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Livestock-MRSA in Danish pig farms

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Abstract

This study looks at factors contributing to LA-MRSA prevalence in Danish pig farms. LA-MRSA is a pathogenic bacteria that are resistant to methicillin (penicillin) and tetracycline antibiotics found in livestock such as pigs, poultry and cattle. By using logistic regression analysis, this study investigates which factors that might have increased the likelihood of having a positive LA-MRSA result in 2010-2011 (n=176 farms). The model consisted of both spatial and in-herd parameters including: *average nearest distance to other farms, pig movement, use of antibiotics and zinc oxide, herd size* and *production systems*. These were set against results from the first Danish cross sectional screening of LA-MRSA in pig herds, during 2010-2011. The results from the logistic regression only showed a modest connection between pig movement (the number of different farms sending pigs to a particular holding) while the other parameters were insignificant and did not indicate increased likelihood.

Keywords

GIS, Epidemiology, MRSA, Denmark, Pig production, Logistic regression.

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Content

List of abbreviations

CHR	**Central Husbandry Register** Database used within Danish agriculture for registration of holdings and animals. It was established in 1992 and run by Ministry of Food, Agriculture and Fisheries of Denmark.
LA-MRSA	**Livestock-associated Methicillin-resistant Staphylococcus Aureus** Pathogenic bacteria that are resistant to methicillin (penicillin) and tetracycline antibiotics found in livestock such as pigs, poultry and cattle.
LA-MRSA CC398	**Livestock-associated Methicillin-resistant Staphylococcus Aureus, clone CC398** The most common strain of Livestock-associated Methicillin-resistant Staphylococcus Aureus in Europe
MRSA	**Methicillin-resistant Staphylococcus Aureus** Pathogenic bacteria that are resistant to methicillin (penicillin) and tetracycline antibiotics.
SA	**Staphylococcus aureus** Pathogenic bacteria that causes minor skin infections in animals and humans.

Pig production terminology

Boar	An uncastrated male pig.
Gilt	A female pig that has not produced offspring
Piglet	Young pig
Sow	A female pig that has produced offspring.
Weaner	A piglet that has been separated from its mother.

List of tables

List of figures

1. Introduction

Livestock-associated Methicillin-resistant Staphylococcus Aureus (LA-MRSA) is a pathogenic bacteria that causes minor skin infections in animals and humans. If the bacterium is transmitted to individuals with reduced immune systems, it can lead to severe and even deadly infections. During the last decade, LA-MRSA has become a major concern worldwide. Pigs are the primary hosts and the prevalence of the strain MRSA CC398 is most predominant in countries with a large-scale pig production.

Denmark is the largest pig producer in the EU per capita and has experienced a dramatic increase in LA-MRSA positive herds. The first cross-sectional screening in 2010-11, found isolates in 16% of all Danish pig herds. In the most recent screening in 2016, 88% were LA-MRSA positive (MEFD 2019). In the wake of this, an ongoing debate on the severity of LA-MRSA has emerged in Denmark as well as internationally. Reports on infected pork, zoonotic transmission and community onset has received substantial media attention. The outbreak has also tapped into the ongoing crisis of antimicrobial residence and animal welfare issues.

Although a majority of pig farms in Denmark are infected at this stage, it is important to trace the onset to prevent similar outcomes in other countries. While Norwegian authorities managed to trace the onset and stop the transmission (Grøntvedt 2016), it has not been fully established how LA-MRSA gained a foothold in Denmark. Several studies have looked into the transmission modes of LA-MRSA among pigs (van Duijkeren 2008; Cuny et al., 2015; Schulz 2019). However, there are few studies covering the onset of the bacterium in Denmark. What made some farms more susceptible to LA-MRSA is still not established.

Another factor that has not been fully covered is the spatial dimension. Most research has revolved around in-herd factors (the use of antimicrobials and zinc oxide, herd size, holding type). Spatial factors such as proximity between farms, movement of pigs and population density have only been covered in a handful of publications (Anker et al.; 2018; Schulz, 2019; Sørensen, et al., 2019).

1.1 Aim of the study

The primary aim of this study is to investigate the significance of the early LA-MRSA-positive pig farms. By combining in-herd level parameters and spatial parameters, this study seeks to investigate which factors that might have contributed to positive test results, at the time of the early onset (2010-2011). Although it is impossible to trace *patient-zero* at this point, comparing key LA-MRSA factors might give an insight into how the LA-MRSA positive farms were different from the negative farms and what might have sparked the onset.

More specifically, this study seeks to address the following objectives based on the 2010-2011 test results:

- Were farms with high antibiotic and zinc oxide consumption, larger units and conventional production systems, more likely to have positive LA-MRSA results compared to negative farms?

- Were farms with closer proximity to other farms and higher frequency of pig movements more likely to have positive LA-MRSA results compared to negative farms?

2. Background

2.1 MRSA

Staphylococcus aureus (SA) is a pathogenic bacterium found in animals and humans. In most cases, it colonizes individuals without leading to any clinical signs. If a colonized individual suffers from reduced immune system, SA can lead to skin and wound infections and to severe conditions such as sepsis and pneumonia. Without treatment of antimicrobial substances, SA-invasive infections can even lead to a great number of deaths (Schulz 2019). A simplified chart of Staphylococcus aureus and its subgroups can be seen in Figure 1.

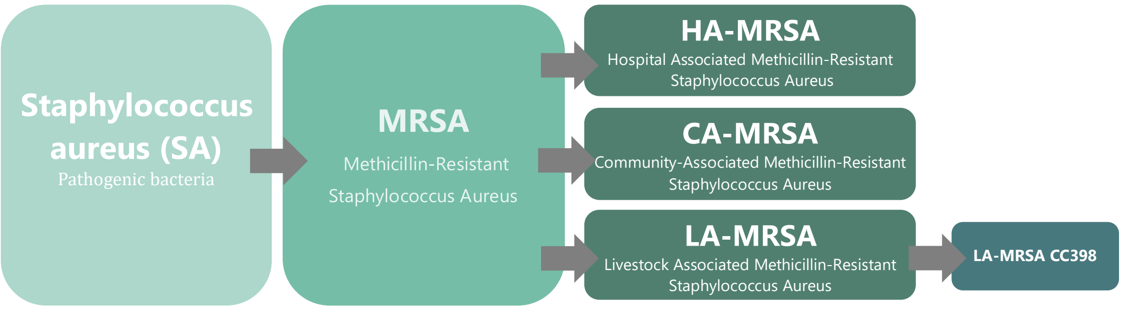

Figure 1: Simplified chart of Staphylococcus aureus and its subgroups

The introduction of penicillin improved the outcome for patients with severe infections, but after a few years, the bacteria gained resistance. MRSA (*Methicillin-Resistant Staphylococcus aureus)* is a group of SA that can resist not only methicillin (penicillin) but also tetracycline antibiotics (Palavecino 2014). MRSA is spread in the same ways as non-resistant *Staphylococcus aureus*, mainly through physical contact with an infected individual. It can also be transmitted through textiles, furniture and dust as the bacteria often binds to skin cells.

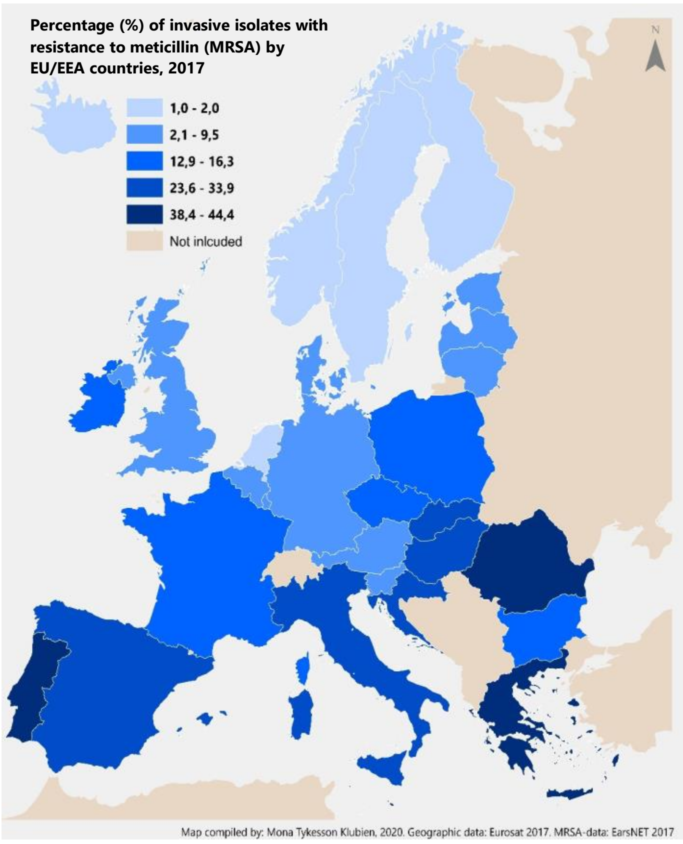

Figure 2: Percentage of MRSA isolates by EU/EEA country 2017

Not all individuals are at risk of catching and becoming infected. Although the reasons for this have not been completely understood, it is believed to be a complex combination of exposure frequency, immune system condition, blood sugar level and differences in microbiome that impact the risk (MEFD 2017).

MRSA was detected in England in 1961. At first, MRSA was almost exclusively prevalent in hospitals (HA-MRSA), but gradually spread and appeared as community onset (CA-MRSA) in the late 1990s. MRSA is now considered to be at an epidemic level globally. The highest (measured) rates are found in United States, Canada, Japan & Indonesia (WHO, 2015). As seen in Figure 2, the situation in Europe is diverse. Southern European countries reported high levels (Portugal 39.2%; Romania 44.4%) in 2017 while Northern European countries reported low levels (Norway 1%; Sweden 1.2%). The population weighted mean for Denmark was 2.5%, which was slightly higher than the other Scandinavian countries (EARS-Net, 2017).

2.2 Livestock MRSA

Livestock MRSA (LA-MRSA) is a clone found in various livestock such as cattle, poultry and pigs in particular. LA-MRSA was first described in 1972 when it was found in Belgian cattle (Devriese, 1972 in Sharma et al., 2016). However, it was not until 2005, when Voss et al. published a study on infected pigs and farmers in the Netherlands, that the type gained full attention (Voss et al. 2005). The most prevalent strain of LA-MRSA among European livestock is called MRSA CC398. Several studies suggest that the strain stems from the human clone MSSA CC398 (Methicillin Sensitive Staphylococcus Aureus). Shortly after MSSA CC398 was transmitted to animals, it mutated and became resistant, first to tetracycline and eventually to methicillin (MEFD 2017).

