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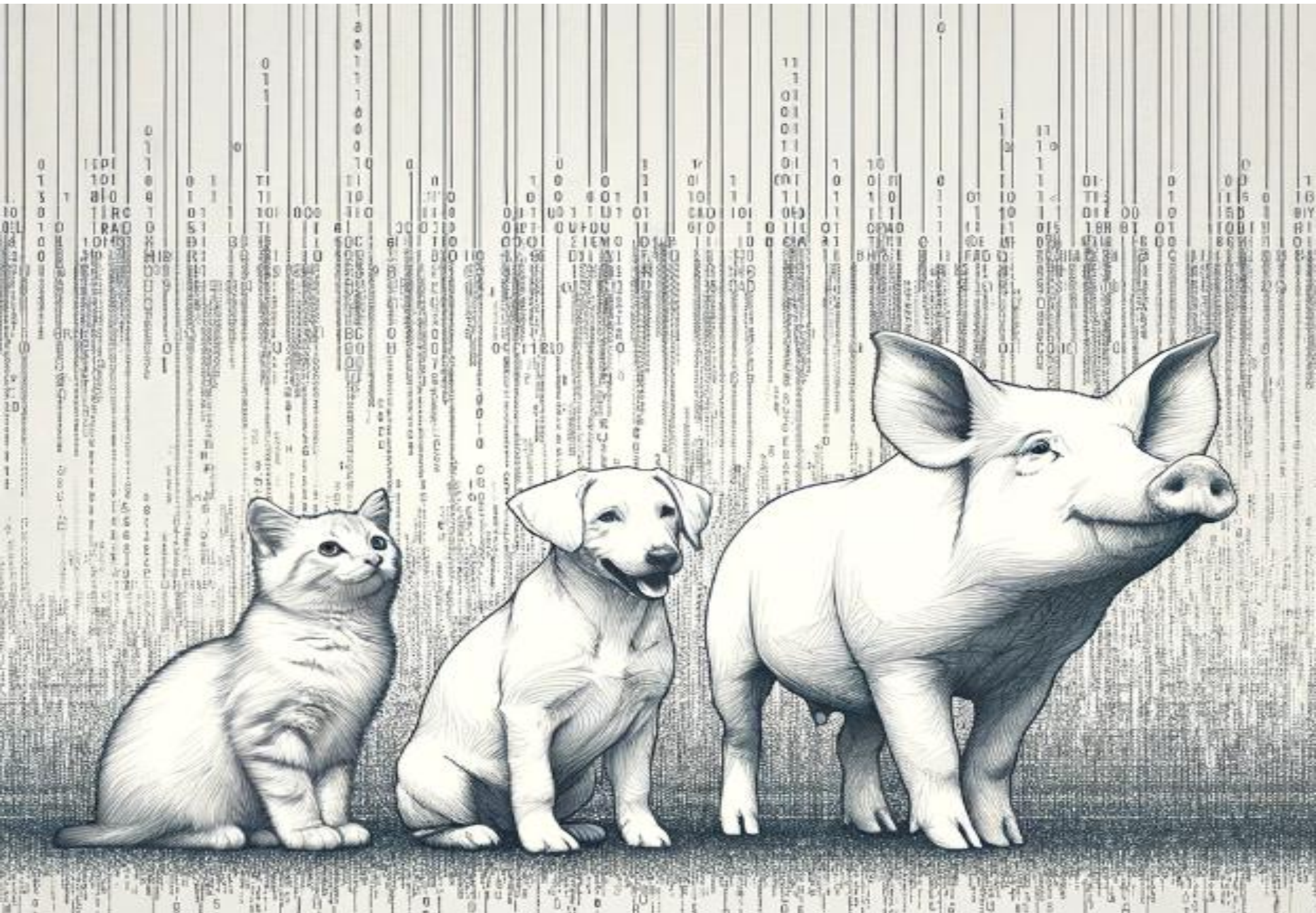
Data-based health surveillance in pigs and trends in antimicrobial use in companion animals



PHD THESIS · 2024

ANNE-SOFIE GLAVIND

This thesis has been submitted to the Graduate School of Health and Medical Sciences, University of Copenhagen, on November 8th, 2024.

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PhD Thesis 2024

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Preface

In 2020, the University of Copenhagen and Statens Serum Institute (SSI) took over the responsibility for the Veterinary Public Authorities Agreement. This marked the beginning of the VetStat cluster from which this PhD project is derived. The VetStat cluster aims to develop new competencies and improve the evidence-based use of the Veterinary Statistics database (VetStat) for research purposes. The PhD project was financed by the Faculty of Health and Medical Sciences, the Danish Food and Veterinary Administration and the Danish Pig Levy Foundation. Part of the scientific work was done in close collaboration with the Danish Agriculture and Food Council and Danish pig producers, who dedicated their time to providing invaluable knowledge to this project.

