" model can be interpreted to be $\sqrt{0.525/508} \approx 0.03$, indicating that the accepted particles in the final generation of the model produced average prevalances within 3% of the expected values over the 508 comparison points. Similarly, the "medium" and "high" models were within $\sqrt{6.7/508} \approx 0.11$ and $\sqrt{2.135/508} \approx 0.06$ of the expected values.

Supplementary Material S3

Sow production cycle

Removal of sows during gestation

The average farrowing rate in Swedish herds is 86.8% according to the production statistics (Farm and Animal Health, 2020). When this was combined with the probability of pregnancy failure (5.5%) at the pregnancy check in breeding unit, 7.7% of the sows and gilts are required to exit the normal breeding cycle during gestation. The most common reasons for this were estimated to be the false-positive detection of pregnancy and resorption of the embryo, and the likelihood of this failure would be higher within the first 28 days spent in the gestation unit.

The daily probability of reproductive failure per sow per day during the first 28 days in the gestation unit was calculated from the exponential growth function:

$$N(t) = N_0 e^{-\lambda t} \tag{13}$$

where N(t) is the number of animals left at time t, N_0 is the number of animals at t = 0 and λ the rate constant.

The rate (λ) was calculated for N(t) = 1 - 0.077 at t=28 as follows:

$$\lambda = -\frac{log(1 - 0.077)}{t} = 0.0029 \tag{14}$$

Removal of sows at weaning

According to Swedish production statistics, 24.2% of the litters are born from gilts (Farm and Animal Health, 2020). The proportion of gilt litters is controlled by the sow replacement rate which was set to 42% per year. The removal rate used in the model at the time of weaning (0.184 per weaned sow) was based on the average annual number of litters per sow (2.23) and the average annual removal rate without reproductive disorders (36.18%) as seen in Engblom et al. (2007).

Replacing removed sows and gilts

In Sweden, it is a common practice for the farms to raise their own gilts (expert opinion). In the model, gilts from the gilt unit were brought into the breeding unit when more animals were needed to supplement the removed sows. The number of new gilts (n_{new}) was dependent on the farrowing section animal capacity (n_{FA}^{pens}), the average farrowing rate (FR), the number of sows returning from farrowing to breeding (n_{FA}^{sow}), as well as the number of previously non-pregnant sows and gilts in breeding buffer pens (n_{BRB}^{sow} and n_{BRB}^{gilt}, respectively):

$$n_{new} = n_{FA}^{pens} \cdot FR - (n_{FA}^{sow} + n_{BRB}^{sow} + n_{BRB}^{gilt}) \tag{15}$$

Excess growing gilts were removed from the herd to slaughter when they reached the maximum time spent in the unit (Supplementary Table S2).

References

Farm and Animal Health, 2020. Smågrisproduktion årsmedeltal (in Swedish). Retrieved on 20 August 2021 from https://www.gardochdjurhalsan.se/wp-content/uploads/2020/05/smagrisprod-medel-2019_25.pdf.
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Supplementary Material S4

Removal of pigs

The mortality rates were calculated from exponential growth function (Eq. 13 in Supplementary Material S3), where t = time spent in the respective unit (Supplementary Table S2) and the reduction of animals as described in subsection Removal of pigs in the main article.

Growing pig mortality rate:

$$\lambda = -\frac{log(1 - 0.02)}{56} = 0.0004 \tag{16}$$

Finishing pig mortality rate:

$$\lambda = -\frac{log(1 - 0.017)}{99} = 0.0002 \tag{17}$$

Piglet mortality rate:

$$\lambda = -\frac{log(1 - 0.177)}{35} = 0.006 \tag{18}$$

II

Perspectives of on-farm biosecurity and disease prevention among selected pig veterinarians and pig farmers in Sweden

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Submitted manuscript

Abstract

Background
Biosecurity is important in preventing the spread of infectious diseases in animal production. Previous studies have identified a disparity between the biosecurity recommendations provided by veterinarians and the actual practices implemented by farmers. This study compares group discussions with a few key actors among Swedish pig veterinarians and farmers on their perceptions of pig farm biosecurity.

Methods
Two separate focus group discussions were conducted, one with five Swedish pig veterinarians and one with three pig farmers, to explore their views on pig farm biosecurity and efficient biosecurity measures. The discussions were analysed to identify differences and similarities in how biosecurity was perceived in these two groups.

Results
The study identified differences between these veterinarians and pig farmers in how they perceived good biosecurity and the level of biosecurity in Swedish pig herds. The veterinarians perceived that adhering strictly to the farming system and its barriers is essential for good biosecurity. The biosecurity in the pig farms was often considered inadequate, and the veterinarians described difficulties in biosecurity-related communication with the farmers. The pig farmers valued the flexibility of the farming system over strict barriers and described that the level of biosecurity is good in Swedish pig herds. However, both groups also shared similar views regarding to the challenges in farm biosecurity, and they highlighted that biosecurity measures with proven efficacy are important for farmer motivation.

Conclusions
This limited study suggests that different perspectives on biosecurity can contribute to the communication difficulties between pig farmers and veterinarians. Acknowledging both the differences and similarities of the different perspectives may help improve cooperation and communication in biosecurity-related questions.

Introduction

Biosecurity plays an important role in preventing infectious animal diseases. Voluntary programmes, as well as legal requirements for biosecurity plans on animal holdings, are becoming increasingly common [1, 2]. In pig production, many diseases can be prevented and controlled by internal and external biosecurity measures [3]. Although these measures are biologically well-founded [4], the value of each individual measure is difficult to assess, and risk assessment models have been developed to address this challenge [5, 6]. These models confirm that combinations of measures are required to reduce the risk of introduction and spread of infections within pig farms. The variability in risk for different diseases, different farms and different transmission routes presents a challenge in itself when it comes to motivating individual farmers to implement preventive measures [4]. The implementation is further affected by several factors, such as the farmer's personality, gender, age, education level and access to information [7-9]. Successful disease prevention is difficult to measure, as its result is the absence of an event that might or might not have occurred without the preventive effort. Hence, motivating biosecurity routines is challenging, and several studies indicate that the implementation of on-farm biosecurity measures is often inadequate [10-12]. Different drivers and priorities have been noted in farmers' perspectives of biosecurity [13, 14] and that of veterinarians and farm advisors [15].

Social science researchers have argued for a multifaceted understanding of farmers' biosecurity practices, which take into account the farmers' local knowledge [16-18]. Although farmers tend to be very concerned about diseases, their practices do not always follow veterinary advice [19]. One aspect of this pertains to conflicting ideals of 'good farming'. Shortall et al [20] show how the ideals of the

traditional and independent stockkeeper tend to be in conflict with the ideals of the large commercial farmer, who in turn tend to align more with the veterinarians' ideals of farm biosecurity. Moya et al [18] argue that an understanding of farmers' biosecurity practices as inadequate is reductionist, since it does not account for how traditions are combined with the implementation of official recommendations. Veterinary advice is only one of many elements that farmers take into account in their work. Shortall [17] writes, 'It is not the case that farmers operate in a "knowledge vacuum" that vets attempt to fill'. In the farmers' everyday practice, veterinary advice is combined with other sources of knowledge, their own values and what is actually doable on the farm. Moreover, several researchers argue that what is 'doable' is to recognise and, to some extent, accept the existence of biosecurity threats [21, 22].

Summing up, previous research shows that discrepancies between veterinary biosecurity recommendations and farmers' practices are common, which might create tensions in the relationship between these actors. To explore this, the current paper compares group discussions with some key actors among Swedish pig veterinarians and pig farmers faced with similar challenges relating to pig health.

Methods

Two focus group discussions [23] were organised with key actors among Swedish pig veterinarians and Swedish pig farmers, respectively. The original purpose was to elicit information about feasible interventions against livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA), as a basis for selecting interventions to test in a model of this zoonotic pathogen. Therefore, we chose to talk to key actors with vast knowledge of Swedish pig production. The veterinarians were selected by direct invitation to pig veterinarians working with the veterinary advisory services Farm and Animal Health. This is the main advisory organisation for pig farmers, covering the majority of all commercial pig farms in Sweden, and their veterinarians have a strong contact net among Swedish pig veterinarians and a large impact on their field practices. The organisation runs a national biosecurity scheme, in addition to several disease-specific control programmes. The farmers were selected by inviting the entire board of the Swedish Pig Farmers Association, that is, the national representatives of this farmer group. The final selection of participants depended on who could participate on the date and time that suited most of them, resulting in five veterinarians (two female, three male) and three pig farmers (one female, two male). All the veterinarians had long experience in working with pig health and participation in the development and implementation of national pig health programmes. The farmers were all large pig producers with farrow-to-finish herds, with long-standing knowledge of Swedish pig production in general, and were used to representing their profession in different discussions on national level. The participants lived in different parts of Sweden, in the areas where most pig farms are located.

Both meetings were conducted via Zoom (Zoom Video Communications, Inc. San José, USA), on two consecutive days. The meetings were facilitated by the first author, based on a predetermined discussion guide (Supporting Information S1). Before the start of the discussion, all participants were informed about the purpose of the discussion, that the meeting would be recorded, that they would remain anonymous in all publications and presentations of the results, and that they could withdraw from the study at any time. The recording was only started after consent had been given by all participants.

As the aim was to understand what interventions would be feasible in pig farms, the discussions were initiated around this subject, specifically mentioning LA-MRSA and the possibility of its detection in Swedish pig herds. Participants were given a few examples of potential interventions to stop the within-herd spread and asked to think of other means to achieve this and discuss the feasibility of different measures in Swedish pig herds.