Today, MRSA CC398 is a major concern in European livestock production, especially in pig production. Large numbers of pigs are either colonized or infected. However, carrying pigs rarely show any clinical symptoms. Nonetheless, MRSA CC398 poses a zoonotic risk as the bacteria regularly transmits to humans. Farmers, veterinarians, slaughterhouse workers and their household members are the most affected human group (Grøntvedt et al., 2016). Nasal carriage of MRSA CC398 has been reported in 77-86% of humans with occupation exposure to pigs in a German study (Cuny, 2015). A similar study from Taiwan reported 34 % (Fang et al., 2014). Several studies have also concluded that MRSA CC398 has spread into the wider community, to persons without livestock contact (Larsen et al., 2015; van Cleef et al., 2011). A Danish study by Anker et al. (2018) is also pointing towards community onset and The Danish Ministry of Environment and Food (MEDF) concludes that LA-MRSA poses a great threat to humans with severe health conditions and weak immune systems (MEDF, 2017).

There are no recent figures covering the extent of MRSA CC398 on EU-level, neither among pigs nor in the general human population. However, a survey conducted, based on 2013-data from hospital serving laboratories, found MRSA CC398 isolates in 17 out of 19 participating countries. The Netherlands, Denmark and Spain reported the largest numbers of LA-MRSA in humans: (n = 164, 157 and 52, respectively) (Kinross et al., 2016). These three countries are also leading pig producers in the EU (Table 1).

Table 1: Pig production - slaughters in tonnes and per capita, per EU-country in 2018 (European Commission, 2019)

EU-country	Tonnes	EU-country	Tonnes
Germany	5343000	Sweden	249000
Spain	4530000	Czechia	211000
France	2182000	Finland	167000
Poland	2082000	Greece	82000
Denmark	1581000	Bulgaria	80000
Netherlands	1536000	Croatia	75000
Italy	1471000	Lithuania	64000
Belgium	1073000	Slovakia	57000
United	927000	Estonia	43000
Austria	510000	Cyprus	42000
Hungary	436000	Latvia	37000
Romania	365000	Slovenia	22000
Portugal	362000	Luxembourg	13000
Ireland	303000	Malta	4000

EU-country	Tonnes/cap	EU-country	Tonnes/cap
Denmark	0.273473	Sweden	0.024604
Spain	0.097089	Italy	0.024320
Belgium	0.094134	Lithuania	0.022785
Netherlands	0.089401	Luxembourg	0.021595
Germany	0.064535	Czechia	0.019887
Ireland	0.062728	Latvia	0.019128
Austria	0.057808	Romania	0.018689
Poland	0.054823	Croatia	0.018268
Cyprus	0.048598	United Kingdom	0.013987
Hungary	0.044588	Bulgaria	0.011347
Portugal	0.035176	Slovenia	0.010644
France	0.032603	Slovakia	0.010472
Estonia	0.032597	Malta	0.008409
Finland	0.030291	Greece	0.007634

2.3 Studies on LA-MRSA transmission

In the wake of the rapid increase, a considerable body of research has emerged. Several veterinary, medical and epidemiological studies have investigated how to detect it, how it is transmitted and what control measures to implement (Schulz, 2018; Grøntvedt, 2016).

The spread of LA-MRSA has also caused stirred discussions on animal welfare, food security and the overuse of antimicrobial substances. LA-MRSA is more prevalent in industrial farm systems where antibiotics has been used as preventive measure (van Duijkeren 2008; Cuny et al., 2015). The overuse of antimicrobial substances is one of the main drivers behind all strains of MRSA and has been announced as one of the most staggering future crisis by WHO (WHO, 2019). As a consequent, a fair share of MRSA-research has revolved around the link between in-herd dynamics such as the use of antimicrobial substances and zinc oxide and LA-MRSA incidents (Cuny et al., 2015; Slifierz et al., 2015a). There has also been a focus on production systems. Some studies have concluded that LA-MRSA is more common in conventional herds compared to organic production units (Slifierz et al., 2015b; van de Vijver et al, 2012). A more recent study found a connection between the absence of sows and the prevalence of MRSA CC398 (Sørensen et al., 2018).

Even though previous research has contributed to a vast body of knowledge – especially in the case of Denmark, there are still areas up for further investigation. The first incident of Danish LA-MRSA in pigs was detected in 2006 but it took years before the authorities acted and carried out screenings (MEFD 2017). While Norwegian authorities managed to trace the onset and stop the transmission (Grøntvedt 2016), it has not been fully established how the LA-MRSA gained a foothold in Denmark. There are no specific studies on the significance of the early LA-MRSA positive farms.

2.3.1 Spatial studies on LA-MRSA

Another dimension that has been widely overlooked in connection to LA-MRSA is geography. Spatial dimensions have been a central part of epidemiological inquiry ever since John Snow published his cholera map. Proximity and environmental conditions are important factors behind the introduction and spread of bacteria. In this light, a surprisingly small body of LA-MRSA literature has studied spatial factors.

Most of the work has revolved around clusters and distance between human MRSA-carriers. Only a handful of studies have investigated spatial aspects of Livestock associated MRSA. One of the few is Mroczkowska et al. (2017) which examined the prevalence of LA-MRSA on 123 Polish pig farms. They found bacteria on almost two-thirds of all pig farms and in 14 out of 16 provinces but could not establish any differences between positive and negative farms based on spatial proxies. Nor could the study find any significant differences based on farm type and size of herd. In another study by Sørensen et al., (2018), Euclidian proximity is included in the initial model but not presented in the results or the conclusion.

By contrast, there are several studies which include spatial proxies to model the prevalence in humans. A study by van Rijen et al. (2014) looked at the proportion of LA-MRSA CC398 in Dutch hospitals and concluded that more cases were found in pig-dense areas. Another Dutch study by Zomer et al. (2017) looked at the proximity between human carriers of LA-MRSA CC398 and pig farms. The results showed that carriers on average lived much closer to pig farms compared to non-carriers (median: 184 versus 402 meters). It should be noted that the study only included persons not living or working on a pig farm. This could indicate that living near farms increases the risk becoming infected but Zomer et al. (2017) call for further research to investigate the modes of transmission. A third study conducted along the Dutch-German border by Paget et.al (2015), found that living near a farm (<5km) was associated with MRSA carriage.

A Danish study by Anker et al. (2018) corresponds with the Dutch results as it concludes that the prevalence of human carriers was significantly higher in rural, pig dense areas. Anker et al. used a set of GIS-methods to investigate the spatial connections. Home address coordinates of human carriers were plotted against pig farms to model the distance from farms.

The above-mentioned studies all focus on the proximity between human carriers and pig farms. The association between geographic distance and transmission from farm to farm is covered in fewer studies. LA-MRSA has been detected in dust and soil close to farms in a few studies (Friese 2013, Rosen et al 2018). In one of the earliest studies by Gibbs et al (2006), organisms resistant to antibiotics were found up to 150 meters downwind of a pig farms in the US. However, according to Schulz (2019), remote herd-to-herd transmission via air is unlikely. It is more likely that spread between farms is linked to zoonotic (human-to-animal and animal-to-human) transmission. As mentioned before, high rates of MRSA CC398 among farmers and their family members, veterinaries and slaughterhouse workers have been found in several studies (van Cleef 2010; Cuny 2015; Fang et al. 2014). These groups are especially susceptible and are likely to be the source of human LA-MRSA in groups without known animal contact (Anker, 2018). The transmission is likely to occur when a human carrier visits a farm or animal market and thereby transmitting the bacteria on to animals. Since human carriers are likely to visit farms and animal markets in close proximity to their own resident or neighbourhood, geographical distance might be a key factor.

Schulz (2018; 2019) has modelled this indirect transmission between farms in Denmark in an extensive way. Her studies have looked into pig movements and calculated the distance-dependent probabilities. According to this model, contact probabilities starts off by 0.12 at 1 km distance and drops to 0 after 100 km. However, the concluding remarks from Schulz is that pig movements and distance alone, cannot fully explain the increase of LA-MRSA. Other factors such as within-herd dynamics, indirect transmission via humans and unexplained introduction is more likely to be contributing (Schulz 2018). Another study from the same research group, concluded that holdings that received animals from negative farms had a significantly reduced risks of LA-MRSA CC398 onset (Sieber et al., 2018).

2.4 MRSA CC398 in Denmark

As mentioned earlier, the first case of MRSA CC398 in Danish pig herds was reported in 2006. By that point, 60-80% of Dutch herds were already estimated carriers (van Alen et al. 2016). Despite the risk of similar transmission rates, Danish authorities waited until 2010 to conduct the first cross-sectional screening. As seen in Table 2, it showed a prevalence of 16% in Danish pig herds. Results from the most recent screening in 2016, found 88% of the herds to be MRSA CC398-positive (MEFD, 2017).

Table 2: Results from the Danish MRSA CC398-screening (MEFD 2017)

Year	2010-2011	2014	2016
Prevalence	16%	68%	88%

The rapid increase is also reflected in the rate of MRCA CC398 found in humans. The number of new MRSA CC398 incidences increased by 200% between 2007 and 2016. It should be noted that the increase also depends on new guidelines and surveillance programs. Nonetheless, a study by Schulz (2018) point toward an actual and substantial increase. Almost 5000 cases of LA-MRSA (27.4% of all MRSA cases) were registered in Denmark between 2007 and 2016. About 85% of these had direct or indirect contact with livestock. The remaining 15% was not reported to have any links to livestock (MEFD, 2017). Figure 3 is based on MRSA CC398-data from 2016. Although these data are aggregated, they reflect the correspondence between pig density and human MRSA CC398 incidents.