The foundation for this PhD was laid by a chance meeting with Amanda at a VetStat workshop where I represented the Veterinary Inspection Unit East of the Danish Veterinary and Food Administration. Through my work as an official veterinarian, I have met many Danish pig producers and witnessed their daily commitment to the animals and their work to balance health, productivity and antimicrobial use. I have also listened to their challenges and occasional frustrations regarding the legal requirements placed on them. I would therefore like to begin with a big thank you to Amanda. Thank you both for introducing me to this project and for all the help you've given me. I am grateful that this project can be finished by working closely with you.

The start of my PhD was characterised by the coronavirus pandemic, which struck almost immediately. This resulted in some long years at the home office in Slagelse and, therefore, not spending as much time in Frederiksberg as I had initially planned. Fortunately, I took revenge in the last part of my PhD, which took place at Axelborg.
In this regard, I thank Lis for your great commitment to my project. I feel incredibly privileged that you have devoted so much time to mentoring and guidance and for being so committed to making me part of the work community and patiently listening to my frustrations with data processing.

A big thank you to Helle for your always wonderful support and all the compliments I didn't always feel I could live up to. I felt very safe knowing that your support was always available. It has been rewarding to share the pig weeks with you, and I have enjoyed every single time I have made the trip to Roskilde - even if it was in the middle of a snowstorm.

Thank you to Liza for stepping in at the start of my project. You have been invaluable in overcoming the challenges of my first paper.

Jeanette, it's been quite a journey the two of us have embarked on. Thank you so much for your great support and for listening to all my concerns and frustrations, first regarding the project, then my new baby and then again regarding the project. Thank you to you and Alice for your invaluable help and for patiently assisting with my frustrations with R and VetStat.

A big thank you to Jan Dahl and Bjørn Lorenzen for help with data and to Charlotte Sonne and Signe Stricker for help with questions regarding pig production.
I am very grateful for all the pig producers who took the time to talk to me about their production - without them, my project would not have been as interesting.

Finally, a big thank you to my family for putting up with my very stressful state of mind. A heartfelt thank you to Mikael, who patiently listened to my frustrations and endless talk about pigs and databases. I tend to forget that you're not as passionate about pigs as I am. This project would not have been possible without your support, especially since the last 4 years have been with our little fantastic Liva.

Slagelse, November 2024

Summary

The Danish Veterinary Statistics database (VetStat) covers sales of all veterinary medicinal products and holds a central position in national antimicrobial monitoring systems. One prominent initiative derived from VetStat data is the Yellow Card scheme. This legal intervention sets limitations on the maximum permitted limit values for antimicrobial use (AMU) in Danish pig production, promoting prudent antimicrobial stewardship among Danish veterinary practitioners and pig producers. The use of VetStat data for companion animals is also becoming increasingly important. With the Veterinary Medicinal Products Regulation put in force in January 2022, EU Member States are now requested to report antimicrobial sales or use data to the Antimicrobial Sales and Use Platform instead of the voluntary data reporting to the European Medicines Agency. Part of the initiative requires data to be compiled for companion animals (dogs and cats) starting in 2030. Therefore, the existing national systems must be validated to adhere to the EU requirements. Because VetStat data is fundamental in the veterinary sector for monitoring antimicrobial sales data, it is important to strengthen the evidence-based use of VetStat data in research.

The aim of this PhD project was to improve the practical use of VetStat data in data-based health surveillance by extending its usage across different animal species and improving the knowledge of the relationship between AMU, herd characteristics and animal health in pig production.

The association between herd characteristics and AMU levels in Danish weaned pigs were examined in a case-control study. Data was collected through telephone interviews with 24 pig producers who received a Yellow Card in weaners between 2016 and 2020 and 28 pig producers who, for 12 consecutive months, had an AMU in weaners below the national average of 10.7 – 8.8 ADD/100 animals/day. Herds with a Yellow Card in weaners were more likely to treat gastrointestinal diseases (OR = 4.8) than herds with a low AMU and were more likely to use flock medication (peroral preparations) (OR = 10.5). The results of the multivariable regression model indicate that herds with a Yellow Card in weaners were less likely (OR = 0.04) to have strict routines working from youngest to oldest, tending to sick pigs last and having sufficient room for all newly weaned pigs to eat (OR = 0.08).