The recorded discussions were transcribed and the transcripts were analysed manually. First, the transcripts were read in full and empirical codes were created. As the codes differed between veterinarians and farmers and constituted two different ways of describing good biosecurity, we ordered data according to these two approaches, conceptualised as: *Staying true to the system* and *Flexibility*. In addition, the detailed issues which were described similarly in the two groups were coded and organised into the following themes: *Developments in the pig industry, Motivating actions, and Individual drivers*.

Results

Different perspectives on biosecurity

Veterinarians: stay true to the system

In the veterinarians' discussion, an initial narrative of Swedish pig farming as characterised by several shortcomings in biosecurity emerged. The veterinarians described it as difficult to communicate with farmers about these shortcomings; that the pig farmers don't understand them. For example, one veterinarian described a feeling of speaking a completely different language. A recurrent theme in the veterinarians' descriptions was that biosecurity is challenged by farmers' tendency to not stay true to the 'system'. With the term 'system', the veterinarians referred both to how

pigs are organised into different age groups that are kept separate and how production is carried out in a batchwise 'all in, all out' principle. One veterinarian stated, and the others agreed:

> 'This, upholding a batchwise and sectioned breeding, that is what I see that they are sloppy with everywhere today.'

The veterinarians described the system-breaking practices as occurring on all kinds of pig farms. Even on the farms described as 'aware' and the 'best' in relation to biosecurity, the system is continuously challenged:

> 'It was great, it was like super, they are excellent. They have this fantastic production and very good biosecurity in many ways. It's just that they still move pigs around.'

Throughout the discussion, the veterinarians described 'the system' as a set of rigid rules that should never be broken. One veterinarian said:

> 'When you choose a system then you can't just say that I want other rules than the system requires… You can't have stragglers all the time that have to be moved backwards in the system. You can't have one sow farrowing at the wrong time… I think this applies to many infectious diseases, we manage them by having a very controlled production. So the foundation, or part of it, is to really control the production and not accept any exemptions.'

For the veterinarians, the primary function of the system is to stop the spread of disease:

> 'I also think that we have the system with sectioned, batchwise, production to streamline, but most of all to keep diseases at bay.'

A common breach of the system, as described by the veterinarians, is to assemble pigs that have not reached the expected slaughter weight in a 'buffer section'. By this, some farmers create a separate system that opposes the original system. As the quote below indicates, these buffer sections were described as extremely problematic for biosecurity:

> 'We reduced the respiratory problems in a holding, where we've sampled a lot and now it was negative practically all the way to the fattening units. But they also have buffer units, and it was like a bomb, all of it.'

The veterinarians brought up another example of challenging the system, bringing in nursing sows when the litters are too large. Staying true to the system would mean that the piglets that cannot be fed by their biological mother would instead be euthanised, and the pigs that do not gain weight like the others would be culled or placed in a separate production line.

> 'I'm thinking that one intervention that would be very effective is to euthanise all that deviate from the norm…at every stage. … Generally, it's a very economic attitude.'

Staying true to the system is central for veterinarians, which was evident when they discussed vaccinations. While vaccinations reduce the risk of disease, they can be problematic since they might encourage farmers to ignore the separation of animals.

> 'More vaccines, that's both an advantage and a disadvantage: "A little mycoplasma, we no longer need to keep sectioning because of that, we have good vaccines. Or lawsonia, we'll vaccinate so it goes away".'

The veterinarians also described that farmers did not only challenge the system by moving pigs, but also that the staff tend to ignore hygiene routines and thereby spread pathogens between different stables. The veterinarians described such routines as easy to follow, thus the non-compliance was perceived as irrational.

> 'I have a good example where we had problems with respiratory infections in the growing units, and we introduced a total change of clothing when they entered and the respiratory problems basically vanished... Quite simple measure, really. Some boots hanging outside, pants that they put on, easy… You don't have one employee per section… it can be practically an autostrada back and forth. They go and help each other and whatever between sections and that will not turn out well… But what they have in place now is that they finish all the time. We've tried to find simple things.'

Moreover, the veterinarians described how farmers tend to implement hygiene routines after a disease outbreak has occurred when it, according to the veterinarians, is too late.

> 'Making them understand the internal biosecurity… it's very, very, unusual, I feel, that they change clothes and boots between different age groups, and it almost takes an outbreak… to make it happen. Even though we know it would be desirable.'

The veterinarians also mentioned their different roles:

> 'We're both inspector and advisor and it's about the balance. But there is still a difference to when a salesperson comes and tells you things and when it's actually your own vet. '

The veterinarians felt alone in prioritising biosecurity and disease prevention, while other actors are focusing on production output and short-term financial gains.

> ‘It’s our own agricultural experts, in our own organisation, they don’t understand us, we don’t speak a common language.’

Farmers: flexibility of the system, safeguarding other values

In the discussion with pig producers, a different perspective on biosecurity emerged. Biosecurity was described as generally good in Swedish pig production:

> ‘I think… that the awareness among us pig producers about the benefits of good biosecurity, it’s probably, like, very high.’

The producers described prevention of disease spread as always present in their own and their colleagues’ minds:

> ‘But you always carry the thought with you: How can I avoid transmission of the infection.’

The producers described that practices of mixing animals from different sections and groups exist on their farms. However, this was expressed in very different terms compared to the veterinarians: Firstly, the producers described themselves as well aware of the disease risks.

> ‘But everything must also work practically. Everything must run smoothly, so one does as well as one can… you never move pigs that have a problem. You mustn’t move pigs backwards in the system.’

Secondly, they emphasised how disease control sometimes conflicts with other values. They stated that it is sometimes necessary to mix pigs despite the risks. A recurring perspective in the producers’ discussion was their other priorities in addition to disease control, and thus they sometimes make conscious deviations from biosecurity routines.

One example was when a producer described balancing the risk of disease against the gains from buying animals.

> ‘We chose the risk of getting something contagious against the benefit of upgrading our breeding stock… after discussing back and forth we arrived at, if we implement quarantine and choose holdings with a good health status the risks should be relatively low, but we’ll see if this was right.’

Importantly, as the quote below illustrates, the disease control might, according to the producers, conflict with the ‘optimal system for the pigs’.

> ‘Sometimes it may be that… biosecurity is in conflict with the optimal system for the pigs… If you have a gilt that is a little thin and has large piglets then you may want to wean them a week early and mix the piglets with another group to, well, make it as good as possible for the animals… we do those things every now and then but we’re aware of that it isn’t good for the internal biosecurity. But that’s a balance you must make.’

Like the veterinarians, the producers described that sometimes a disease outbreak is needed to follow hygiene routines strictly. However, in contrast with the veterinarians, the producers did not describe this as a flawed form of practice, but as natural:

> ‘Of course, the more infections that circulate the more you care, it’s quite natural. Then it’s like… maybe you don’t care as long as everything works and you don’t have any major problems. But perhaps… the details maybe you address first when you have the knife against your throat and there is a problem.’

Moreover, the farmers described that following basic hygiene routines is not always easy. For example when disease prevention conflicts with the staff’s social need to have breaks together:

> ‘Well we have top status but it’s not always easy to comply with all the time in practice… it’s mainly the external biosecurity that I find difficult, for example the staff wouldn’t be allowed to have coffee together… without changing clothes, and it feels like the risks of getting a disease may be larger elsewhere.’

Topics of concern for both groups

Developments in the pig industry

Although the discussions in the two groups were characterised by differences, we could also identify similarities. Both groups highlighted challenges related to steadily increasing farm sizes and genetic advances leading to larger litters. But while the veterinarians worried about shorter farrowing intervals jeopardising the fundamental idea of the batchwise system, the farmers mentioned the opportunities to mix batches and create better welfare for the sows.

As mentioned above, the veterinarians presented the genetic developments leading to larger litter sizes as a challenge to the system as it creates a need for nursing sows. The farmers also mentioned this, but they primarily described it as an issue related to older buildings. They talked about possible solutions in new systems, with larger pens and milking cups for supplementary feeding. Both groups expressed concerns about the genetic development leading to higher animal density.

> Veterinarian: ‘It gets full, full, full, they have growing pens built for ten pigs but now there are fifteen… more pigs that are all nice and even and grow at the same rate.’

> Farmer: 'If you look at the production the pigs are 10 kg heavier than 15 years back in time. The slaughter weight has increased… so that you have almost one more pig in the pens than what you did back then.'

Both farmers and veterinarians also mentioned the challenges of trying to expand and increase herd size with old buildings, as compared to building new houses with larger pens.

Motivating actions

Both groups highlighted the importance of being able to show the positive effects of biosecurity. The farmers mentioned follow-up of indicators during veterinary herd visits, using their own data to show their staff how biosecurity breaches may be detrimental, and to celebrate when good work paid off.

> 'Those are things we can celebrate, when we have broken the record in number of weaned, when we had zero treated sows, like, and the numbers of lameness, and piglet diarrhoea, and treatments of diarrhoea among the growers. You follow up and see "Why was there a peak there"… just that you have an awareness and can discuss about why it is like this or that.'

The veterinarians acknowledged that it might be hard to motivate improved biosecurity when the results are not visible. They wished for a 'litmus test' to demonstrate how infections spread through a herd, and shared experiences of successful herd interventions with demonstrable results and redeemable problems discovered by simple follow-up of disease data.

The veterinarians proposed that individual production indicators could be problematic, e.g. striving for low piglet mortality might lead to keeping runt pigs that must be managed by either mixing with subsequent batches or in a buffer pen:

> 'Again, we're talking about mortality, that is, mortality up until weaning. We're talking percentages and you're bad if you have a high mortality. So, it's wrong, counterproductive goals that are set up, in my opinion.'

Paradoxically, as suggested by one of the veterinarians, the good animal health status in Sweden may reduce the incentives for biosecurity. As described above, both groups recognised a disease outbreak as one of the strongest incentives for strict biosecurity measures.