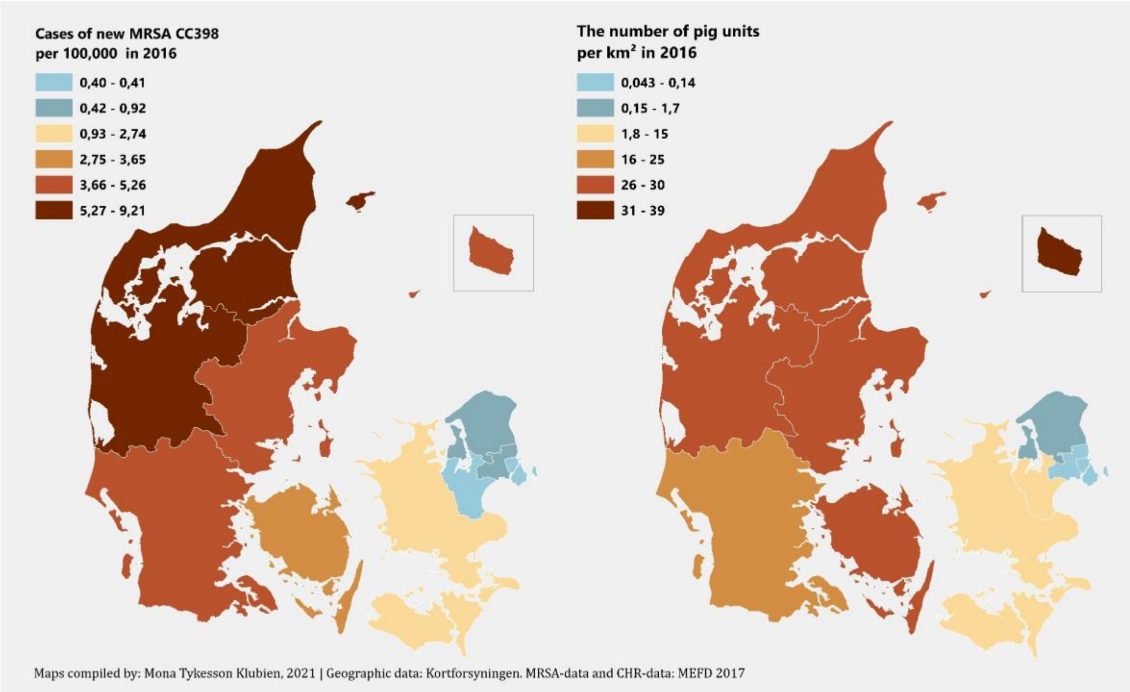

Figure 3: MRSA CC398 in humans and pig density at regional level in 2016

2.5 Pig production in Denmark

As seen in Table 1, Denmark is the largest European pig producer per capita. To get a sense of what these numbers mean, Denmark had a human population of 5.8 million and a pig population of 12.8 million in December 2018. When looking at the annual production, about 28 million pigs are produced on Danish soil, making Denmark into the most pig dense country in Scandinavia (Figure 4) (Eurostat, 2020; SSB, 2020). Further, Danish pig industry has a substantial economic importance. About 90% of the produced pigs and meat are sold abroad which in turn accounts for 5% of the total Danish export (DAFC 2020).

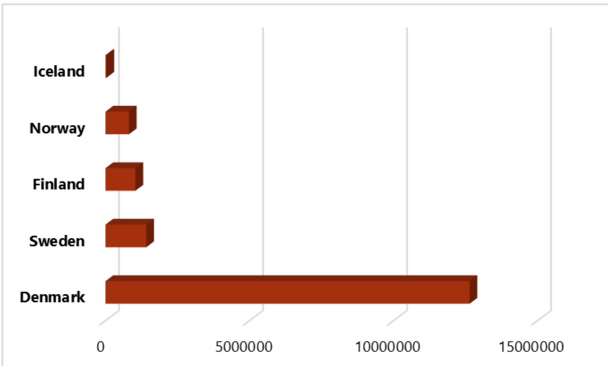

Figure 4: Pig population (number of pigs) in Scandinavia (2018)

In line with other modern pig production systems, Denmark has a pyramidal structure (Figure 5). The top is formed by *breeding units* (breeding herds, multiplier herds, quarantine stations, boar stations) the centre by *production sites* (production herds, weaner

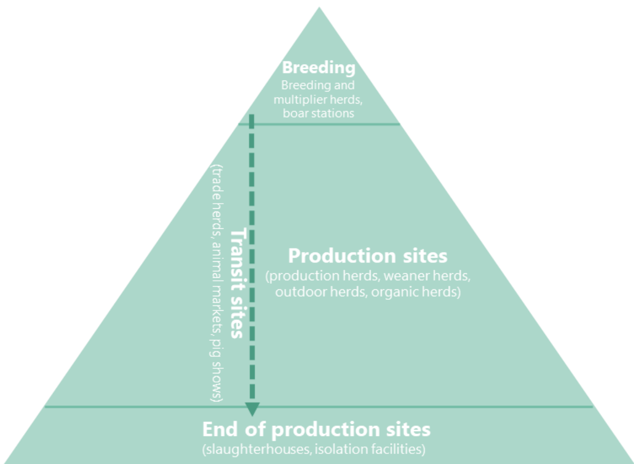

Figure 5: Structure of the Danish pig production system (Schulz 2018)

herds, outdoor herds, organic herds) and the bottom by *end of production sites* (slaughter houses, export isolation facilities). In addition, there are transit sites (trade herds, animal markets, pig shows) operating on a vertical level (Schulz 2018).

The pyramidal structure increases the risk of disease transmission as herds are intertwined through a network of interactions and trade (Broens et al., 2011; MEFD, 2017). Despite the obvious risks, Danish authorities did not conduct any MRSA-screenings among breeding herds until 2014. The results from the first scan revealed MRSA CC398 among 63% of the breeding herds.

2.5.1 Animal welfare

Even though Danish pig meat is consumed in more than 140 countries (DAFC, 2020), the production has periodically gained negative attention due to deficiency in animal welfare and the extensive use of antimicrobials. By law, Denmark is required to follow the EU Council Directive *Minimum standards for the protection of pigs*[1]. The country also has its own animal welfare legislation *Bekendtgørelse om beskyttelse af svin*[2]. When comparing Danish and Swedish requirements, Danish demands are less rigorous (see Table 3). Fixation of lactating sows is allowed in Danish pig production while prohibited in Sweden. The purpose of this practice is to prevent the sow from stepping on or crushing her piglets. The sow is thus separated from the piglets and placed in a narrow-crated box. The total space requirements of a lactating sow with piglets is also smaller compared to the Swedish standards. Other differences are the practices of castrating mail piglets without anaesthesia and tail-docking which still are allowed in Denmark while banned in Sweden.

Table 3: Comparison of animal welfare regulation of pigs between Denmark and Sweden (SSNC 2018)

Practise	Denmark	Sweden
Fixation of lactating sows	Allowed 150 days per year	Prohibited
Castrated without anaesthesia	Allowed	Prohibited
Space of sow with piglets	4 m^2	6 m^2
Tail-docking	Allowed 90 % of the tails are docked	Prohibited

2.5.2 Antimicrobial consumption

Another area up for scrutiny is the consumption of antimicrobials. As seen in Figure 6, the use of prescribed antimicrobials in relation to pig production has decreased in absolute numbers since 2014 and in relative numbers since 2009 (Danmap, 2001-2018). Danish authorities and farms have made joint efforts to reduce the usage of antimicrobials (FAO, 2019).

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[1] (2008/120/EC)
[2] BEK nr 17 af 07/01/2016

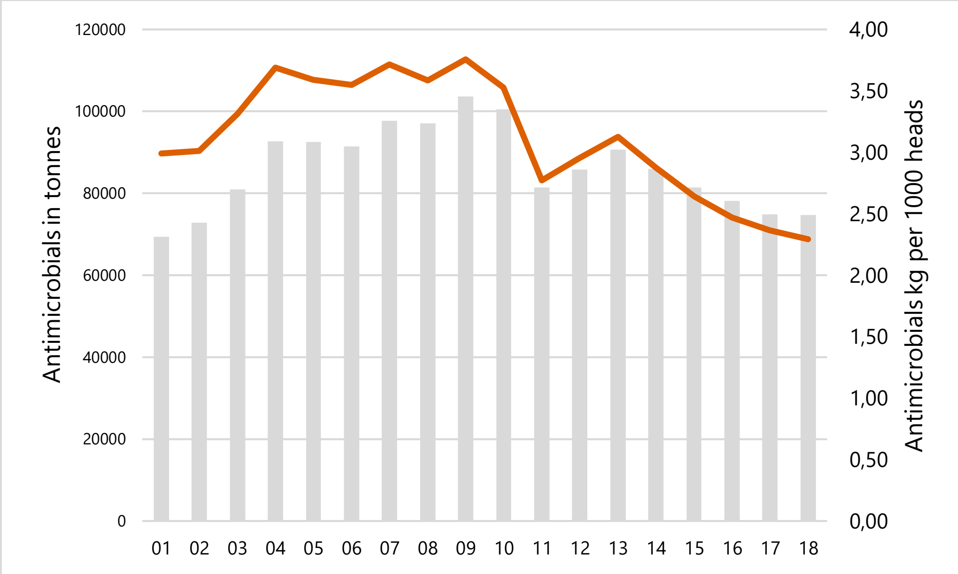

Figure 6: Antimicrobial consumption Danish pigs 2001-2018

A general trend towards reduced usage can also be seen in most European countries (Table 4). The estimated PCU (population correction unit) is not normalized in this case, which results in high sales for countries with a large pig production. Nonetheless, the total amount of antimicrobials can be a risk factor. During the study period (2010-2011), antimicrobial prescription for pigs were 1856 tonnes in Denmark alone (Esvac 2020).

Table 4: Estimated PCU (in 1,000 tonnes) of the population of food-producing species by country (Esvac 2020)

2010				2017			
Country	**PCU 1000 Tonnes[3]**	**Country**	**PCU 1000 Tonnes**	**Country**	**PCU 1000 Tonnes**	**Country**	**PCU 1000 Tonnes**
Austria	403	**Latvia**	33	**Austria**	364	**Latvia**	40
Belgium	901	**Lithuania**	67	**Belgium**	842	**Lithuania**	70
Bulgaria	0	**Luxembourg**	..	**Bulgaria**	84	**Luxembourg**	11
Croatia	0	**Malta**	..	**Croatia**	88	**Malta**	4,4
Cyprus	0	**Netherlands**	1493	**Cyprus**	45	**Netherlands**	1616
Czech Republic	240	**Norway**	125	**Czech**	198	**Norway**	129
Denmark	1856	**Poland**	..	**Denmark**	1752	**Poland**	1472
Estonia	35	**Portugal**	349	**Estonia**	37	**Portugal**	344
Finland	181	**Romania**	..	**Finland**	154	**Romania**	477
France	1887	**Slovakia**	..	**France**	1782	**Slovakia**	46
Germany	0	**Slovenia**	26	**Germany**	3749	**Slovenia**	18
Greece	0	**Spain**	3333	**Greece**	112	**Spain**	3902
Hungary	316	**Sweden**	230	**Hungary**	307	**Sweden**	201
Iceland	5,8	**Switzerland**	0	**Iceland**	5,8	**Switzerland**	200
Ireland	250	**UK**	623	**Ireland**	281	**UK**	766
Italy	1032			**Italy**	813		

2.5.3 Zinc oxide consumption

Apart from the extensive use of antimicrobials, the compound zinc oxide (ZnO) is also widely used in pig production. This inorganic substance is given to weaners to prevent

[3]Estimated PCU (in 1,000 tonnes) of the population of food-producing species, by country

diarrhoea and promote growth. The European Commission has decided to phase out the therapeutic use, since high doses have been linked to antimicrobial resistance (Slifierz et al., 2015a; Cavaco et al., 2010). Another reason is the environmental risk as redundant zinc pollute water and soil (EMA 2020).