The association between AMU levels and animal health was examined in 10.5 million finisher pigs delivered to 9 Danish abattoirs between 2016 and 2020. The study compared lesion prevalence in herds with a high AMU (a Yellow Card in finishers), herds with a medium AMU (above 2.5 ADD/100 animals/day in at least one month out of nine consecutive months), and herds with a low AMU (equal to or lower than 2.5 ADD/100 animals/day in nine consecutive months). Despite differences in AMU, only marginal differences in meat inspection lesions were present. The lesion prevalence differed by less than 0.5% between the three AMU levels. Lung lesions were the most common lesion type (16-17%). Associations between AMU levels and the prevalence of meat inspection lesions were analysed in mixed-effects logistic regression models. Herds with a medium AMU level were associated with the lowest risk of meat inspection lesions. In seven of the eight lesions included in the study, large herds were

associated with a lower risk of meat inspection lesions than small herds. The risk of lung and tail lesions was higher for herds in the western region of Denmark compared to the eastern regions, and the risk of tail lesions, arthritis and osteomyelitis was higher in outdoor herds compared to indoor herds.

The usability of the VetStat database in estimating total national sales of antimicrobials in Danish companion animals was examined by calculating the sales of antimicrobials for use in Danish dogs and cats from raw VetStat data. In 2018, sales of antimicrobials recorded on animal group code 90 amounted to 515 kg active compound. Of this amount, 53% were products licensed solely for use in livestock or horses. Antimicrobials licensed solely for dogs and cats registered on animal group code 0 covered 706 kg active compound (tablets, ointment, ear- and eye drops). This means that the estimate relies on how data is extracted from VetStat. In addition, VetStat does not contain any treatment data for Danish companion animals. This means that estimates from VetStat will not accurately reflect how much parenteral antimicrobials are used in Danish dogs and cats.

The number of assigned Yellow Cards between 2015 and 2020 represents less than 4% of the total number of Danish pig herds. Danish pig herds with a Yellow Card between 2015 and 2020 complied with the regulatory restrictions imposed by the DVFA in the Yellow Card scheme when considered as a whole. There was a significant increase in the number of Yellow Cards assigned in 2019 and 2020 compared to the previous years. It coincides with a reduction in the permitted limit values for all age groups in 2019 and brings the limit value closer to the national average for all three age groups. AMU levels were reduced for all three age groups during the nine months that followed a Yellow Card. The median ADD/100 animals/day was reduced across all age groups.

The results of this thesis provide knowledge for future antimicrobial reduction measures in the veterinary sector. A Yellow Card in weaned pigs was associated with only a few herd characteristics compared to herds with low AMU in weaned pigs. However, the herds differed in the most frequently treated herd health diseases and treatment methods. In finishers, both a high and a low AMU could be linked with health and welfare issues. These results indicate that the advisory role of the herd veterinarian could be pivotal in preventing adverse effects of antimicrobial reduction measures on animal health and welfare. For companion animals, VetStat data was not applicable as a proxy for AMU in dogs and cats. With the current structure of the database, a calculated AMU from VetStat data will only be an approximation. Estimating national AMU in dogs and cats is not possible until antimicrobial treatment data from veterinary practitioners in small or mixed practices is transferred to VetStat via the billing system.

Sammendrag (Danish summary)

Den danske Veterinære Statistikdatabase (VetStat) dækker salget af alle lægemidler til veterinært brug. Databasen har en meget central position i de systemer der i Danmark overvåger salg af antibiotika til dyr. Et fremtrædende initiativ der bygger på brugen af VetStat data er Gult Kort-ordningen. Gult Kort-ordningen fungerer som et lovindgreb hvori der er fastsat grænseværdier for den maksimale tilladte brug af antibiotika i danske grisebesætninger. Ordningen blev indført for at fremme fornuftig brug af antibiotika blandt danske griseproducenter og dyrlæger.

Fokus rettes dog også i højere grad mod brugen af VetStat data til kæledyr. Med den nye veterinærlægemiddelforordning der trådte i kraft i januar 2022, skal medlemslandene i EU rapportere data om brug og salg af veterinære lægemidler til en fælles EU lægemiddeldatabase frem for de frivillige indberetninger til Det Europæiske Lægemiddelagentur. De ændrede krav medfører samtidig at der nu stilles andre krav til både format og indhold. Det betyder at fra 2030 skal indberetningerne også indeholde information om salg og brug af lægemidler til brug for hund og kat. Disse ændringer betyder at de eksisterende nationale systemer nu skal leve op til EU-kravene.