Regarding the original purpose of the meetings, which was to gain knowledge about feasible interventions to reduce LA-MRSA, the participants found it difficult to propose any measures. However, although some challenges were mentioned, the farmers stated that almost any intervention could be implemented if a positive effect could be expected.

> 'If only someone gives a clear directive, I think any farm can do it… if you know what result you can expect. Of course, if you know that you really can eradicate something by making a strong effort then I think most people could do very much.'

Despite the assurance that this was purely theoretical, the veterinarians were hesitant to suggest any interventions of which they had no experience or evidence, reflecting the guiding principles of veterinary practice.

Good life beyond biosecurity

While recognising the need for economic profit, both groups acknowledged the fact that biosecurity is not the only priority for the farmers. Preventing disease was described as important, but sometimes in conflict with a 'good farmer's life', which was also important to the farmers.

> 'It's a balance. We have to live with this as well… it mustn't become… it may be secure, but it must also be nice…'

The veterinarians also to some extent recognised the need for the farmers to prioritise other things in life than animal disease control. As described above, this was presented mainly as a challenge, but also as an opportunity.

> 'I like it in a way, this that "Yes I want a good life, that's better than lots of money"… It's us who need to find out, what are the goals on this holding? … how can I incentivise my advice?

Discussion

In this small study, the farmers described Swedish pig herds as having good biosecurity. This statement was supported by the generally low disease prevalence and low use of antimicrobial drugs. Still, they acknowledged that there might be room for improvement. Previous studies have pointed out that on a general level, Swedish farms do have room for improvement in their biosecurity [24, 25]. In contrast to the farmers, the veterinarians took a more negative stance on the current status of farm biosecurity, describing it as inadequate and that communicating with farmers about biosecurity as difficult.

The seemingly different views in the two groups could be due to within-group dynamics during the discussions. It could also be related to the veterinarians perceiving biosecurity as an essential tool in animal disease control, which is one of the main interests of the veterinary profession, while for the farmers it is simply one of many aspects of good animal husbandry. The participating veterinarians are experienced animal health advisors, and hence expected to be highly aware of biosecurity aspects in animal production [26]. For the farmers, many other

things beyond disease prevention and control, for example, to 'have a good life', are important and influence their decisions. However, the diverging perceptions on the state of biosecurity, and the communication challenges, can be linked to veterinarians and farmers having different ways of describing good biosecurity. For the veterinarians, staying true to the system and not breaking barriers between different groups of pigs were framed as key. The farmers described flexibility as crucial, both for securing overall biosecurity and preserving other values. In line with previous research, producers thus described a tendency to accept and 'live with' biosecurity threats, in a way that is not recognised by official recommendations [21, 22]. That farmers need to be flexible and adaptive, and that rigid rules are problematic for them, have been described previously [27].

Although the discussions were characterised by differences in perspectives on biosecurity and biosecurity-related issues, we also identified similarities. For example, the veterinarians to some extent appreciated farmers' desire to have a good life and recognised that the farmers saw the importance of healthy, happy pigs. Consequently, their discussion also highlighted the need to come up with biosecurity advice that is not too onerous, and that the health and well-being of the pigs could be used as incentives.

The farmers implied that they were willing to implement any necessary disease control measures if the measures were proven to be effective. It is likely that farmers are concerned about the time or monetary investments that interventions might require and the costs of biosecurity and disease prevention measures have been identified as barriers to implementing disease prevention measures [8, 28]. However, guaranteeing the success of any control measure is impossible.

The small number of interviewed participants limits the conclusions that can be made based on the discussions and this may be regarded as a pilot investigation. We chose to include representatives from the veterinary advisors and producers who are key actors in the Swedish pig industry, to elicit information from actors used to discuss biosecurity on the industry level as well as on individual farms. The original purpose was to understand what was seen as feasible on-farm interventions in case of an infectious disease outbreak and hence we were seeking informants with experience from both farm and national levels. The veterinarians were experienced pig veterinarians working with preventive animal health and the farmers were experienced producers and representatives of the pig producers' organisation.

The focus of the discussions turned quickly to various aspects of biosecurity, partly guided by the facilitator but also governed by the participants, indicating that this is a topic of high interest for both parties. Despite the highlighted diversities, there was an underlying agreement about important challenges and end goals, but these were phrased differently in the respective groups. These observations may provide inspiration for future research and discussions about communication between veterinarians and farmers.

Conclusion

This limited study may form the basis for future investigations. On the one hand, the discussions in the two groups were characterised by differences in perspectives on biosecurity and biosecurity-related issues. In particular, the two groups described what good biosecurity is in different ways. For the veterinarians, staying true to the system and never breaking the barriers separating groups and sections of pigs was framed as key, while farmers described flexibility towards such systems as crucial, both for securing overall biosecurity and for preserving other kinds of values.

On the other hand, we also identified similarities between the groups, especially regarding how improvements to biosecurity could be motivated. Taken together, we suggest that the different perspectives on biosecurity can partly explain difficulties in communication between farmers and veterinarians, as well as the lack of implementation of official biosecurity policies in farms. However, we also argue that it is important to acknowledge not only the differences between farmers' and veterinarians' perspectives, but also identify the similarities, as these can provide the common ground for cooperation and improvement.

Author contributions

All authors contributed to the planning and execution of this study. All authors participated in the focus group discussions, which were facilitated by Hedvig Gröndal. Susanna Sternberg Lewerin and Hedvig Gröndal analysed and interpreted the data. All authors contributed to authorship and approved the final version.

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Conflicts of interest

The authors declare they have no conflicts of interest.

Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. According to national legislation, no ethical approval was required. All participants were informed of the purpose and the recording prior to the discussions. Consent was recorded for all participants.

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

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ABSTRACT

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

Implications

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

Introduction

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

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

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

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

Material and methods

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

Model structure

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

Model extensions

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

Between-pen transmission

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

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

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

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

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

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

Distribution of the infectious time period

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

Model parameters

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

Control measures

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

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

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

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

Improved biosecurity

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

Disease surveillance

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

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

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

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

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

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

Cleaning the environment

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

Mixing of pigs

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

Extended empty period in pens

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

Data analysis

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

Results

Parameterisation of transmission rates and model validation

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

Control measures

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

Improved biosecurity

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

Disease surveillance

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

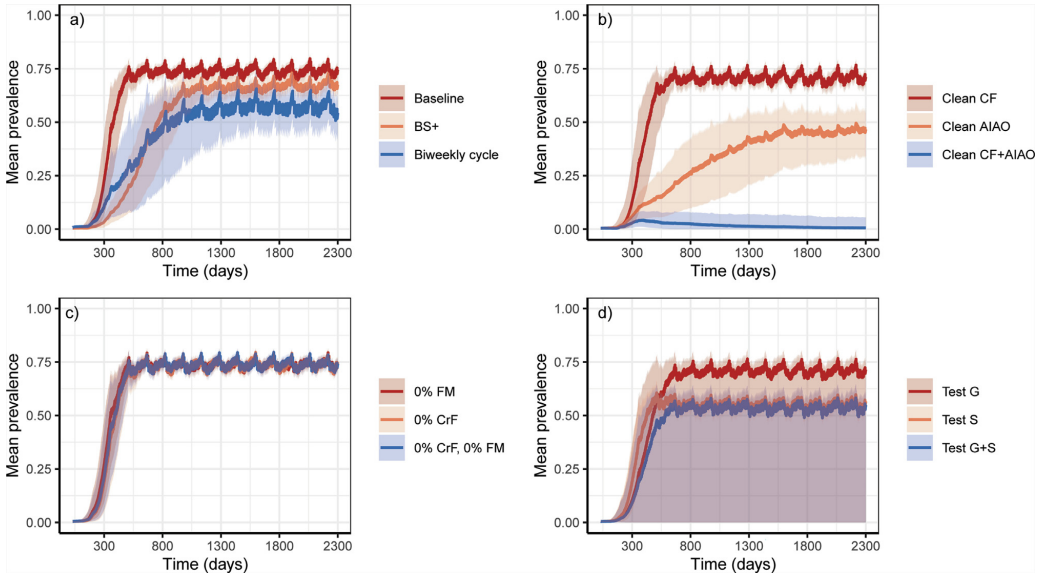

Fig. 1. The model-predicted mean prevalence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in a pig herd when single control measures were introduced in the disease outbreak phase. The lines represent the within-herd prevalence and the ribbons the corresponding 95% credible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

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

Cleaning the environment

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

Mixing of pigs

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

Extended empty period in pens

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

Probability of disease elimination

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

Discussion

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

Control measures

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

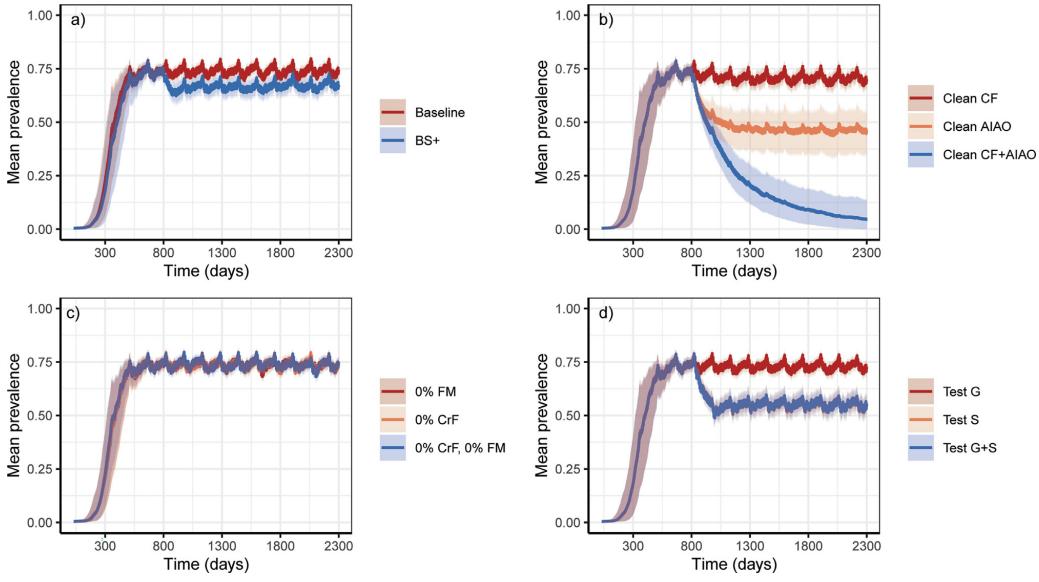

Fig. 2. The model-predicted mean prevalence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in a pig herd when single control measures were introduced in the endemic phase of disease spread. The lines represent the within-herd prevalence and the ribbons the corresponding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