3. Data

This section describes how each dataset was retrieved, managed and modified. It also entails a discussion on sample size and geographic coverage.

3.1 MRSA-test results

The MRSA-screening data were made public through a judgment from the Supreme court in Denmark in 2016 (Højesteret 2016) and were requested by email from the Ministry of Environment and Food of Denmark (MEFD). The tests had been carried out during three different time periods: 2010-11, 2012 and 2014. The geographic distribution of the 2010-11 test locations results can be viewed in Figure 7. Each test is tied to a unique CHR-number (*Central Husbandry Register*) that in turn is tied to a specific herd. Only results from the 2010-11 screening were used in this study since the aim was to study the early onset.

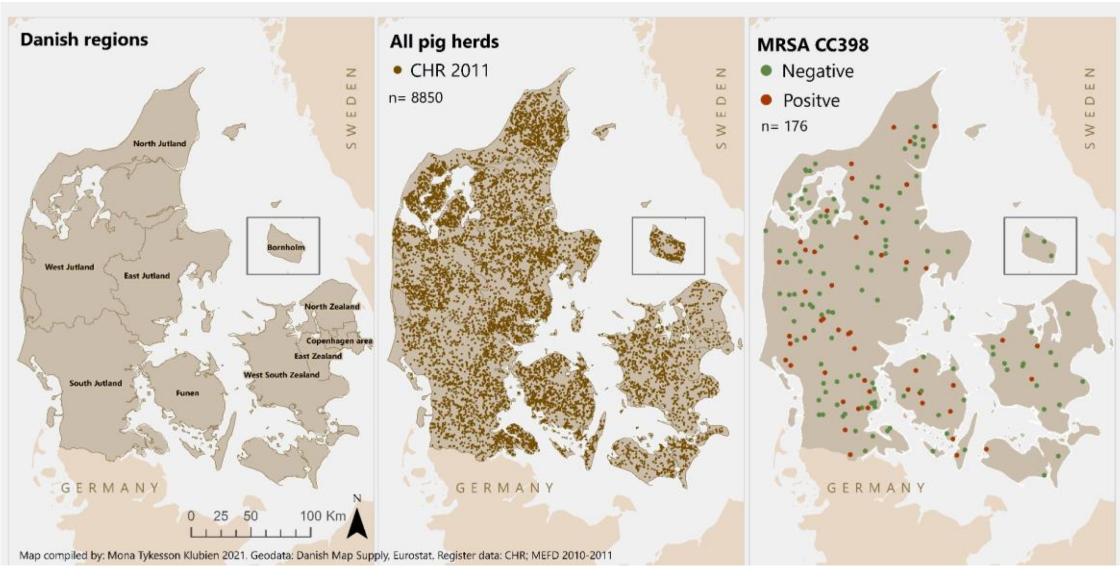

Figure 7: Map of Danish regions, all registered pig herds in 2011 and MRSA CC398-test herds in 2010-2011.

The MRSA CC398 screening results of 2010-2011 were processed in the following way: *Methicillinresistente S. aureus* tests were selected and all the other types of tests (*E.coli, salmonella*) were excluded. Only tests performed on pigs were included, all other animal types were excluded. Duplicate CHR-cases were excluded. When two tests from the same herd (CHR) were found both positive and negative, the herd was typed as positive. Only when all samples from the same CHR were registered as negative, the CHR was typed as negative. The remaining results were georeferenced to fit the CHR-spatial data (UTM EUREF89, zone 32). Only results with sufficient CHR-numbers were kept.

Table 5: MRSA CC398-screening observations used in this study

2010-2011	MRSA-tested pig holdings	Pigs holdings in total	Number of positive tests
MRSA-tested pig holdings	176	8850 (2010)	52

3.2 In-herd parameters

In-herd parameters include data that are specific to each individual herd such as size, holding type and use of antibiotics. Following Cuny et al. (2015), in-herd parameters may be linked to spontaneous emergence of LA-MRSA. The included parameters will be outlined in detail below.

3.2.1 Size of herds

Georeferenced data on pig holdings were obtained from the Central Husbandry Register (CHR), a database for livestock holdings controlled by the MEFD. All data are open and accessible to the public. The database contains information on the *holding-id, geographical position, name of owner, type of production* and *animal units*. The two latter variables were used in the analysis and are briefly described below.

The size of herds is measured in animal units [*Dyreenheder*]. The pig farmers report the units into the database themselves. Each unit is based on number and type of pigs combined. For example, 208 piglets constitute 1 animal unit while 39 finisher pigs also equal 1 pig unit (MEFD 2014). The data retrieved from CHR did not entail the proportion within the summarized units. Therefore, the animal unit-variable does not reflect the size of each herd in absolute numbers. Instead, it measures the production capacity of each herd. The data ranged from 0.33 to 818.14 animal units.

3.2.2 Holding type

The CHR-database contains three different production type-categories: *Organic, conventional,* and *conventional/outdoor*. To label a holding as *organic* and for that matter as *conventional/outdoor,* Danish pig holdings must meet stricter animal welfare and feed demands (SEGES 2017). These include segregation between sow and piglet after at least seven weeks compared to four for conventional breeding. At least 90% of the feed need to be organically produced and GMO-free. The indoor space for a finisher pig of 100 kilos, is minimum 2.3 m^2 compared to 1.2 m^2 for conventional/outdoor and 0.65 m^2 for conventional. In addition, all organic and conventional/outdoor pigs need to have access to outdoor areas. The maximum amount of transportation hours also vary. Organic and conventional/outdoor pigs can be transported up to eight hours while conventional has a maximum of 24 hours (illustrated in Figure 8).

Figure 8: Differences between the three production types, two examples.

The three types of holdings were included as categorical data in the analysis. The distribution of types between the 176 cases were: 10 organic, 2 conventional/outdoor and 164 conventional.

3.2.3 Use of antimicrobial substances

Data on the use of antimicrobial substances were derived from the database VetStat (2020). This database contains information on prescribed veterinary medicine tied to unique CHR-numbers. The consumption of all prescriptions was processed in the following way: Antibiotic substances were selected manually by consulting the Veterinary Product Database (2020) and the EU Veterinary Medicinal Product Database (2020). A complete list of substances can be found in Appendix 1.

The sum of the prescribed substances was divided by the *Animal Unit* to obtain a relative measure. A more common approach is using the ADD (animal daily dose) approach (Jensen et al., 2004) which divides the active antimicrobial substances by the average weight of the livestock. Since the CHR-data did not contain information on average weight or specific figures on the share of fattening pigs, sows, gilts or piglets, this approach could not be chosen.

3.2.4 Zinc oxide consumption

Apart from the extensive use of antimicrobials, the compound zinc oxide (ZnO) is also widely used in pig production. This inorganic substance is given to weaners to prevent diarrhoea and promote growth. The European Commission has decided to phase out the therapeutic use, since high doses have been linked to antimicrobial resistance (Slifierz et al., 2015a; Cavaco et al., 2010). Another reason is the environmental risk as redundant zinc pollute water and soil (EMA 2020).

There are no open comparisons available between EU-countries. However, Danish figures can be accessed from 2009 and show a slight relative decrease in recent years (Figure 9) (Danmap, 2018).

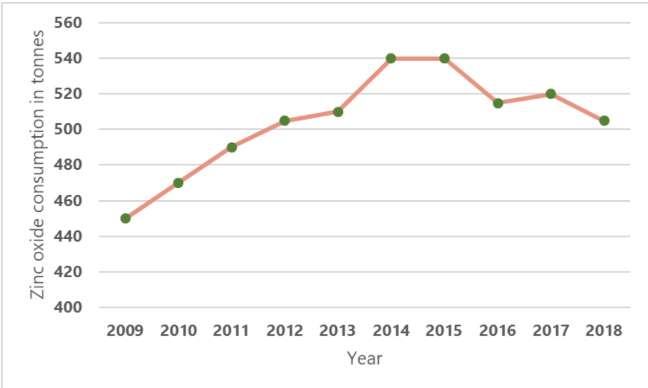

Figure 9: Zinc oxide (Zn0) consumption in tonnes

3.3 Spatial parameters

Spatial parameters incorporate distance between pig farms and pig movements. These parameters are included to capture contact-transmission. The movement of pigs have been linked to MRSA transmission in several studies (Schulz 2018; Sørensen et al., 2018). Fewer studies have incorporated distance as proxy. While movement mainly reflect transmission between pigs, distance is more likely to capture zoonotic transmission. How the two parameters were calculated is outlined below.

3.3.1 Pig movements

The pig movement data were also retrieved from the CHR-database but from a restricted part called the *Pig Movement Database [Svineflyttedatabase]*. The database can be accessed through Danish e-identification and contains all movements of Danish pigs.

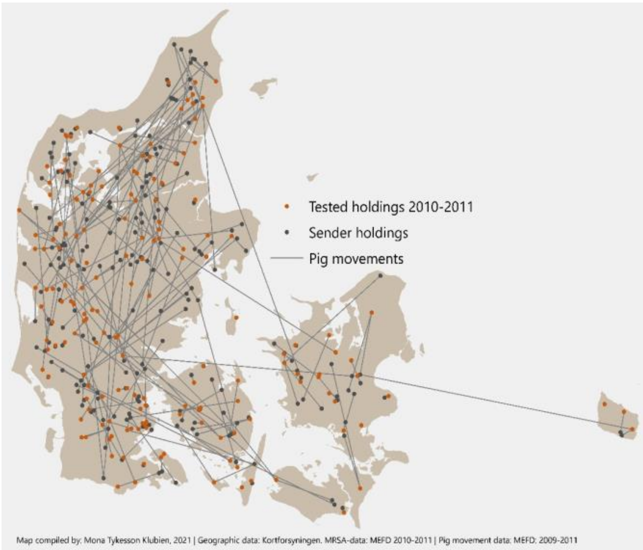

Figure 10: Movements between pig farms, 2010-2011.

The CHR-data were processed in the following way: All the movements one year prior to the MRSA-screening date were retrieved. The CHR-number of the sender holding(s) and the number of pigs were kept. Both the number of senders that a tested farm received pigs from and the total number of pigs were initially included in the model. An illustration of the complex web of sender and receiver holdings can be seen in Figure 10.