I den danske veterinærsektor er VetStat data en vigtig datakilde i den løbende overvågning af salget af veterinære lægemidler. Det bevirker at det er vigtigt løbende at styrke den evidensbaserede brug af VetStat data i forskning.
Formålet med dette PhD-projekt er derfor at styrke anvendelsen af VetStat-data i databaseret sundhedsovervågning i griseproduktion ved at forbedre kendskabet til forholdet mellem antibiotikaforbrug, besætningskarakteristika og dyresundhed. Et yderligere formål med dette PhD-projekt er også at udvide brugen af VetStat-data på flere dyrearter ved at undersøge anvendeligheden af VetStat-data hos kæledyr.

Sammenhængen mellem besætningskarakteristika og antibiotikaforbruget i danske grisebesætninger med en produktion af fravænnede smågrise blev undersøgt i et case-kontrolstudie. Data blev indsamlet via telefoninterviews med 24 smågriseproducenter, der mellem 2016 og 2020 alle modtog et Gult Kort og med 28 smågriseproducenter der alle havde et antibiotikaforbrug der i 12 på hinanden følgende måneder lå under landsgennemsnittet på 10,7 - 8,8 ADD/100 dyr/dag.
Producenter med et Gult Kort i smågriseholdet var mere tilbøjelige til at behandle mavetarmlidelser (OR = 4,8) end producenter med et lavt antibiotikaforbrug. De var også mere tilbøjelige til at anvende flokmedicinering (perorale præparater) (OR = 10,5). Resultaterne af den multivariable regressionsmodel viser, at smågriseproducenter med et Gult Kort var mindre tilbøjelige (OR = 0,04) til at have faste rutiner der består i at arbejde fra de yngste til de ældre dyr og tilse syge grise til sidst. De var samtidig også mindre tilbøjelige til at have tilstrækkelig med ædeplads til, at alle nyligt fravænnede smågrise kan æde samtidig (OR = 0,08).

Betydningen af antibiotikaforbruget for dyresundhed blev undersøgt for 10,5 millioner slagtegrise leveret til 9 danske slagterier mellem 2016 og 2020. Studiet sammenligner

forekomsten af udvalgte slagtelæsioner og antibiotikaniveauet i slagtegrisebesætninger med et højt antibiotikaforbrug (et Gult Kort i slagtegriseholdet), i slagtegrisebesætninger med et middel antibiotikaforbrug (over 2,5 ADD/100 dyr/dag i mindst én måned ud af 9 på hinanden følgende måneder) og i besætninger med et lavt antibiotikaforbrug (lig med eller lavere end 2,5 ADD/100 dyr/dag i 9 på hinanden følgende måneder). På trods af markante forskelle i antibiotikaforbrug, var der kun marginale forskelle i forekomsten af kødkontrollæsioner. Prævalensen af læsionerne varierede med mindre en 0.5% mellem de tre grupper. Lungelæsioner var hyppigst forekommende (16-17 %) i alle tre grupper.
Sammenhængen mellem antibiotikaniveauet og forekomsten af kødkontrollæsioner blev undersøgt i logistiske regressionsmodeller.
Risikoen for fund af slagtelæsioner var lavest i besætninger med et middel antibiotikaforbrug. I syv ud af de otte slagtelæsioner, der indgik i studiet, var store besætninger forbundet med en lavere risiko for slagterifund sammenlignet med små besætninger. Risikoen for lunge- og halelæsioner var højere i besætninger i den vestlige del af Danmark sammenlignet med de østlige regioner, og risikoen for halelæsioner, led- og knoglemarvsbetændelse var højere i udendørs besætninger sammenlignet med indendørs besætninger

Anvendeligheden af VetStat-data til at estimere det samlede forbrug af antibiotika til danske katte og hunde blev undersøgt ved at beregne salg af antibiotika med forventet forbrug hos hund og kat ud fra rådata fra VetStat databasen. I 2018 udgjorde salget af antibiotika registreret på dyreartskode 90 samlet set 515 kg aktivt stof. Af denne mængde udgjorde produkter der udelukkende var godkendt til brug hos heste og produktionsdyr 53%. Derimod udgjorde antibiotikapræparater registreret på erstatningskoden 0 men godkendt til hund og kat (tabletter, salve, øre- og øjendråber) 706 kg aktivt stof. Denne skæve fordeling udgør en risiko for upræcise estimater af salg af antibiotika til hund og kat såfremt data trækkes fra VetStat databasen. På nuværende tidspunkt indeholder VetStat databasen heller ingen data om hvilke præparater der er anvendt til at behandle hunde og katte på danske dyreklinikker. Det betyder i sidste ende at det ikke er muligt at få et komplet overblik over antibiotikaforbruget til hund og kat.