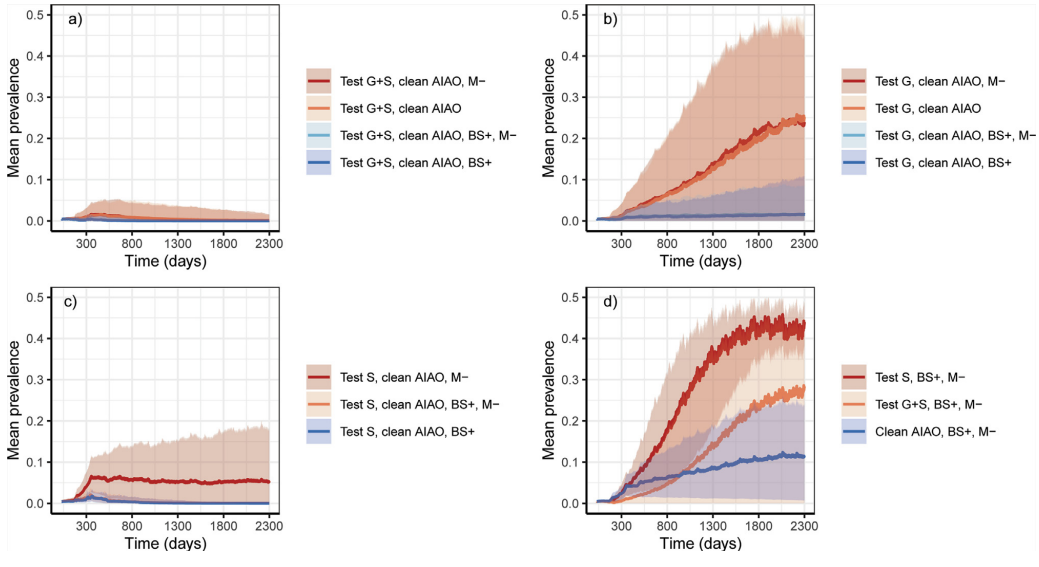

Fig. 3. The model-predicted mean prevalence of livestock-associated methicillin-resistant *Staphylococcus aureus* in a pig herd when combined control measures were introduced in the outbreak phase of disease spread. The lines represent the within-herd prevalence and the ribbons the corresponding 95%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

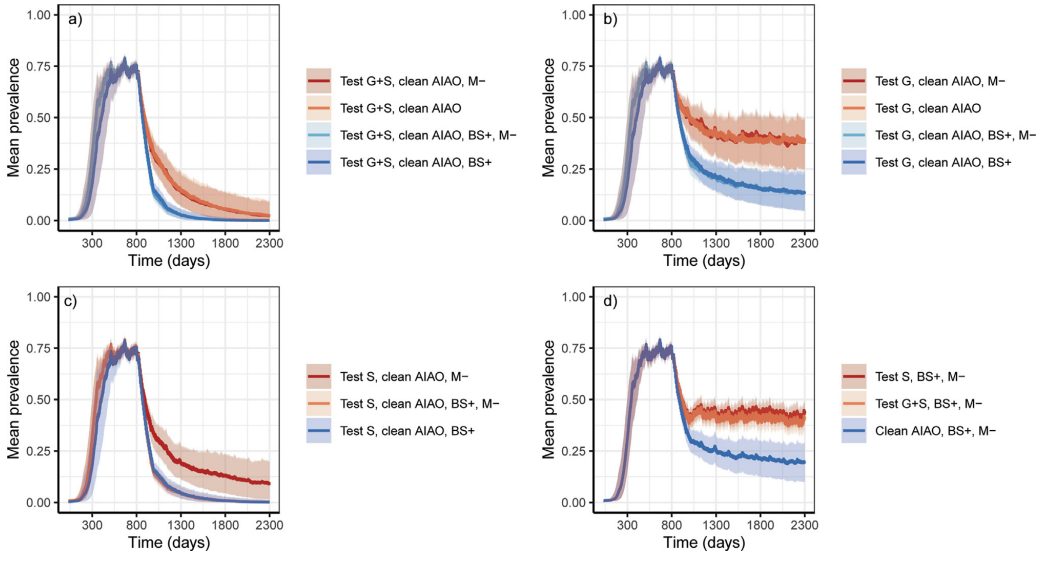

Fig. 4. The model-predicted mean prevalence of livestock-associated methicillin-resistant *Staphylococcus aureus* in a pig herd when combined control measures were introduced in the endemic phase of disease spread. The lines represent the within-herd prevalence and the ribbons the corresponding 95% credible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

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

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

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

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

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

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

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

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

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

Time to disease elimination

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

Limitations

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

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

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

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

Overall aspects

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

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

Supplementary material

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

Ethics approval

Not applicable.

Data and model availability statement

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

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Declaration of interest

None.

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

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Supplementary Figure S1

Model fit

The fit of the parameterised livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) transmission rates was evaluated by comparing the model-predicted prevalences at each time point and pig category against the expected prevalences from the literature (Broens et al., 2012). The comparison was done by running 1 000 trajectories of the fitted model and for each trajectory subtracting the expected prevalence from the model prevalence.

The compared time points were:

1. Sows when they enter the farrowing unit
2. Sows three days after entering the farrowing unit
3. Piglets three days after entering the farrowing unit
4. Sows three weeks after entering the farrowing unit
5. Piglets three weeks after entering the farrowing unit
6. Pigs 7 days after weaning
7. Pigs 35 days after weaning
8. Pigs 84 days after moving to the finishing unit

The results are presented in Supplementary Figure S1 below. This indicates that the model fit varies between categories of pigs and ages. The model fits well at the herd level but the model-predicted prevalence is higher than expected in both piglets and sows prior to weaning (3 weeks after entering the farrowing unit).

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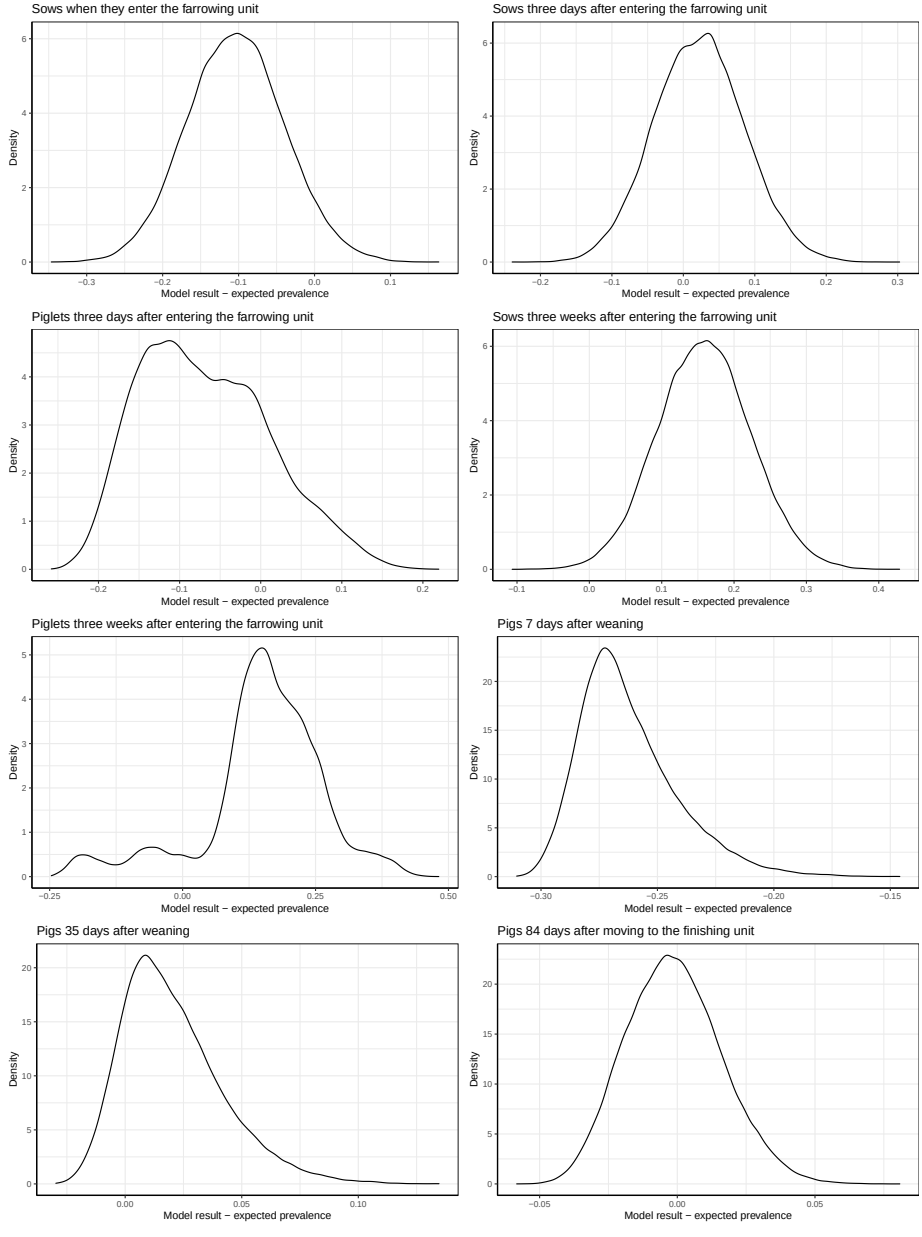
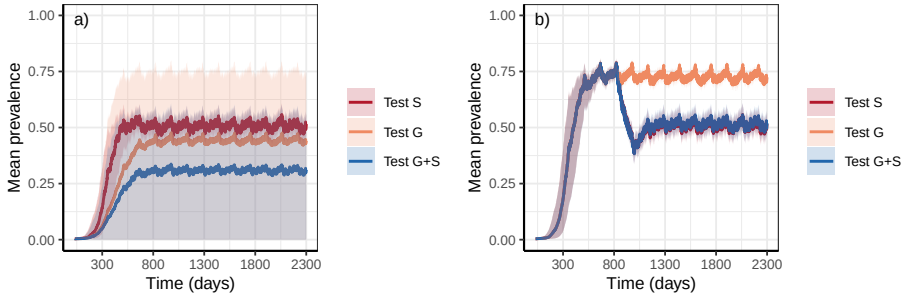