The tested holdings received in total 851021 pigs from 271 different holdings. 22 of the senders delivered pigs to more than one tested holding. Three delivery holdings also belonged to the MRSA-tested farms, whereof two were MRSA-positive. A majority (68%) of movements where short distance (<50 km). Only 12% of the exceeded 100 km. Two specific variables were derived: *number of received pigs* (*Movement a*) and number of senders (*Movement b*).

3.3.2 Proximity between farms

The proximity between tested farms and all other pig farms were calculated by using the Euclidian distance. As mention in section 2.31, only a handful of studies have considered distance as proxy (Sørensen et al., 2018; Anker et al., 2018). Since distance is included to capture zoonotic transfer, driven by the mobility of humans (humans visiting farms and the interaction between farm workers and veterinarians) it would have been ideal to model network as opposed to Euclidian distance. However, due to Denmark's many island and overall small size, it would not add validity in this particular study.

3.4 Sample size

Only a small share of the pig holdings were tested in the 2010-2011 sampling (176/8850). The documentation from MEFD (2010) states that holdings were selected in connection to other types of controls or sometimes even randomly. When evaluating the sample size through the standard formula where:

$$\frac{\frac{Z^2 * p(1-p)}{e^2}}{\div \atop 1 + \frac{Z^2 * p(1-p)}{e^2 N}} = 191{,}8$$

Z² equals the squared confidence level (in this case 95%)
p equals the estimated proportion of the population (in this case 0.5)
e² equals the margin of error (in this case 7%)
N equals the sample size

it shows an ideal sample size of 192.

Thus, 176 cases is not too far off if we accept a 7% confidence interval. Also, even though the selection of test farms were made randomly, the locations reflect the spatial distribution of all farms relatively well. Figure 11 depicts 100 km^2-hexagons around each pig farm that existed in 2011. The darker the colour, the higher the number of farms in each hexagon. As seen, locations of tested farms correspond with high concentrations of farms in general. However, there are some uncovered high-concentration spots. In the southern parts of East Jutland (3) no tests were carried out despite a high concentration of farms.

Another concern is the type of holding that was tested. As outlined in section 2.5, the Danish pig production system is pyramidal. Including *Place in the pyramid* as an in-herd parameter would have been beneficial but since none of the breeding herds were tested before 2014, inclusion was not possible for the 2010-11 sample.

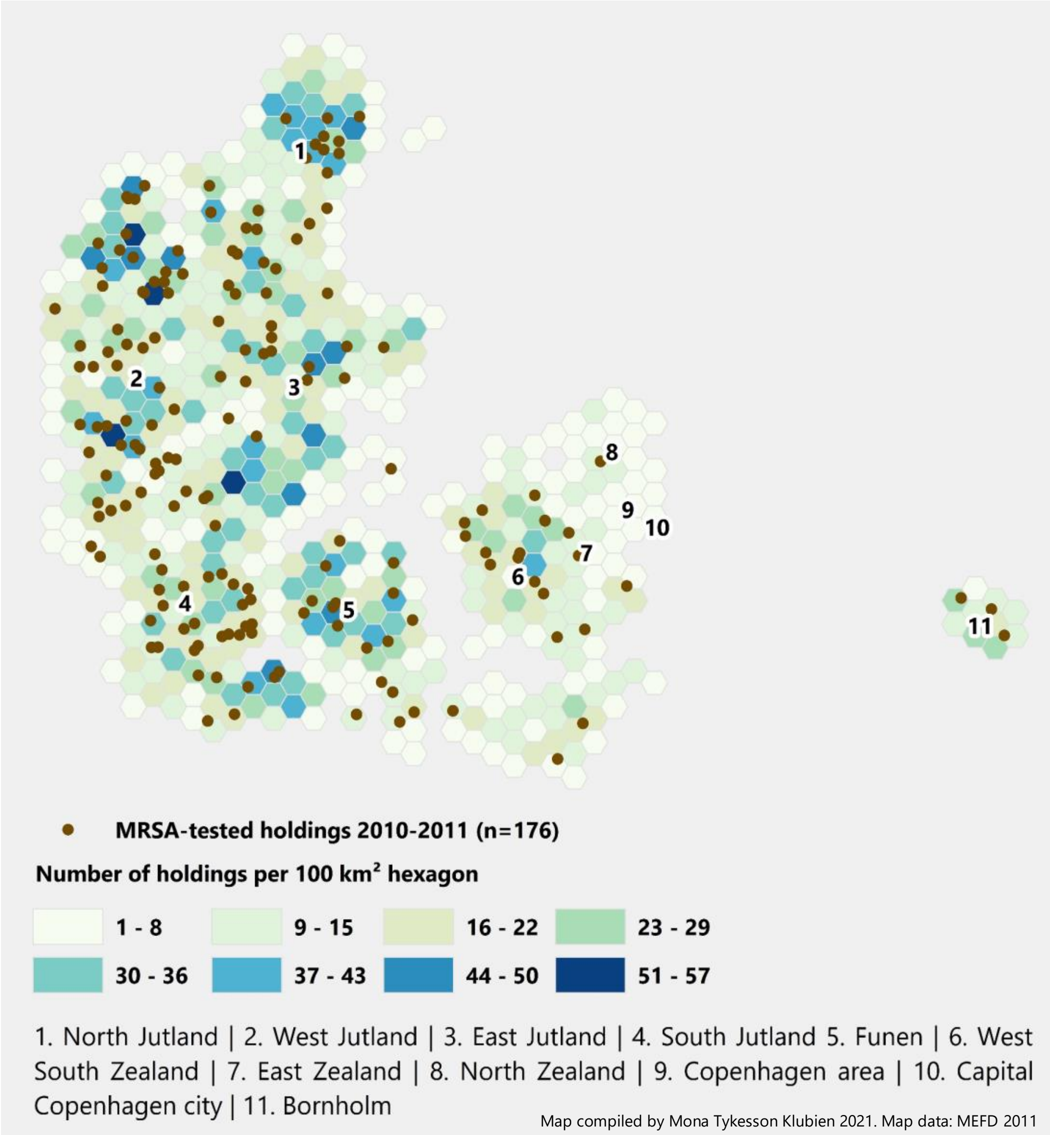

Figure 11. Tested holdings plotted against concentration of all holdings.

3.5 Causal links

The parameters discussed above, have all been identified in previous studies and selected on their priori associations. As seen in Figure 12, their links to LA-MRSA prevalence have roughly the same mediators: *transmission between pig*s or *mutations*. The spatial distance proxy has another mediator: *zoonotic transmission* as human mobility is likely to cause the bacterium to transfer in this case. These parameters constitute the base model of many LA-MRSA-studies. However, as seen in the ongoing research on Covid-19 susceptibility, there are complicated casual chains and few reliable models.

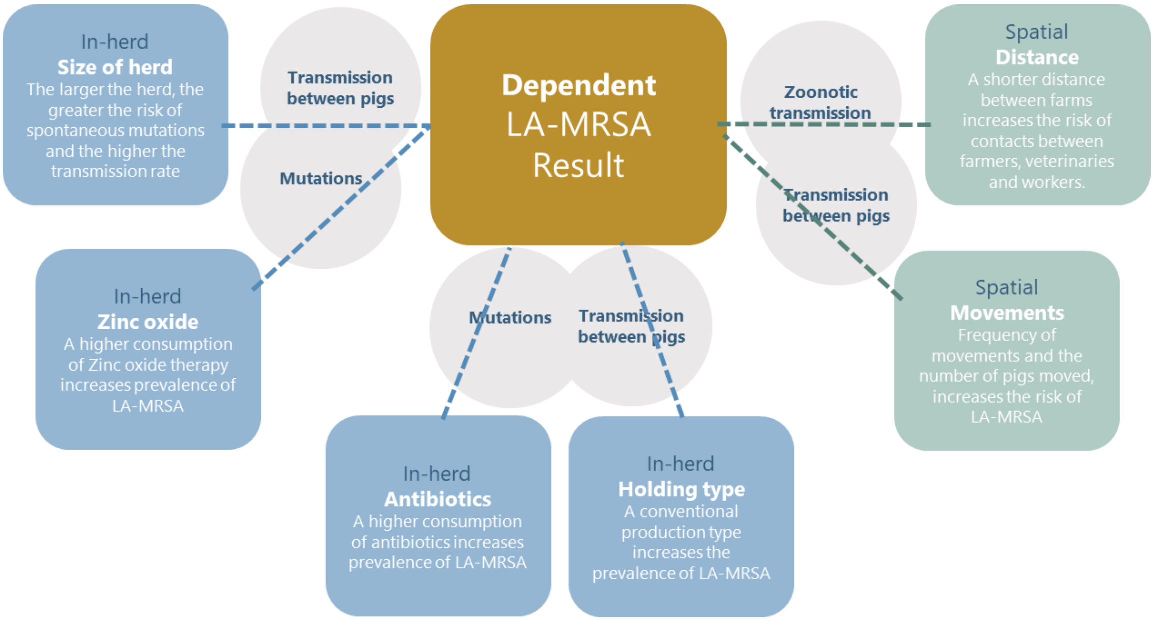

Figure 12. Causal links between explanatory parameters and LA-MRSA-results

4. Methodology

The methodology section consists of two parts. The first section contains an explorative analysis of the variables. The second, outlines how the likelihood was tested using a logistic regression model.

4.1 Explorative analysis

The model consisted of seven independent variables. Six out of these were continuous and one categorical (Table 6). To test whether the positive MRSA-test results were independent from each other spatially, a stepwise autocorrelation test was performed (4.1.1). The data distribution has been outlined in section 4.1.2 and a multicollinearity tests have been described in section 4.1.3.

Table 6: Data descriptives

Variables	Description	Data characteristics	Range	Mean/Mode
LA-MRSA result	Result from MRSA CC398 screening of 176 pig herds, 2010-2011	Binary	1: Positive 2: Negative	Negative
Size of herd	Number of animal units (number of pigs related to type of pigs)	Continuous	0.33-818	190.5
Holding type	Type of production: Conventional, conventional/outdoor, organic	Categorical	1: Conventional. 2: Conventional/outdoor 3: Organic	Conventional
Antibiotics	Prescribed antimicrobial substances one year prior to test date normalized by animal unit.	Continuous	0-19696.9	399.8
Zinc oxide	Prescribed antimicrobial substances one year prior to test date normalized by animal unit.	Continuous	0- 2936.1	201.1
Movements a Number of received pigs	Total number of received pigs one year prior to the testing.	Continuous	0-97187	4794.3
Movements b Number of senders	The number of senders that supplied the tested farms with pigs, during one year prior to the testing	Continuous	0-12	1.58
Distance Distance to all other farms	Average distance to all other pig holdings (Euclidean distance)	Continuous	99 km- 348 km	134,6

4.1.1 Autocorrelation

The dependent variable was constituted by LA-MRSA CC398 tested farms from the 2010-2011 screening. The data were binary – either LA-MRSA-positive (1) or LA-MRSA-negative (0). Out of the 176 farms that were tested, 52 (29.5%) had a positive LA-MRSA result.