Antallet af Gule Kort der er blevet givet til danske grisebesætninger mellem 2015 og 2020 udgør mindre end 4% af det samlede antal danske grisebesætninger. Overordnet fulgte de danske grisebesætninger der havde modtaget et Gult Kort de restriktioner de blev pålagt som følge af Gult Kort ordningen. Der var en betydelig stigning i antallet af tildelte Gule Kort i 2019 og 2020 sammenlignet med de foregående år. Det falder sammen med den reduktion i de tilladte grænseværdier for alle aldersgrupper der blev indført i 2019 hvor grænseværdien for alle tre aldersgrupper blev bragt betydeligt tættere på landsgennemsnittet. For alle tre aldersgrupper blev antibiotikaforbruget reduceret i perioden efter et Gult Kort.

Resultaterne af dette PhD-projekt giver input til fremtidige antibiotikareducerende tiltag i veterinærsektoren. Et Gult Kort hos fravænnede smågrise var kun forbundet med nogle få besætningskarakteristika sammenlignet med besætninger med lavt forbrug. Besætningerne adskilte sig dog med hensyn til de hyppigst behandlede besætningssygdomme og behandlingsmetoder. Hos slagtegrise kunne både et højt og et lavt antibiotikaforbrug kædes sammen med sundheds- og velfærdsproblemer. Disse resultater indikerer, at besætningsdyrlægens rådgivende rolle i fremtiden kan være afgørende for at forhindre negative virkninger af antibiotikareducerende tiltag på dyrenes sundhed og velfærd. For kæledyr var VetStat-data ikke anvendelige som en proxy for antibiotikaforbruget. Med den nuværende struktur i VetStat databasen vil et beregnet forbrug kun være en tilnærmelse. Estimering af nationalt antibiotikaforbrug hos danske hunde og katte er således ikke mulig før behandlingsdata fra dyrlæger i smådyrs eller blandet praksis overføres til VetStat på samme måde som det bliver gjort for stordyrspraktiserende dyrlæger.

Abbreviations

ADD	Animal daily doses
AGP	Antimicrobial growth promoter
AMR	Antimicrobial resistance
AMU	Antimicrobial use
ASU	Antimicrobial Sales and Use Platform
CHR	Central Husbandry Register
CNS	Central Nervous System
DANMAP	The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme
DVA	Danish Veterinary Association
DVFA	Danish Food and Veterinary Administration
EMA	European Medicines Agency
ESVAC	European Surveillance of Veterinary Antimicrobial Consumption
EU	European Union
MRSA	Methicillin resistant Staphylococcus aureus
MS	Member States
OR	Odds ratio
PWD	Post Weaning Diarrhea
SPF	Specific Pathogen Free System
VASC	Veterinary Advisory Service Contracts
VetStat	The Danish Veterinary Medicines Statistics Program

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Figure 16. The number of weeks it takes to produce a pig from 7 to 30 kg for 66 herds assigned a Yellow Card between 2015 and 2020 (cases) and 28 herds with a low AMU in weaners (controls). The dotted line indicates the average of 8 weeks to 30 kg in standard production.

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1. Introduction

The concept of "antimicrobial stewardship," introduced in 1996, highlights the importance of appropriate and prudent use of antimicrobials in human and veterinary medicine (Dyar et al. 2017). While antimicrobial resistance (AMR) can naturally emerge through errors in bacterial replication and the exchange of resistance genes among microorganisms (European Medicines Agency, 2024a), the misuse and overuse of antimicrobials have significantly accelerated its spread. As a result, antimicrobial stewardship has become crucial in addressing the growing threat of AMR in animals and humans (Velazquez-Meza et al., 2022).

In human healthcare, infections caused by antimicrobial-resistant pathogens are estimated to cause 35,000 deaths annually in Europe (European Medicines Agency, 2024a). AMR also presents significant risks in veterinary medicine, especially when it jeopardises animal welfare due to the limited availability of effective treatments or restrictions on veterinary antimicrobials prioritised for human use. The European Union, through the Animal Health Law (AHL), actively promotes the balance between safeguarding animal health and preventing the development of antimicrobial resistance (European Parliament and Council, 2016). Beyond the EU, global organisations such as the World Health Organization (WHO) and the World Organisation for Animal Health (OIE) have implemented policies, including the Global Action Plan on AMR, aimed at reducing antimicrobial use (AMU) and combating AMR across all sectors (World Health Organization, 2015; OIE, 2016).

The economic implications of AMR also add urgency to the issue. Treating antimicrobial-resistant infections increases healthcare and veterinary costs, and disease outbreaks can result in significant economic losses, especially in livestock production (O'Neill, 2016; World Bank, 2017).