Supplementary Figure S1: The posterior densities of the difference between model-predicted and expected prevalences in 8 pig categories. The x-axis represents the difference in model-predicted prevalence and expected prevalence (e.g. 0 = no difference in the model-predicted prevalence and expected prevalence; 0.1 = the model-predicted prevalence is 10% higher than the expected prevalence; -0.1 = the model-predicted prevalence is 10% lower than the expected prevalence.)

Supplementary Figure S2



Supplementary Figure S2: The model-predicted mean prevalence of livestock-associated methicillin-resistant *Staphylococcus aureus* in a pig herd when disease surveillance with 100% test sensitivity was used. The disease testing was scheduled 2 days before sows and/or new gilts were moved to the breeding unit. The lines represent the mean within-herd prevalence and the ribbons the corresponding 95% credible interval. Each model was run for 10 000 trajectories.
a) control measures introduced at disease outbreak phase
b) control measures introduced at endemic phase of disease spread
Abbreviations: S = sows; G = gilts; G+S = gilts and sows.

Supplementary Table S1

Supplementary Table S1: The probability of elimination of livestock-associated methicillin-resistant *Staphylococcus aureus* and the mean time to elimination in the pig herd model when different control measures were introduced at endemic phase of disease spread. Only control measures that had >0% probability of elimination are included in the table. Each control measure was run for 10 000 trajectories per scenario. The mean time to elimination was calculated from the day of disease introduction to the herd. For surveillance control measures (testing), diagnostic test sensitivity was 70%.

Control measure	Mean time (days) to elimination	Probability of elimination (%)
Test G+S, clean CF and AIAO, BS+, M-	1392	100
Test G+S, clean CF and AIAO, M-	1794	94.25
Test G+S, clean AIAO, BS+, M-	1786	92.6
Test G+S, clean AIAO, BS+	1768	94.4
Test G+S, clean AIAO, M-	2092	16.13
Test G+S, clean AIAO	2077	15.35
Test sows, clean AIAO, BS+, M-	1979	64.53
Test sows, clean AIAO, BS+	1972	66.23
Test sows, clean AIAO, M-	2138	0.17
Clean CF and AIAO	2147	2.39

Abbreviations: BS+ = improved biosecurity (between-pen transmission reduced to 0); M- = cross-fostering and finishing mixing reduced to 0; AIAO = all-in all-out pens; G+S = gilts and sows; CF = continuous flow pens.

Supplementary Table S2

Supplementary Table S2: The probability of elimination of livestock-associated methicillin-resistant *Staphylococcus aureus* and the mean time to elimination in the pig herd model when disease surveillance was done with 100% diagnostic test sensitivity. The surveillance control measures were introduced either at the outbreak or endemic phase of the disease spread.

Control measure	Time	Mean time (days) to elimination	Probability of elimination (%)
Test G+S	outbreak	293	40.09
Test G+S	endemic	-	0
Test sows	outbreak	-	0
Test sows	endemic	-	0
Test gilts	outbreak	288	39.14
Test gilts	endemic	-	0

Abbreviations: G+S = gilts and sows.

Supplementary Material S1

Transmission through environment

$$\varphi_{tot}(t) = \varphi_i(t) + C_r[\varphi_r(t) - \varphi_i(t)] + C_r C_f[\varphi_f(t) - \varphi_r(t)] \tag{1}$$

$$S_{sows} \xrightarrow{\varphi_{tot}(t)\cdot\beta_{mature}\cdot S_{sows}} I_{1,\,sows} \tag{2}$$

$$S_{gilts} \xrightarrow{\varphi_{tot}(t)\cdot\beta_{mature}\cdot S_{gilts}} I_{1,\,gilts} \tag{3}$$

$$S_{piglets} \xrightarrow{\varphi_{tot}(t)\cdot\beta_{piglets}\cdot S_{piglets}} I_{1,\,piglets} \tag{4}$$

$$S_{growers} \xrightarrow{\varphi_{tot}(t)\cdot\beta_{growing}\cdot S_{growers}} I_{1,\,growers} \tag{5}$$

$$S_{finishers} \xrightarrow{\varphi_{tot}(t)\cdot\beta_{finishing}\cdot S_{finishers}} I_{1,\,finishers} \tag{6}$$

Erlang distributed recovery

$$I_{1,\,sows} \xrightarrow{\frac{1}{3D}\cdot I_{1,\,sows}} I_{2,\,sows} \tag{7}$$

$$I_{1,\,gilts} \xrightarrow{\frac{1}{3D}\cdot I_{1,\,gilts}} I_{2,\,gilts} \tag{8}$$

$$I_{1,\,piglets} \xrightarrow{\frac{1}{3D}\cdot I_{1,\,piglets}} I_{2,\,piglets} \tag{9}$$

$$I_{1,\,growers} \xrightarrow{\frac{1}{3D}\cdot I_{1,\,growers}} I_{2,\,growers} \tag{10}$$

$$I_{1,\,finishers} \xrightarrow{\frac{1}{3D}\cdot I_{1,\,finishers}} I_{2,\,finishers} \tag{11}$$

$$I_{2,\,sows} \xrightarrow{\frac{1}{3D}\cdot I_{2,\,sows}} I_{3,\,sows} \tag{12}$$

$$I_{2,\,gilts} \xrightarrow{\frac{1}{3D}\cdot I_{2,\,gilts}} I_{3,\,gilts} \tag{13}$$

$$I_{2,\,piglets} \xrightarrow{\frac{1}{3D}\cdot I_{2,\,piglets}} I_{3,\,piglets} \tag{14}$$

$$I_{2,\,growers} \xrightarrow{\frac{1}{3D}\cdot I_{2,\,growers}} I_{3,\,growers} \tag{15}$$

$$I_{2,\,finishers} \xrightarrow{\frac{1}{3D}\cdot I_{2,\,finishers}} I_{3,\,finishers} \tag{16}$$

$$I_{3,\,sows} \xrightarrow{\frac{1}{3D}\cdot I_{3,\,sows}} S_{sows} \tag{17}$$

$$I_{3,\,gilts} \xrightarrow{\frac{1}{3D}\cdot I_{3,\,gilts}} S_{gilts} \tag{18}$$

$$I_{3,\,piglets} \xrightarrow{\frac{I_{3,\,piglets}}{D}} S_{piglets} \tag{19}$$

$$I_{3,\,growers} \xrightarrow{\frac{I_{3,\,growers}}{D}} S_{growers} \tag{20}$$

$$I_{3,\,finishers} \xrightarrow{\frac{I_{3,\,finishers}}{D}} S_{finishers} \tag{21}$$

Descriptions of the parameters used in the transmission functions

Parameter	Description
$\varphi_{tot}(t)$	Total infectious pressure in pen i at time t
$\varphi_i(t)$	Environmental infectious pressure in pen i at time t
$\varphi_r(t)$	Environmental infectious pressure of all pens ($\varphi_i(t)$) in room r at time t
$\varphi_f(t)$	Environmental infectious pressure of all rooms ($\varphi_r(t)$) in the farm at time t
C_r	Scale factor for within-room transmission
C_f	Scale factor for within-farm transmission
D	Duration of carriage
β_{mature}	Indirect transmission rate for adult pigs
β_{piglet}	Indirect transmission rate for piglets
$\beta_{growing}$	Indirect transmission rate for growing pigs
$\beta_{finishing}$	Indirect transmission rate for finishing pigs
S_{sows}	The number of susceptible sows in a node
$I_{x,\,sows}$	The number of sows in infected category x in a node
S_{gilts}	The number of susceptible gilts in a node
$I_{x,\,gilts}$	The number of gilts in infected category x in a node
$S_{piglets}$	The number of susceptible piglets in a node
$I_{x,\,piglets}$	The number of piglets in infected category x in a node
$S_{growers}$	The number of susceptible grower pigs in a node
$I_{x,\,growers}$	The number of grower pigs in infected category x in a node
$S_{finishers}$	The number of susceptible finisher pigs in a node
$I_{x,\,finishers}$	The number of finisher pigs in infected category x in a node

Supplementary Material S2

Calculation of the acceptance function for the approximate Bayesian computation

The Approximate Bayesian Computation (ABC) was applied at the model steady state after the initial burn-in period. For each generation of the ABC, particles (sets of parameters) were tested against published data (the expectation). A particle was accepted if the simulated trajectory for the particle received a score in the acceptance function that was below the tolerance level. The score for a trajectory was calculated by using the equation (22):

$$\sum_{j}^{t} \sqrt{\frac{\sum_{i}^{npens_j} (\frac{ob_{ij} - ex_{ij}}{ex_{ij}})^2}{npens_j}}, \tag{22}$$

where t represents the time points for comparison (the time points are presented in Supplementary Figure S1), j the age categories for which the comparison was made and $npens_j$ the number of pens in each age category. The ob_{ij} describes the individual observed value per age category and ex_{ij} the corresponding expected value per age category.