To test whether positive results were independent from each other, *spatial autocorrelation* tests were applied. If neighbouring observations have similar values (in this case either positive or negative) there is a positive spatial autocorrelation (clustering). On the contrary, if neighbouring observations have divergent values, there is a negative spatial autocorrelation (dispersion). When similar values show no spatial pattern, autocorrelation is random. Since the data were binary it was not possible to use the common *Moran's I spatial autocorrelation* approach. The degree of clustering or dispersion was instead measured by a stepwise combination of Getis-Ord Gi* statistics and aggregation (Scott & Janikas 2010) (Figure 13).

First, an optimized hot spot analysis was run on all tested holdings (a). A regular sized fishnet was outputted based on the density of the input points. The size of the fishnet cells was 15 414 x 15 414 meters. The size was calculated through default settings in the optimized hot spot analysis tool which are based on the average nearest distance and median nearest distance on all of the unique location points (b). The positive points (c) were thereby aggregated to the created fishnet. A count per grid cell was obtained (d). To calculate the proportion of positive tests within each grid cell, a ratio was calculated (e). In the final step (f) an optimized hot spot analysis was conducted using the ratio score as analytic field.

The results from the final hot spot showed Gi_bin-scores that were not significant. This indicates a random distribution of farms that were tested positive. It also confirms the null hypothesis but could also reflect the fact that merely two percent of all farms were tested and selected randomly.

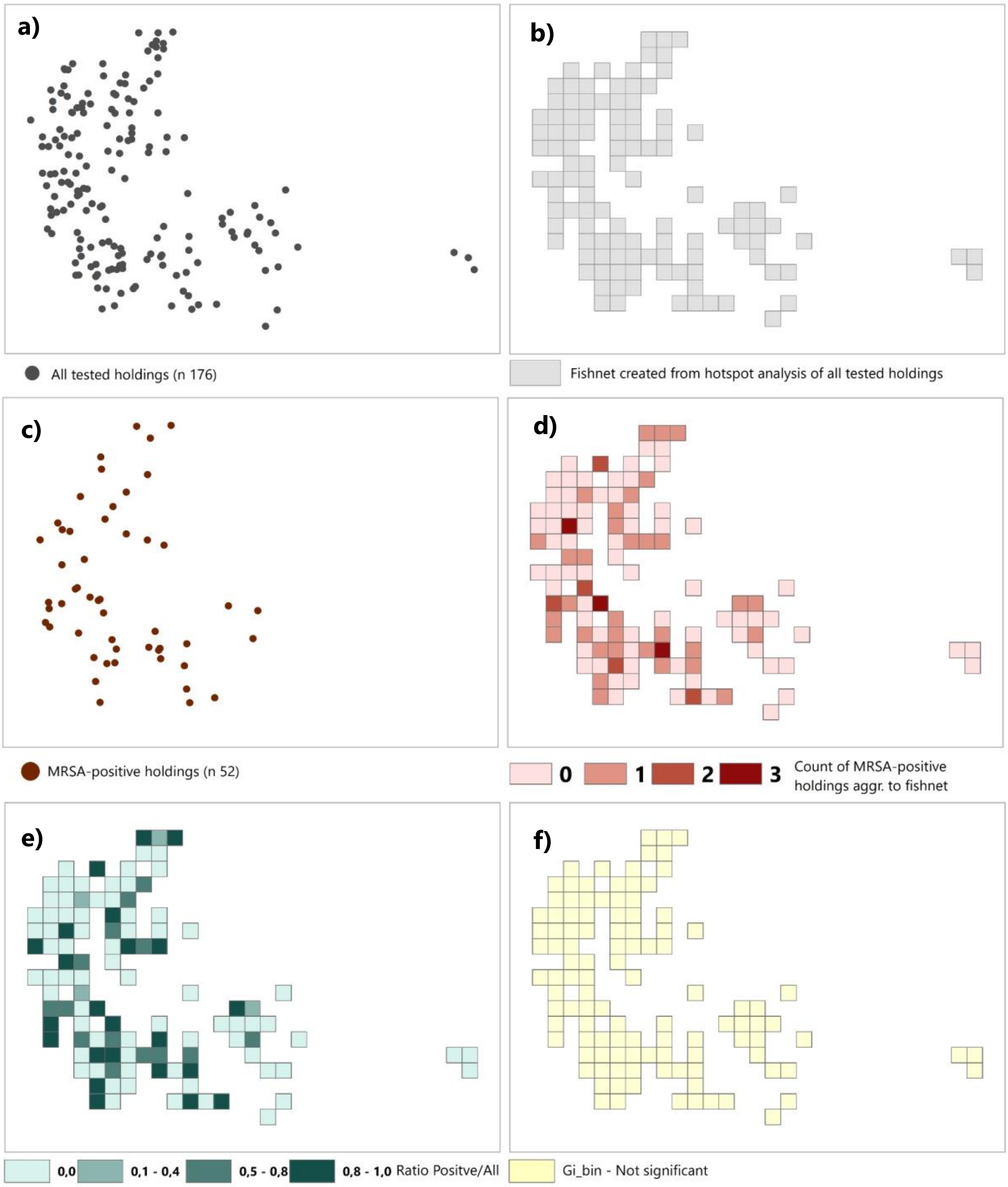

Figure 13. Stepwise combination of Getis-Ord Gi* statistics and aggregation.

4.1.2 Data distribution

When examining the distribution of the continuous, independent variables (Figure 14; Table 7) it is obvious that all six have skewed distributions, several outliers and even extreme values.

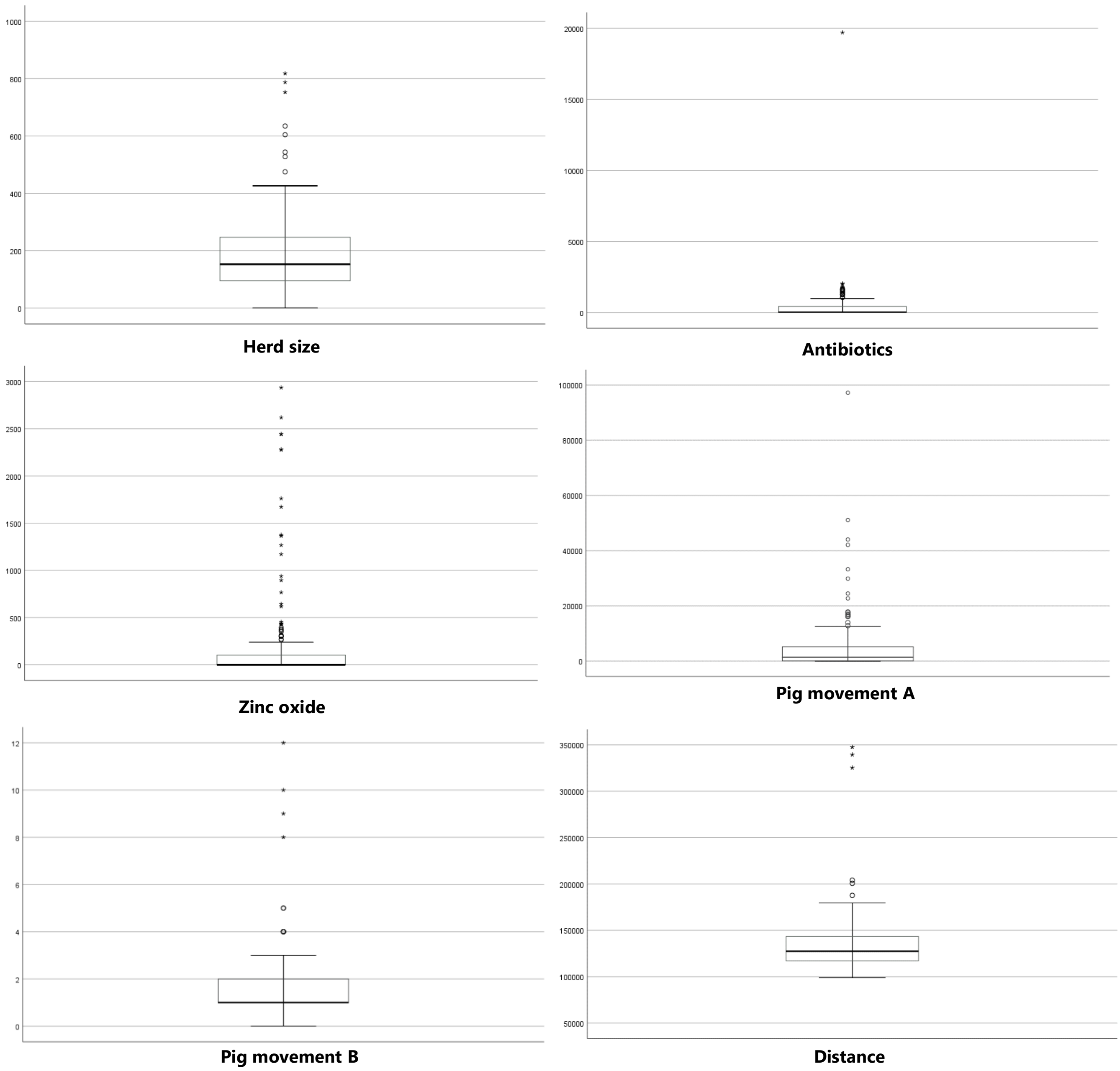

Figure 14. Boxplots of continuous, independent variables – all cases included (*Model a*)

Table 7: Data characteristics of continuous, independent variables

Variable	Mean	Median	Variance	Std. Deviation	Skewness	Kurtosis
Herd size	190.5	152.77	19632.78	140.12	1.91	4.98
Antibiotics	399.77	27.04	2352487.58	1 533.78	11.545	145.16
Zinc oxide	201.04	0	279146.97	538.34	3.431	11.77
Distance (m)	134643.27	127486.87	1097525579.73	33128.92	4.08	22.6
Pig movement A	4 794.38	1 395	109 944 041.28	10485.420	5.302	37.68
Pig movements B	1.58	1	2.66	1.63	3.35	15.64

Since it was difficult to determine if this was caused by natural variations or measurement errors, two different models were conducted:

- **Model a)** All cases were kept
- **Model b)** All outliers (cases above the top whisker) were removed in the variables *Herd size* and *Pig movement A*. Extreme values were removed in *Antibiotic, Zinc oxide and Distance*. All cases were kept in the variable Pig movement B.