One significant issue is the overlap between antimicrobial classes used in livestock production and human medicine, which increases selective pressure on infectious pathogens and reduces the efficacy of treatments (Palma et al. 2020). This overlap has transformed once treatable infections into serious public health challenges, threatening the progress of modern medicine (Velazquez-Meza et al. 2022; World Health Organization, 2020). Recognising this, the WHO declared AMR one of the most urgent global health challenges of the decade (World Health Organization, 2020). The WHO advocates for a One Health approach, promoting collaboration across the human, veterinary, and environmental sectors to mitigate the effects of AMR (Palma et al. 2020).

Since the 1990s, Danish official authorities and the pig industry have worked closely to promote a prudent AMU in Danish pig production. As a result, several measures have shaped Danish pig production over the last decades. In 1995, restrictions were placed upon the profit margin for veterinary medicinal products, limiting the revenue earned by the veterinary practitioner to a maximum of 5% above market value (DANMAP, 2023; Ministry of Food, 2023a). Instead, Veterinary Advisory Service Contracts (VASCs) between the pig producer and the veterinary practitioner were introduced (Ministry of Food, 2021). Here, the veterinary practitioner commits to regular herd visits as a prerequisite for the producer being allowed to initiate antimicrobial treatment independently. The veterinary practitioner must document recurring diseases and collect diagnostic samples before prescribing antimicrobials (Ministry of Food, 2021).

The use of avoparcin as an antimicrobial growth promoter (AGP) was banned in 1995, and the industry followed with a voluntary phase-out of all AGPs in finishers and weaners in 1998 and 1999, respectively (DANMAP, 2023; Ministry of Food, 2023a).

In 1998, the need for systematic monitoring of AMU in livestock production was laid down in "*The Copenhagen Recommendations",* published following the conference on "*The Microbial Threat*" held in Copenhagen (Frimodt-Møller, 2004). The main message urged the European Union (EU) Member States (MS) to monitor national AMU as a measure to promote prudent AMU and mitigate AMR (Frimodt-Møller, 2004). This was immediately recognised in Denmark when the Veterinary Statistics (VetStat) database was launched in 2000 following a collaborative effort between the Danish Veterinary Institute, the Danish Medicines Agency and the Danish Veterinary and Food Administration (DVFA), a government administration under the Ministry of Food, Agriculture and Fisheries (Stege et al., 2003).

The VetStat database holds detailed information on all sales of veterinary medicinal products and brings transparency to national AMU in the veterinary sector (Ministry of Food, 2023a; Stege et al., 2003). VetStat data also enables national reports on trends in veterinary AMU and AMR. The reports are drafted and published annually by the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) (DANMAP, 1997). VetStat data are also submitted to the European Medicines Agency (EMA) and published in European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) reports (European Medicines Agency, 2022a).

The continuous monitoring of veterinary AMU facilitated by the VetStat database spurred changes in the use of several antimicrobial classes in Danish pig production. These changes included restrictions on the use of fluoroquinolones in 2002, a voluntary ban on 3rd and 4th generation cephalosporins in 2010 and restrictions on polymyxins in 2016 (DANMAP, 2023; Moura et al., 2023). However, the most prominent initiative derived from the VetStat database is undoubtedly the Yellow Card scheme. The Yellow Card scheme was launched by the DVFA in 2010 to fulfil a national action plan to reduce AMU in pig production by 10% by 2014 (compared to the level in 2009) (DANMAP, 2023). It serves as a legal intervention, setting limitations on the maximum permitted limit values for AMU in Danish pig herds (Ministry of Food, 2018a). The initiative was supported by the Danish pig industry, which developed a guideline aimed at Danish pig producers, intending to promote a prudent AMU by encouraging "*as little as possible, but as much as necessary*" when using antimicrobials (Danish Agriculture & Food Council, 2023a). The Yellow Card scheme has also been instrumental in promoting responsible behaviour among Danish veterinary practitioners and pig producers. The veterinary practitioner is obligated to prescribe antimicrobials for pigs with infectious diseases while also ensuring that the AMU is below the permitted limit values. Producers must treat the affected pigs and consider the overall AMU for the age group.

Globally, AMU in veterinary medicine exceeds that in human healthcare, a trend seen in Denmark as well (DANMAP 2023; Velazquez-Meza et al., 2022). In 2022, 93.2 tonnes of active compounds were sold in Denmark for use in veterinary medicine. Of these, 75.8 tonnes were sold for use exclusively in Danish pig production. By comparison, the human healthcare sector used 15.7 tonnes of active compounds during the same period (DANMAP, 2023).