IV

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RESEARCH

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Survival of livestock-associated methicillin-resistant *Staphylococcus aureus* CC398 on different surface materials

Krista Tuominen[1*], Sara Frosth[1], Karl Pedersen[2], Thomas Rosendal[3] and Susanna Sternberg Lewerin[1]

Abstract

Background Zoonotic livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is widely spread in pig herds in many countries. However, the knowledge regarding the survival of LA-MRSA in the pig farm environment is currently limited. The aim of this study was to assess the survival of LA-MRSA on different surface materials found in the farm environment. The study investigated the survival of two different LA-MRSA strains belonging to the clonal complex (CC) 398 on four different surfaces: stainless steel, polypropylene plastic, K30 concrete and commercial concrete disk coupons. The survival of the bacteria over time was determined by the viable count method and, where possible, fitting a model to the observed data by using nonlinear least squares method to calculate the half-life ($t_{1/2}$) for different strain and material combinations.

Results The study showed that the half-life of the bacteria was longer on polypropylene plastic ($t_{1/2}$=11.08–15.78 days) than on stainless steel ($t_{1/2}$=2.45–7.83 days). On these materials, both LA-MRSA strains survived through the 14 week observation period. The bacterial decay was fastest on the concrete surfaces, where LA-MRSA became undetectable after 3–9 weeks.

Conclusions The survival of LA-MRSA in the pig farm environment may be affected by different surface materials. A more frequent sampling protocol (< 7 days) is needed to determine the half-life on concrete surfaces.

Keywords Bacterial decay, Concrete, Environment, Half-life, LA-MRSA, Plastic, Steel

Background

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) belonging to clonal complex (CC) 398 is a zoonotic pathogen that colonizes several animal species with pigs being one of its main reservoirs [1, 2]. Even though LA-MRSA rarely causes clinical infections in pigs, it poses a public health risk especially to those working with livestock [3, 4]. However, the spread of LA-MRSA is not limited to direct contact between humans and pigs as spillover to persons without livestock contact has also been reported [5–7]. While the relative contribution of indirect transmission within a pig herd is uncertain, previous studies have proposed that transmission through the environment plays a part in the spread of LA-MRSA [8–10]. Bioaerosols have been proposed as one possible route of environmental transmission of LA-MRSA [9] as well as the contamination of the environment, feed and material [11]. Additionally, humans working at or visiting pig farms are possible sources of LA-MRSA introduction to the herd [12]. A study by Feld

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et al. [13] assessed the survival of LA-MRSA in the dust of pig farms, but the survival on different surface materials commonly found in a pig farm is largely unknown.

The survival of bacteria is dependent on nutrients and external factors such as temperature, humidity, pH and oxygen concentration, but the optimal environment varies between different bacteria. The temperature and pH range for the growth of *S. aureus* in general is 7–48 °C and 4.0–10.0, respectively [14]. According to previous studies, *S. aureus* survives better in lower relative humidity (34%) and the survival declines as the relative humidity increases [15, 16]. *Staphylococcus aureus* is also capable of forming biofilms, which improves its survival in challenging conditions [17, 18].

The survival can vary between different *S. aureus* strains, which suggests that the intrinsic factors of the bacteria are also important. In a study of nosocomial MRSA by Wagenvoort et al. [19], outbreak isolates were found to survive longer than isolates from sporadic cases, where the outbreak isolates were reported to survive up to 6–9 months in *in-vitro* environment. In another study, a nosocomial MRSA strain was found to survive longer than a methicillin-susceptible *S. aureus* (MSSA) strain and the presence of dust of hospital origin was found to increase the survival times for both MRSA and MSSA [20]. The effect of surface materials on the survival of *S. aureus* has been previously studied in a hospital setting, where *S. aureus* remained viable for at least 1 week on all tested hospital surface materials [21]. However, whether or not the survival of LA-MRSA differs from the hospital-associated MSSA and MRSA strains is largely unknown.

This study focused on investigating the survival time of LA-MRSA strains belonging to CC398 on different surface materials (polypropylene plastic, stainless steel and concrete) that are commonly found in indoor pig facilities. Plastic, such as polypropylene, is used in surfaces such as slatted floors, pen dividers and equipment, while stainless steel is used in crate structures as well as in feeders and water nipples. Concrete is widely used as a flooring material in pig farms and can be considered as a harsh material to bacteria due its alkaline, dry and salty properties [22].

The aim of the study was to provide input parameters for an ongoing modelling study and to support decision making regarding the control and sanitation practices against LA-MRSA. To achieve this, the survival of LA-MRSA CC398 was measured on different materials commonly used in the pig farm environment.

Methods

Surface materials

Four different surface materials were used in the study: stainless steel, polypropylene plastic (PP) and two types of concrete. The stainless steel was in the form of sterile 15 mm diameter custom made disks (EN 1.4301; DHinox AB, Uppsala, Sweden) and the polypropylene plastic was screw caps from sterile 15 ml centrifuge tubes (Sarstedt, Nümbrecht, Germany). The two types of concrete were 12.7 mm diameter concrete coated polycarbonate disc coupons (BioSurface Technologies, Bozeman, Montana, USA) and large concrete cylinders. The concrete in the disk coupons was type ½ Portland cement with 200-micron foundry sand as aggregate material. The concrete cylinders were type K30, which is the most common type of concrete used in Swedish pig farms (personal communication, president of Swedish Pig Farmers' Association). The cylinders were broken into pieces of approximately 5–7 cm in diameter. All materials were autoclaved prior to inoculation.

Bacterial strains

Two different *S. aureus* CC398 strains were used to contaminate the surface materials. Both strains (S1 and S2) originated from two Danish field studies: the S1 strain was obtained from the SPACE project [23] and the S2 strain from the BioVir project [24]. The identifiers for the strains were "SPACE sek. 1 gr. 3 hold 1 3/10-19 B10 MRSA 16/10-19" and "BioVir 5b/S3/control W52 sow 6 pig 2", respectively. Both strains were LA-MRSA CC398 and belonged to spa-type t034.

The strains had been stored at − 70 °C in Brain Heart Infusion (BHI) broth (CM1135; Thermo Fisher Scientific Inc., Waltham, MA, USA) with 15% glycerol, before they were subcultured twice on 5% bovine blood agar (B341960; National Veterinary Institute, Uppsala, Sweden) and on selective Oxoid Brilliance MRSA 2 agar (PO5310A, Thermo Fisher Scientific Inc.).

Bacterial counts

The plates were incubated at 37 °C for 24 h. One colony of each subcultured strain was inoculated into 50 mL of BHI broth (Thermo Fisher Scientific Inc.) and incubated at 37 °C for 24 h. A viable count was performed by plating 100 µL of each broth culture as ten-fold serial dilutions on the selective agar as well as on the 5% bovine blood agar to compare the results between different media. The plates were incubated at 37 °C for 48 h (read after 24 h and 48 h).

From each broth culture, 100 µL was applied to the different surface materials. The bacterial concentrations of the broths are presented in Table 1. The contaminated steel, plastic and concrete disk coupons were placed on petri dishes (92 × 16 mm; Sarstedt). The large concrete pieces were stored in 1000 mL polypropylene sample containers (VWR International, Leuven, Belgium). All samples were air-dried for 4 h and then stored on a

Table 1 The estimated concentration of LA-MRSA in broth and in the initial material samples

Material	Strain	Plate[a]	Broth (CFU/mL)	Sample (CFU/mL)
Steel	S1	BA	8.80×10^9	6.60×10^9
		MRSA	7.20×10^9	1.34×10^9
	S2	BA	1.33×10^{10}	8.23×10^9
		MRSA	5.50×10^9	1.48×10^9
Plastic	S1	BA	8.80×10^9	1.33×10^{10}
		MRSA	7.20×10^9	1.47×10^9
	S2	BA	1.33×10^{10}	7.43×10^9
		MRSA	5.50×10^9	8.57×10^8
Concrete disk	S1	BA	1.59×10^{10}	3.60×10^9
		MRSA	7.30×10^9	1.86×10^9
	S2	BA	1.48×10^{10}	4.40×10^9
		MRSA	1.03×10^{10}	1.53×10^9
Concrete piece	S1	BA	1.06×10^{10}	2.16×10^9
		MRSA	7.70×10^9	2.18×10^8
	S2	BA	1.17×10^{10}	5.90×10^8
		MRSA	6.60×10^9	2.26×10^8

The livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) concentrations for two strains (S1 and S2) were estimated in broth and in the initial material samples (steel, plastic, concrete disk and concrete disk). The estimated broth concentrations were from single value and the estimated sample concentrations were mean concentrations from triplicate samples. The concentrations were calculated as viable counts on both 5% bovine blood agar and selective Oxoid Brilliance MRSA 2 agar

[a] BA = 5% bovine blood agar, MRSA = selective Oxoid Brilliance MRSA 2 agar

laboratory bench (room temperature 20–22 °C, relative humidity 66–68%) throughout the experiment, covered with the petri dish lids.