The reason behind removing all outliers in the CHR-based variables *Animal units* and Pig *Movement A* was the suspected recording errors. Owners of holdings report numbers into CHR themselves on a yearly basis (MEDF 2021). It is likely that farmers might overestimate the number of pigs that have been received. Besides overestimation, it could also be a case of simple typing errors. The same error is likely to occur when it comes to the actual transmission between farms.

In the case of antibiotics, extreme values might also be caused by typing errors. It could also be a case of normalization errors. Dividing large doses of antibiotics with small animal units, might cause these extremes. The extreme values removed in *Distance* were all tied to farms located in Bornholm, a remote island, located 180 km from Copenhagen.

4.1.4 Independent variables – multicollinearity

To control for multicollinearity (high correlations among the independent variables), linear regression was carried out on the continuous variables to retrieve *variance inflation factor-measures* (VIF).

$$VIF = \frac{1}{1 - r^2}$$

Both models show VIF-values close to 1 (Table 8), indicating none-existing correlations between the variables.

Table 8: Variance inflation factor-measures (VIF) of independent variables

Model	Herd size	Antibiotics	Zinc oxide	Pig movement A	Pig movement B	Distance
Model a **(n= 176)**	1.042	1.037	1.042	1.024	1.047	1.020
Model b **(n=138)**	1.040	1.297	1.302	1.011	1.034	1.032

4.2 Logistic regression analysis

To explore the main question - which factors that might have contributed to positive LA-MRSA-results, seven explanatory variables were set against the dependant in a logistic regression analysis. Logistic regression is a common method within epidemiology due to the dichotomous nature of the response variable (positive or negative disease status, exposure or no exposure) (Kleinbaum et.al. 2007).

The most common statistical approach to study the relationship between a dependant and independent variables - *the linear regression model*- could not be applied because the dependant variable was binary. Binary data do not have a normal distribution, nor linearity which makes them unsuitable for linear regression. Instead, logistic regression fits an s-shaped logistic function to the data (Figure 15). The function measures the likelihood that (in this case) a farm has a positive LA-MRSA result based on a set of variables.

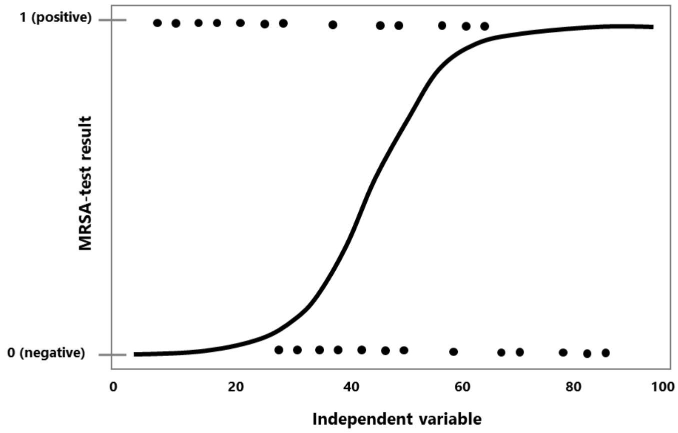

Figure 15: Example of S-shaped logistic function

4.2.1 Coefficients and odds ratio

Coefficients in a linear regression are straightforward and represent the variation observed in the dependant variable connected to the increase of one unit of the independent variable. This cannot be translated into logistic regression since every time x increases by one unit, it represents the logit of Y instead of the variation. In other words, while probability increases by degrees (0-100) in linear regression, probability is measured by odds or maximum likelihood in logistic regression.

The logit transformation (or the log of the odds ratio) is the proportion of a certain outcome in the dependent variable and can be described as follows:

$$l = \text{logit}(p) = \ln(p/(1-p))$$

In this particular case it intends that the mean of an independent variable will be the proportion (p) of positive MRSA-test results. Conversely, the probability of negative test result will be calculated as 1-p.

4.2.2 P-value and standard error

Similar to other statistical analyses, obtaining p-values, or statistical significance is crucial when interpreting the logistic regression results. To be able to reject the null hypothesis, a low p-value (<0.05) needs to be obtained. Conversely, if an independent variable has a high p-value (>0.05) it might indicate that the changes in the independent variable are not associated with the changes in the dependant, making the variable insignificant.

On the same note, the *standard error* measurement, is also a means to control the precision of the variables and to determine whether there is a true relationship between the model and the response variable. It measures the distance between the values and the regression line. An acceptable standard error should fall within plus/minus two standard errors.

5. Results

The results from the two logistic regression models (Model A: All cases kept and Model B excluded cases) were presented in this section. The p-values, odds ratios, standard errors and coefficients of each variable were outlined descriptively below and further analysed in the Discussion part (section 6).

5.1 Model A

The results from the logistic regression Model A show a general lack of fit. Even though the omnibus test indicated a significant results for the model as a whole (p-value 0.028), the results were weak when looking at the individual variables. As seen in Table 8, only one variable, *Pig movement B; p=* 0.027, has a p-value that is smaller than the alpha (0.05) and thereby significant. The variable *Distance* is close to the boundary (p=0.064). However, the other variables are far from being significant as they display rather high p-values, *Pig movement A* even shows a very high one (0.880). If a confidence interval of 90% were chosen, *Herd size* and *Distance* would have been significant. However, if the confidence interval 99 % were chosen, none of the included variables would have been significant.

Table 9: Results from the logistic regression analysis of Model A (all cases kept)

Variables	*P-value sig*	*Coefficients (B)*	*Standard error*	*Odds ratio Exp (B)*
Herd size	0.097	-0.002	0.001	0.998
Antibiotics	0.460	0.000	0.000	1.000
Zinc oxide	0.517	0.000	0.000	1.000
Distance	0.064	0.000	0.000	1.000
Pig movement A	0.880	0.000	0.000	1.000
Pig movements B	**0.027**	0.235	0.106	1.265
Farm type	0.265	-1.205	1.081	0.300

Values in bold are significant at $P < 0.05$

When looking at the coefficients, which reflect the relationship between the independent variables and the MRSA-result, four (*Antibiotics, Zinc oxide, Distance and Pig movement A*) out of seven variables show no relationship (ß=*0*). *Farm type* and *Herd size* display negative values *(*ß *=-1.205; -0.002*) which could indicate that there is a decreased likelihood between these two and the prevalence of MRSA.

The odds ratio values correspond with the previously mentioned proxies, as only *Pig movement B* showed a positive odds ratio. For every one unit increase on *Pig movements B*, the odds of having a positive MRSA-result, change by a factor of 26.5%. The odds ratio for *Herd size* showed and *Farm type* showed the reverse logic. For every one unit increase on *Herd size*, the odds of having a positive MRSA-result decreased by 2 %. For *Farm type* it was as much as a 70 % decreased likelihood if the farm was conventional.

5.2 Model B

The results from the logistic regression Model B has similarities with Model A, but were slightly poorer in most aspects. The omnibus test showed a non-significant result (0.58) and when investigating each independent variable, the relationships were weaker in general (Table 9).

While *Pig movement B* exhibited a significant p-value (0.027) in Model A, it showed a non-significant value in Model B (p=0.065). The only significant variable in this model was *Distance* (p-value 0.038). The other variables showed non-significant p-values, where *Herd size, Antibiotics* and *Pig movement A* displayed fairly high values. If a confidence interval of 90% were chosen, *Pig movement B* would have been significant. However, if the confidence interval 99 % were chosen, none of the included variables would have been significant.

Four (*Herd size, Antibiotics, Zinc oxide and Farm type*) instead of two variables showed negative coefficients in Model B. Again, only *Pig movement B* showed a fairly strong relationship. This was further reflected when looking at the odds ratio where this variable was the only one with a positive odds ratio (28.3%).

Table 10: Results from the logistic regression analysis of Model B (cases excluded)

Variables	*P-value sig*	*Coefficients (B)*	*Standard error*	*Odds ratio Exp (B)*
Herd size	0.666	-0.001	0.002	0.999
Antibiotics	0.642	-0.001	0.001	0.999
Zinc oxide	0.438	-0.002	0.003	0.998
Distance	0.038	0.000	0.000	1.000
Pig movement A	0.688	0.000	0.000	1.000
Pig movements B	0.065	0.249	0.135	1.283
Farm type	0.328	-1.085	1.109	0.338

6. Discussion

The results from both Model A and B did not show any strong connections between the independent variables and a positive MRSA-results. Contrary to the hypothesis, none of the chosen parameters did increase the likelihood of having a positive LA-MRSA result. The only variable that were pointing towards a slight increase was *Pig movement b*. The outcome and limitations of the study are further elaborated below.

6.1 In-herd parameters

6.2.1 Outcome

The probability that a farm has a positive LA-MRSA result was not higher based on any of the in-herd variables. None of the theoretically plausible variables (high antibiotic and zinc oxide consumption, larger units and conventional production systems) could be tied to a higher likelihood of a positive LA-MRSA result, in either of the models. Farm type even had a reverse likelihood. The only potentially interesting result was the significance of *Herd size* using a 90% confidence interval in Model A. However, the significance was not confirm in Model B and since the odds ratio had a reverse logic in both models, this result could not be trusted.

The most surprising non-connection was the use of antibiotics. The (over)use of antibiotics is linked to resistance in most pathogenic bacteria and especially in the case of LA-MRSA. Previous studies on the use of antibiotics have shown a higher prevalence of LA-MRSA in farm systems where antibiotics were used as preventive measure (van Duijkeren, 2008; Cuny et al., 2015). Schulz (2018) also confirmed this link but differentiated between high-risk antibiotics (containing β-lactams or tetracycline) and other types of antibiotics.

When it comes to the other in-herd variables, several studies have confirmed a link between production system (organic vs. conventional), size and LA-MRSA prevalence (Slifierz et al., 2015b; van de Vijver et al, 2012). While production system is a rather straightforward measure (certain requirements need to be met to classify as organic) it is harder to determine what constitutes a large and a small farm. In this case, animal unit[4] was deployed, but whether this measurement captures density and contact between pigs is harder to determine.

[4] 208 piglets constitute 1 animal unit while 39 finisher pigs also equal 1 pig unit.