The high AMU in the Danish pig sector is attributable to the extensive pig production. Denmark is one of the top European pig-producing countries, second only to Germany, Spain, and France. Despite having a human population of 5.8 million (DANMAP, 2023), approximately 33 million pigs are produced annually in Denmark (**Fig. 1**). This includes 18.5 million finisher pigs slaughtered in Danish abattoirs every year, export of 0.3 million finisher pigs and sows, and 14.2 million weaners exported for rearing or breeding purposes (Danish Agriculture & Food Council, 2022). The high AMU in Danish pig production is largely driven by the extensive use in weaners (DANMAP, 2023). This age group is mostly treated with peroral antimicrobials for gastrointestinal disorders (DANMAP, 2020; Moura et al., 2023). The second highest AMU is for sows and piglets. They are primarily treated with parenteral preparations prescribed for disorders in limbs, skin, and central nervous system (CNS). However, the AMU in finishers is nearly as high. Here, peroral preparations are mostly prescribed for gastrointestinal disorders (DANMAP, 2020; Moura et al., 2023).

Despite the high AMU in the pig sector, Denmark has successfully reduced AMU over the past decade. Since introducing the Yellow Card scheme, sales of antimicrobials for use in Danish pig production decreased from 100.5 tonnes of active compound in 2010 to 71.4 tonnes in 2022 (DANMAP, 2023), placing the Danish livestock sector in the lowest third compared to the 31 EU MS who report national antimicrobial sales or use data to the EMA (European Medicines Agency, 2022a).

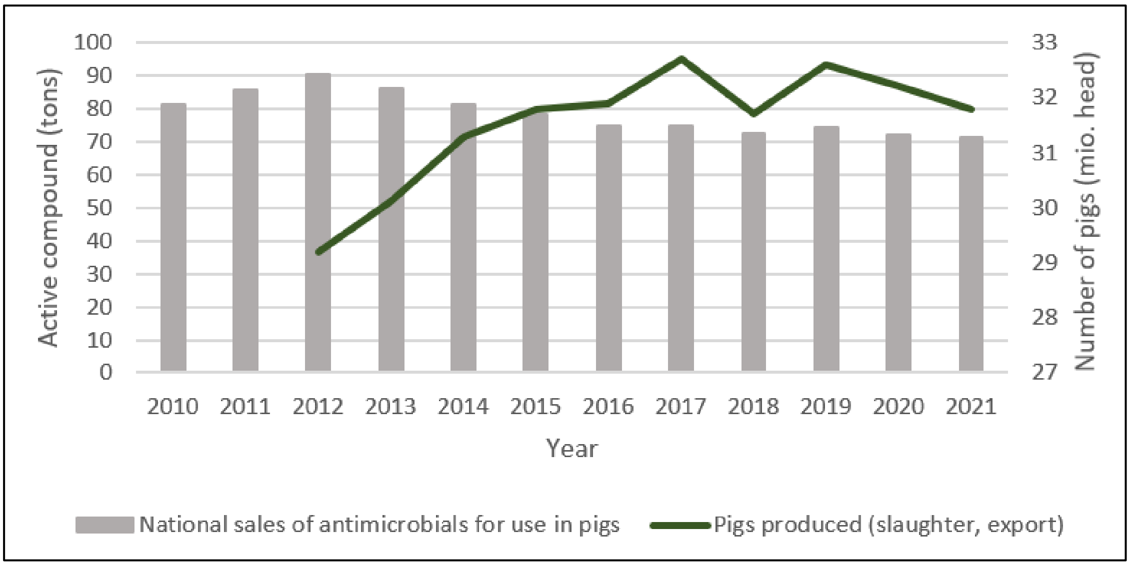


Figure 1. *Timeline of the changes in sales of antimicrobials measured in tons of active compound for use in Danish pigs between 2010 and 2021, including the changes in the number of Danish pigs produced (for slaughter and export) between 2012 and 2021. (Source: DANMAP and Statistics Denmark)*

In addition to sales of veterinary antimicrobials, meat inspection findings are monitored for all finisher pigs delivered to Danish abattoirs. Following the introduction of the Yellow Card scheme, the prevalence of chronic peritonitis, chronic enteritis and umbilical hernia increased between 2010 and 2011 (Alban et al., 2013). During the same period, the prevalence of tail bites, chronic pericarditis and pneumonia decreased. Pigs delivered to Danish abattoirs are subject to thorough ante- and postmortem inspections carried out by veterinarians and trained inspectors using a comprehensive coding system to record lesions found during meat inspection (Ministry of Food, 2022a). Meat inspection is vital for food safety and ensures that meat unfit for human consumption does not enter the food chain (Ministry of Food, 2022a).