An initial viable count for each material was performed after the samples had dried. This was done by placing one material sample into a 50 mL centrifugation tube (Sarstedt) with 5 g of 3 mm glass beads (soda-lime glass; Merck KGaA, Darmstadt, Germany) and 10 mL of buffered peptone water (1% peptone, 8.5% NaCl), followed by shaking for 3 min in 660 rpm in an orbital shaker (Yellow line OS 2 basic; IKA-Werke GmbH & Co. KG, Staufen, Germany). The large pieces of concrete were immersed in 100 mL of peptone water with 50 g of glass beads. From the suspension, ten-fold serial dilution was prepared using a Dilushaker III (LabRobot, Stenungsund, Sweden). From the serial dilution, 100 µL was plated on selective MRSA plates and 5% bovine blood agar plates and incubated at 37 °C for 48 h (read after 24 h and 48 h). For the following viable counts, all sample triplicates were plated on selective MRSA plates and one triplicate from each sample was also plated on 5% bovine blood agar. Using selective media was seen as suitable for this study as it reduces the risk of contamination of the plates by other bacteria. The viable counts for plastic and steel were continued at weekly intervals for a total of 14 weeks. For the concrete samples, the first two viable counts were performed at 1 week intervals and the subsequent counts once every 2 weeks for a total period of 5 and 11 weeks for the concrete disks and the large concrete pieces, respectively.

Confirmation of the strains

Suspected MRSA colonies from the beginning and the end of the study for each material and strain combination were confirmed to be MRSA by using a qPCR assay detecting *mecA*, *mecC*, *nuc* and PVL genes as previously described by Pichon et al. [25]. The qPCR was run with the following modifications: two duplex assays were run instead of one quadruplex assay (*nuc*/PVL and *mecA*/*mecC*, respectively) and the TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific Inc.) was used instead of the QuantiFast Multiplex PCR kit from Qiagen. The assays were run on the CFX Opus 96 Real-Time PCR Instrument (Bio-Rad Laboratories Inc., Hercules, CA USA) and analysed by the CFX Maestro Software version 2.0 (Bio-Rad Laboratories Inc.) with default settings. The strains CCUG 60578 and CCUG 63582 (Culture Collection, University of Gothenburg, Sweden) were used as positive controls and DNase- and RNase free water (W4502; Merck KGaA) as negative control. Prior to the qPCR, the strains were subcultured on blood agar and incubated at 37 °C for 24 h. Approximately 3 µL of each culture was suspended to 180 µL of lysis buffer G2 (Qiagen, Hilden, Germany) in a 2 mL microcentrifuge tube. Ten µL of lysozyme (100 mg/mL; Merck KGaA) and 10 µL lysostaphin (5 mg/mL; Merck KGaA) was added to the microcentrifuge tubes and vortexed prior to incubation in a ThermoMixer C (Eppendorf, Hamburg, Germany) at 37 °C and 300 rpm for 90 min. The DNA was extracted with EZ1 Advanced XL (Qiagen) and EZ1 DNA tissue kit (Lot. No. 169044160) using the bacterial protocol and 100 µL elution volume.

Bacterial decay models

The observed bacterial counts on the selective MRSA plates were analysed by using nonlinear least squares regression (NLS). This was performed by using the nls-function (Nonlinear Least Squares) in R version 4.2.0 [26]. The bacterial decay was described with an exponential model $N(t) = N_0 e^{-\lambda t}$, which can be linearized to $\ln N(t) = \ln N_0 - \lambda t + \varepsilon_t$, where N(t) represents the CFU number (CFU/mL) of bacteria at time t, N_0 is the CFU number of bacteria at $t = 0$, λ is the rate of decay and ε_t represents the error term. The half-life ($t_{1/2}$) was obtained from the decay rates by $t_{1/2} = \ln(2)/\lambda$. The model was fit-

ted separately for the different bacterial strain and surface material combinations.

Results

Initial bacterial concentrations

The LA-MRSA concentrations in broth and the initial mean concentrations recovered from samples are presented in Table 1. In most cases, the mean bacterial concentrations obtained from the sample materials were lower than the concentrations of the corresponding broth that was used for preparing the samples. The initial mean bacterial concentrations on selective LA-MRSA plates were consistently lower than the corresponding samples on 5% bovine blood agar plates.

Bacterial counts on plastic and steel over time

The viable cell counts on plastic and steel samples are presented in Fig. 1. The observed bacterial counts followed an expected exponential decay up to week 14 when an increase was observed on both steel and plastic samples. During the observation period, complete die out of the bacteria was not observed for either strain on these surface materials. The blood agar plates yielded larger number of colonies than the corresponding selective MRSA plates that were prepared from the same sample. Mixed culture growth (suspected contamination, i.e., not *S. aureus*) was observed in some of the blood agar plates, which were excluded from the results.

Bacterial counts on concrete over time

The bacterial counts on concrete disks and concrete pieces are presented in Fig. 2. The observed counts of both bacterial strains decreased very rapidly on both concrete surfaces. Due to the rapid decrease, the time interval for sampling was unable to capture the exponential nature of bacterial decay even when the bacterial counts below quantification limit (< 30 CFU) were included in the results. The blood agar plates yielded larger numbers of colonies than the corresponding selective MRSA plates that were prepared from the same sample. Mixed culture growth (suspected contamination) was observed on some of the blood agar plates, which were excluded from the results.

LA-MRSA became undetectable on all concrete material and strain combinations during the observation

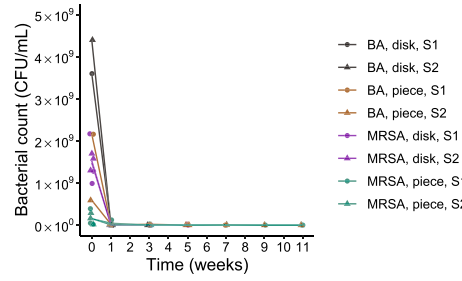

Fig. 2 The livestock-associated methicillin-resistant *Staphylococcus aureus* counts (CFU/mL) on concrete surfaces over time. Two different concrete surfaces were used (concrete disks and concrete pieces). Concrete disks were observed for 4 weeks and concrete pieces for 11 weeks. All strain and material combinations were plated in triplicates on Oxoid Brilliance MRSA 2 agar (MRSA) and as a single sample on 5% bovine blood agar (BA). The points represent the observed viable counts; counts with less than 30 CFU are also included in the figure. If a sample had a count below the quantification limit (< 30) on multiple dilution plates, the count from lowest dilution is presented. The lines represent the mean viable count of each concrete type, strain and plate combination

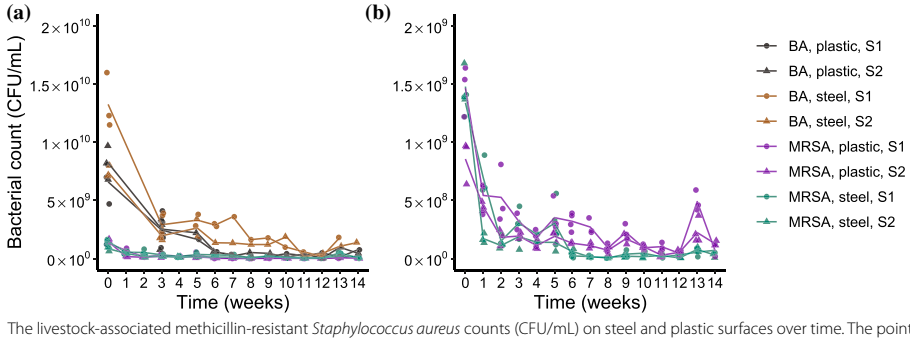

Fig. 1 The livestock-associated methicillin-resistant *Staphylococcus aureus* counts (CFU/mL) on steel and plastic surfaces over time. The points represent the viable counts within the quantification range (30–300 CFU) that were obtained from the samples; the lines represent the mean viable count of each material, strain and plate combination. **a** 5% bovine blood agar (BA) and selective Oxoid Brilliance MRSA 2 agar (MRSA). **b** selective MRSA plates from the same data. The counts from strain S1 steel sample on blood agar at week 14 are missing due to an error in sample preparation

Table 2 Time until LA-MRSA became undetectable (die out) on viable count plates prepared from concrete samples

Material	Strain	Plate[a]	Time to die out (weeks)
Concrete disk	S1	BA	5
Concrete disk	S1	MRSA	5
Concrete disk	S2	BA	3
Concrete disk	S2	MRSA	3
Concrete piece	S1	BA	5
Concrete piece	S1	MRSA	7
Concrete piece	S2	BA	9
Concrete piece	S2	MRSA	9

Two different types of concrete (concrete disk and concrete piece) were used as sample materials. The livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) were determined to have died out when the bacteria had become undetectable on all viable count plates. The viable counts were prepared on 5% bovine blood agar and selective Oxoid Brilliance MRSA 2 agar. The first two viable counts were performed at 1 week intervals and the subsequent counts at biweekly intervals for a total period of 5 weeks for the concrete disks and 11 weeks for the large concrete pieces

[a] BA = 5% bovine blood agar, MRSA = selective Oxoid Brilliance MRSA 2 agar

period. The observed die out for each combination is summarized in Table 2.

Confirmation of the strains

The suspected LA-MRSA colonies recovered from the prepared samples were confirmed to be LA-MRSA by using qPCR. All of the material and strain combinations were determined to be *mecA* and *nuc* positive and *mecC* and PVL negative.

Bacterial decay models

The estimated decay constants and half-lives for steel and plastic surfaces are presented in Table 3. When the exponential decay models were fitted to the bacterial count data for these surface materials, the residuals of the fitted models for each material and strain combination were not normally distributed for any of the strain and material combinations. The fitted intercepts and coefficients were significantly different from 0 ($P \leq 0.05$). On concrete, the quick die out of the bacteria provided only a limited number of points to fit the NLS model and the data did not have equal variance over all ranges of the explanatory variable (time). Therefore, it was not possible to fit a model to this dataset.