6.2.2 Limitations

Even though the model included previously confirmed in-herd parameters, there is still a risk of unreliable measurements. The discussion on animal units above, is one example of how key parameters can be obtained very differently. The use of antibiotics and zinc-oxide share a similar bias. In this study, the sum of prescribed antibiotic and zinc oxide substances was divided by the *Animal Unit* to obtain a relative measure. This was done since using the absolute unit of measure per farm would not have been adequate since the size of the farms varied to such degree. However, the relative measure used in this case, can also be problematic since animal unit does not reflect the number of pigs or the weight of the herd. Another issue might be connected to the selection of substances. This study looked at all types of antibiotics while Schulz (2018) only included high-risk types. A third issue might be connected to the discrepancy between prescription and usage. During the study period, it was common to use antibiotics and zinc oxide as preventive and growth promoting drugs. That could indicate that the doses prescribed, were not utilized during the study period.

As written above, farm type is more straightforward compared to the use of drugs and farms size. However, the requirements for organic production varies in different countries. Most of the studies confirming a linked between negative results and organic production were set in Germany and Holland. A more stringent way of measuring animal welfare might have been the presence of sows. A recent Danish study (Sørensen et al., 2018) found a connection between the absence of sows and the prevalence of MRSA CC398. This could indicate that later separation between piglet and sow might prevent transmission to a greater extent than increased spacing and organic forage.

6.2 Spatial parameters

6.2.1 Outcome

Farms that on average were located closer to other farms were not more likely to have positive LA-MRSA results. The variable *Distance* did show signs of modest significance in Model B and not in Model A. However, the odds ratio for this variable was not positive in any of the models. Thus, spatial proximity, at least as it was measured here, could not be tied to susceptibility among Danish pig herds in 2010-2011. One of the few studies conducted on spatial proximity between farms and LA-MRSA susceptibility (Mroczkowska et al.; 2017) confirms the lack of connection. The studies on spatial proximity and human prevalence however, have showed more convincing links between living near a pig farm and being infected by LA-MRSA (van Rijen et al 2014; Paget et al. 2015; Zomer et al. 2017; Anker 2018).

One possible reason why spatial factors might be more important for human prevalence, could be the difference in mobility. Infected humans living near positive farms are likely to have their workplace associated with the farm or have close-by associates that are somehow connected to a farm. Zoonotic transmission is likely to occur more often than within-species transmission since pigs' mobility are confined to a specific compound. However, if the pigs are moved at the hands of man, and transported to other farms, the risk of transmission could increase. Modest signs of this could be seen in the results from Model a (Table 9). The number of pig movements (*Pig movement b*), or more specifically put - the number of different farms sending pigs to a particular holding, was the only variable that showed signs of relevance in both models (odds ratio 1.265 in Model A and 1.283 in Model B). Schulz (2018; 2019) has also showed the importance of pig movements in her extensive studies. Her research group has concluded that holdings that received pigs from negative farms had a significally reduced risk of LA-MRSA (Sieber et al., 2018). But, the same studies call for causation and conclude that pig movement alone cannot explain the difference between infected and non-infected farms. Having said that, it could be worth while studying more layers within the *Pig movement B*-variable. It could be interesting to find out if there is a difference between the different senders and at what number of senders it increases the likelihood.

6.2.2 Limitations

The lack of strong connections between spatial proxies and susceptibility also need to be viewed in the light of validity. The average nearest distance parameter was based on Euclidian instead of network distance. Network distance was initially calculated since it theoretically might capture the zoonotic transmission in a more realistic way compared to Euclidian distance. Pigs and humans do not move as the crow flies. Further, as Schultz (2019) points out, transmission of LA-MRSA is unlikely to occur through dust and soil transported in a Euclidian mode.

Despite this logical rationale, network distance was not favoured due to the high risk of measurement errors. If the adequate amount and quality of data cannot be incorporated, a network based dataset might be less appropriate compared to a Euclidian dataset. As Frizelle et al. (2009) argues, building a reliable network dataset requires detailed and updated data which often entails field work and access to extensive databases. In this particular case it also required knowledge of how the farmers usually moved and which farms they usually visited more than ten years ago. The final reason for favouring Euclidian distance was correspondence with other studies. Anker (2018) and Mroczkowska et al. (2017) used Euclidian average distance to measure the proximity effects in their studies.

6.3 Other limitations

The discussion in section 6.1.2 and 6.2.2 highlights issues connected to the choice of variables and measurement errors. It is also important to consider choice of statistical method, the sampling method and size and time period in this matter.

The first major concern connected to logistic regression is sample size. Even though there is no consensus on a minimal number of observations, Hosmer et. al. (2013) suggest that at least 400 observations while others such as Hair et al. (2009) recommend a ratio of 10 cases per dichotomous alternative. The fact that only 176 observations were included and only 52 out of these were positive might lead to uncertain results. The small number of observation also causes general doubts. Even though the sample size was reasonably fair in statistical terms, the risk of not obtaining a representative sample is greater during the onset of an infection. In 2010, there were 8850 registered pig farms in Denmark. Only testing 176 randomly selected finishing farms, is likely to create a less valid result.

Another concern is choosing the right study period for all variables. Since the aim was to investigate the early onset, LA-MRSA results from the first screening in 2010-2011 was chosen. The result was set against data (use of antibiotics and zinc oxide, herd size, production system and pig movement) corresponding to that time period. However, since LA-MRSA might have been prevalent long before the screening, it is impossible to know how well the time series actually coincide.

A third issue which was raised in section 2.5 is the fact that the Danish authorities waited until 2014 to test breeding herds. According to Schulz (2018) breeding herds have played a significant role in the introduction and spread of LA-MRSA in Denmark. If the breeding herds were included in the screening of 2010-2011, it would have been possible to check how proximity and contact to those farms might have influenced the likelihood.

7. Summary and conclusion

LA-MRSA is a pathogenic bacteria that are resistant to methicillin (penicillin) and tetracycline antibiotics found in livestock such as pigs, poultry and cattle. During the last decades it has become a major concern, especially in countries with a large pig production. Denmark is the largest pig producer in the EU per capita and has experienced a dramatic increase in LA-MRSA positive herds. The first cross-sectional screening in 2010-11 found isolates in 16% of all Danish pig herds. In the most recent screening in 2016, 88% were LA-MRSA positive (MEFD 2019).

This study looks at factors contributing to LA-MRSA prevalence in Danish pig farms during the early onset and first screening period in 2010-2011. It attempts to investigate whether a set of in-herd and spatial factors contributed to the likelihood of having a positive LA-MRSA. The likelihood was tested using logistic regression analysis.

The results from the in-herd analysis showed no significant results. Thus, farms with high antibiotic and zinc oxide consumption, larger units and conventional production systems, were not more likely to have positive LA-MRSA results compared to organic, small-sized, low consumption farms.

The outcome from the analysis of the spatial parameters was also weak. Farms that on average were located closer to other farms were not more likely to have positive LA-MRSA results. Thus, spatial proximity could not be tied to susceptibility among Danish pig herds in 2010-2011. However, modest signs likelihood was seen between positive prevalence and the number of different farms sending pigs to a particular holding (variable *Pig movement b*). This was the only variable that showed signs of relevance, which has been confirmed in other studies (Schulz 2018; 2019).

Based on the findings of this study, investigating why some of the farms were more susceptible during the early onset requires a better test result sample and modified measures. To capture the spatial dimensions properly, and to look further into the variable Pig movement, it might be important to add qualitative methods to capture how the zoonotic transmission might have occurred. However, these matters are difficult to address in retrospect which makes it very difficult for future research to pin point the causes of the early onset.

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9. Appendix

Antibiotic substances were selected manually by consulting the Veterinary Product Database (2020) and the EU Veterinary Medicinal Product Database (2020). A detailed list of all prescriptions is presented in Appendix 1. The CHR-numbers were anonymized due to integrity reasons.

ALAMYCIN PRO. VET.
ALAMYCIN VET.
AMOXINSOL VET.
APRALAN VET.
AQUACYCLINE VET.
AUREOSUP VET.
AUROFAC
AUROVET
AUTOVACCINE IMPETIGO VET.
BAYCOX VET.
BETAMOX VET.
BORGAL VET.
CEVAZURIL
CHLOROMED
CIRCOVAC
CLAMOXYL PRO. VET.
CLAMOXYL VET.
COBACTAN VET.
COLINOVINA VET.
COLIPLUS
COLIVET
CURAMOX PRO. VET.
CURAMOX VET.
CYCLO SPRAY VET.
DENAGARD VET.
DIHYDROSTREPTOMYCIN VET.
DOXYCYCLIN "2CARE4" VET
DOXYCYCLIN "BIOVET" VET.
DOXYCYCLIN "SCANVET" VET.
DOXYLIN VET.
DOXYPIG
DRAXXIN
DUMOCOL VET.
DUOPRIM VET.
ENGEMYCIN VET.
ETHACILIN VET.
EXCENEL VET.
FENYLBUTAZON "OBA"
FINADYNE VET.
FLORKEM
FLUNIXIN VET.
FUCIDERM VET.
FUCITHALMIC VET
GENTOCIN VET.
HYDRODOXX
KARIDOX
LINCOCIN VET.
LINCOMIX VET.
LINCO-SPECTIN VET.
MAMYZIN VET.
MELOVEM
MELOXIDYL
METACAM TIL KVÆG OG SVIN
METACAM TIL KVÆG, SVIN OG HESTE
MYCOBAC HYO VET.
MYCOBAC UNO VET.
NAXCEL
NOROBRITTIN VET.
NORODINE VET.
NOROMOX PRO. VET.
NOROMYLIN VET.
NOROPEN PROLONGATUM VET.
NOROPEN VET.
NOROSPECLIN VET.
NOROSTREP VET.
NUFLOR VET.
OCTACILLIN VET
PENICILLINPROK. ROSCO VET.
PENOVET VET.
PIGGIDOX VET.
PRACETAM VET.
PREMEDOX VET.
PULMOTIL VET.
PYREXIN VET.
SELECTAN
SOLACYL
SOLUDOX VET.
STABOX VET.
STREPTIPENPROKAIN ROSCO VET.
STREPTOCILLIN VET.
TERRALON PRO. VET.
TETROXY PRO. VET.
TIAMVET VET.
TILMOVET
TILMOVET VET.
TOLTAROX VET.
TRIBRISSEN FORTE VET.
TRIBRISSEN VET.
TRIMAZIN FORTE VET.
TROCOXIL
TYLAN VET.
TYLMASIN VET.
TYLOSIN "CEVA" VET.
TYLOSIN VET.
VETICYCLIN PROLONGATUM VET.
VOREN VET.
ZEROFEN VET.

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