Discussion

This study investigated the survival of LA-MRSA CC398 on different materials commonly used in the pig farm environment. Surface materials that allow the bacteria to persist in the environment will contribute to the risk of LA-MRSA carriers, not only among the pigs but also among humans working in the farm environment. Identifying such risk surface materials is beneficial for planning effective cleaning and disinfection protocols in farms. While cleaning and disinfection have been shown to be efficient in reducing LA-MRSA, routine cleaning procedures are not sufficient to remove all LA-MRSA from the environment [27]. However, cleaning and disinfection after culling the entire herd has been proven to be efficient in eradicating LA-MRSA from a pig farm [28].

The results of this study suggest that the survival time of LA-MRSA CC398 varies depending on the surface material. Of the materials used in the study, concrete seems to be the most unfavourable surface for LA-MRSA. Even though estimating the half-life using a modelling approach was not possible for the concrete data, this conclusion is supported by the observation that the used LA-MRSA strains became undetectable on the concrete surfaces in 3–9 weeks, while on polypropylene plastic and stainless steel the bacteria were still viable after 14 weeks. However, the bacterial counts on the concrete should be assessed with caution as there remains uncertainty in the proportion of the bacteria that was detectable from the concrete surfaces. As concrete is capable of absorbing water [29], some of the bacterial suspension could have been absorbed and trapped in the concrete samples. Therefore, the bacteria inside the bigger concrete pieces may not have been detected with the methods used in this study. However, LA-MRSA also

Table 3 The decay of livestock-associated methicillin-resistant *Staphylococcus aureus* on steel and plastic surfaces

Material	Strain	Decay constant (λ)/day[a]	SE (λ)[b]	Half-life ($t_{1/2}$) in days[a]
Steel	S1	0.089 (0.070–0.107)	0.009	7.83 (6.45–9.96)
	S2	0.283 (0.212–0.354)	0.035	2.45 (1.96–3.27)
Plastic	S1	0.044 (0.031–0.057)	0.006	15.78 (12.22–22.26)
	S2	0.063 (0.045–0.080)	0.009	11.08 (8.70–15.28)

The presented values were obtained by fitting a model to observed bacterial data by using nonlinear least squares (NLS) method. Separate models were fitted to two bacterial strains (S1 and S2) on two surface materials (steel and plastic)

[a] Corresponding 95% confidence intervals are presented in parenthesis

[b] Standard error for daily decay constant (λ)

diminished quickly from the thin concrete disks, which, together with the radical drop of weekly bacterial counts between the first two observation time points, suggests that concrete is a hostile surface for LA-MRSA. This observation is further supported by a study by Maresca et al. [22] where the microflora on concrete was shown to have relatively low bacterial diversity and most of the identified bacteria belonged to the Actinobacteria and Proteobacteria phyla.

Many textbooks and standards recommend counting only culture plates with colony numbers between 30 and 300 [30, 31]. However, in this study, the viable counts on concrete that were below these quantification limits were included in the results to illustrate the rapid decrease in the bacterial counts between the first and second observation time points. Due to the uncertainty and the low number of quantifiable observations for concrete, the presented bacterial count results need to be interpreted with caution. Further studies with a shorter sampling interval are needed to be able to fit a model and calculate a half-life for the survival of LA-MRSA on concrete.

In this study, the non-linear least squares method was used to fit models to the polypropylene plastic and stainless steel data. The obtained fits for different material and strain combinations were imperfect, but this approach was considered to best represent the bacterial decay which is assumed to be exponential in the absence of active growth. Fitting an NLS-model to the data generated an average decay, but was unable to capture the increase in bacterial counts which was observed at the end of the study for the steel and plastic samples.

Based on results presented in Table 3, LA-MRSA survives longer on polypropylene plastic surface than on stainless steel or concrete. The mean half-life for plastic ($t_{½}$ = 15.8 and 11.1 days for strains S1 and S2, respectively) was also longer than what has been previously reported in dust ($t_{½}$ = 5 days) in the study by Feld et al. [13]. These results indicate that plastic surfaces could allow LA-MRSA CC398 to persist in the environment longer than on the other studied materials or in dust. However, in a pig farm environment the different surfaces can be assumed to be at least partially covered by organic material and therefore the survival of LA-MRSA in the pig farm environment is likely to be an interplay between several factors. These factors also include the environmental conditions (e.g., temperature and humidity) and the intrinsic properties of different bacterial strains. Additionally, surfaces in the barn environment deteriorate over time, making them rough and potentially more favourable for bacterial survival than what was observed in this *in-vitro* study. Nevertheless, these results suggest that considering the properties of the surface materials may improve the results of on-farm cleaning and disinfection routines. From a disease modelling perspective, more data about the survival and decay of LA-MRSA can support accurate model development.

The strains obtained for this study were field samples from Danish studies. As both of the strains belonged to the same spa-type, they do not represent the whole spectrum of different LA-MRSA CC398 strains. Using these particular isolates was seen as justified since they represent LA-MRSA, which have been encountered in a pig farm environment and were recovered from well-known farms with high traceability. The fitted decay constants and the corresponding calculated half-lives indicate that there is a difference in survival between different LA-MRSA CC398 strains. This is an interesting result as one could assume that LA-MRSA strains belonging to the same spa-type would behave similarly. Further research including more diverse strains of LA-MRSA would be required to understand the variation of survival times. In this study the LA-MRSA strain S2 appeared to have a shorter half-life on plastic and steel than strain S1. The variation in survivability of different strains was more pronounced on concrete and steel than on plastic. Interestingly, on concrete the S1 strain stayed detectable longer than the S2 strain, but due to the uncertainties related to the concrete data, interpretations of the concrete results should be made with caution.

The viable count method was used to estimate bacterial concentrations and each strain and material combination was counted in triplicate on selective MRSA agar. However, the selective MRSA plates are commonly used for the detection of the bacteria rather than for quantification purposes, and it is likely to be a more demanding medium for the bacteria to grow on. The purpose of selective media is to inhibit the growth of non-target bacteria, while they may not provide the optimal growth conditions for the target bacteria. This phenomenon is also known for other types of selective media, which means that counts on selective media are underestimating the true number of bacteria. To monitor the possible difference between selective and non-selective media, one of each sample triplicate was also plated on 5% bovine blood agar. The results shown in Fig. 1, Fig. 2 and Table 1 all demonstrate that the viable counts on selective MRSA agar were systematically lower than the counts on the corresponding blood agar plates. However, based on visual observation of the data, the relationship between the different media remained sufficiently stable over time. Therefore, using the selective MRSA agar was seen as justified since the decay is likely to be similar on both media and since it affected all samples equally.

During the study period, fluctuations in the bacterial counts on the plastic and steel surfaces were observed. Generally, this could be seen as normal variation, but

on week 14 all the bacterial counts increased simultaneously. Different technical errors in the laboratory were considered, but no clear reason was identified to explain this increase. This raised questions regarding the possible dynamics of the decay of LA-MRSA; perhaps biological processes such as biofilm formation could have affected the observed viable counts at this time point. The reason for the increased counts remains open and additional studies would be needed to identify whether this phenomenon recurs.

This study did not look into the differences in biofilm formations on different surface materials, but bacterial biofilms are known to be more resilient to environmental challenges than planktonic cells [32]. According to a study by Lee et al. [33], *S. aureus* had a higher level of biofilm formation on hydrophilic surfaces (e.g., stainless steel) than on hydrophobic surfaces (e.g., polypropylene plastic), but the formation increased on hydrophobic surfaces when 1% glucose was supplemented to the growth media. The authors also concluded that the ability to form biofilms varied between the different *S. aureus* strains. This contrast between hydrophilic and hydrophobic surfaces was not observed in the current study, but the results are not directly comparable. It remains uncertain what kind of combined effect the different surface material properties, the LA-MRSA CC398 strains and the BHI broth had on the survival of the bacteria or how much biofilm was formed on the different materials. As the BHI broth was not washed from the bacteria before contaminating the materials, the nutrients of the broth would likely have supported the survival of the bacteria. However, conserving the BHI broth when contaminating the samples was seen as justified, as it was regarded to be more analogous to conditions where bacteria are shed to the environment together with organic material, such as skin cells or secretions, than it would have been if the suspension had not had any nutrients.

Conclusions

The survival of LA-MRSA in the pig farm environment may be different on different surface materials in the farm. LA-MRSA survives longer on polypropylene plastic than on stainless steel or concrete mixture. Concrete seems to be an unfavourable material for LA-MRSA but further research is needed to determine half-life on concrete surfaces. The findings highlight the importance of bacterial contamination of the environment for on-farm persistence of LA-MRSA and the relevance of effective cleaning and disinfection routines for mitigating spread. Additionally, the data presented can help to improve the input parameters of LA-MRSA models.

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Author contributions
KT, SF, KP and SST were involved in planning of the study. KT and SF were responsible of performing the laboratory work. KT and TR performed the data analysis. KT wrote the manuscript drafts of the study, all authors have contributed to the writing process. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was conducted on laboratory samples in compliance with good laboratory practice and the current laws of the country in which they were performed.

Consent for publication
Data have not been published previously.

Competing interests
The authors declare that they have no competing interests.

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Pigs are the primary reservoir of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA). LA-MRSA are zoonotic, multiresistant bacteria capable of causing infections in humans. The work presented in this thesis used disease modelling to assess possible LA-MRSA control measures in a Swedish farrow-to-finish pig herd. Additionally, the survival of LA-MRSA on surface materials commonly found in pig farms was studied.

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