Contrary to Danish pigs, companion animals in Denmark account for only 1-2% of total national sales of antimicrobials (DANMAP, 2023). However, the level of detail at which antimicrobial sales data for companion animals are registered in VetStat is considerably lower than that for pigs, and so are the restrictions on AMU. Companion animals, namely dogs and cats, are often integral to households, leading to close physical contact between owners and their pets (Dickson et al., 2019). This behaviour is considered valuable to the owner but poses a risk of direct transmission of zoonotic AMR pathogens (Dickson et al., 2019; Loeffler et al., 2005).

With the Veterinary Medicinal Products Regulation put in force in January 2022 (European Medicines Agency, 2018), all EU MS are now requested to report antimicrobial sale or use data to the Union Product Database, replacing the voluntary data reporting to the ESVAC reports (European Medicines Agency, 2024b). Now, data on antimicrobial sales or use must be uploaded to the Antimicrobial Sales and Use (ASU) Platform (European Medicines Agency, 2024b). This approach entails specific data collection and formatting criteria. Part of the initiative requires data to be compiled for companion animals (dogs and cats) starting in 2030 (European Medicines Agency 2021; European Medicines Agency 2022b). Additionally, the data must be reported separately for each animal species, accompanied by a biomass measurement (European Medicines Agency, 2023a). Therefore, the existing national systems must be validated to adhere to the EU requirements.

Although VetStat data holds a central position in national antimicrobial monitoring systems in Denmark, it is still important to improve data reliability and usability and to extend the use of data to more animal species. Since VetStat data is also used as a basis for regulatory penalties in Danish pig production, it is important to continue exploring the effects of different data aggregation methods and identifying inaccuracies in estimating AMU. By strengthening the use of VetStat data in research, we can improve its practical use as an important data resource in data-based health surveillance.

1.1. Aim of this thesis

The overall aim of this PhD study was to strengthen the evidence-based use of VetStat data in research by improving the knowledge of the relationship between antimicrobial use, herd characteristics and animal health in Danish pig production and to extend its application across different animal species by improving the usability of VetStat data in companion animals.

The studies in this PhD thesis are structured according to the following hypotheses:

1) Assignment of a Yellow Card is associated with specific herd characteristics that differ from herds with a lower AMU.
2) Assignment of a Yellow Card is associated with poor animal health in Danish pig herds.
3) VetStat data is not a suitable proxy for estimating AMU in companion animals.

The specific objectives were:

1) To examine the association between AMU levels and herd characteristics such as biosecurity, hygiene, feed regimens and antimicrobial treatment routines in Danish pig herds with weaners
2) To examine the association between meat inspection findings and AMU levels in Danish finisher pigs
3) To assess the usability of the VetStat database in estimating total national sales of antimicrobials in Danish dogs and cats

1.2. Outline of the thesis

This PhD thesis consists of 9 chapters and an appendix section (Appendix A, Appendix B and Appendix C). The first chapter includes the introduction. Chapter 2 presents the background, general aim, hypotheses, and objectives for the work included in this thesis, and Chapter 3 is an overview of methods and data collection. Chapter 4 summarises the main findings of this thesis. The results include relevant results from Manuscripts I, II and III and additional results. Chapters 5, 6 and 7 include a general discussion, overall conclusions, and perspectives. All references are presented in Chapter 8. The three manuscripts are presented in Chapter 9. An overview of the calculations of AMU in Danish pig production is presented in Appendix A. An English version of the questionnaire used for collecting data on herd characteristics in Manuscript I are included in Appendix B. The biological associations between meat inspection lesions for each antimicrobial use level in Manuscript II are included in Appendix C.

2. Background

2.1. Danish pig production

2.1.1. Herd structure and productivity

Pig production represents one of the primary industries within the Danish food cluster (Danish Agriculture and Food Council, 2023b). Herds with pigs are mainly located in the Western parts of Denmark, where pig production accounts for over 25% of private employment in many parishes (Danish Agriculture and Food Council, 2023b). Intensive production systems characterise Danish pig production. In 2021, the total pig population reached 33 million pigs, including 18.5 million finishers and sows for slaughter and 14.2 million weaners for export (Danish Agriculture & Food Council, 2022). While the number of pigs produced in Denmark increased steadily each year, the structure changed significantly, trending toward fewer but larger production systems. In 1990, the number of holdings with pigs reached 29,903. This number was reduced to 2,576 in 2021 (Danish Agriculture & Food Council, 2022; Danish Agriculture & Food Council, 2011).

In Denmark, holdings with pigs can comprise multiple buildings or units housing one or more age groups. In this thesis, a "herd" refers to a holding for which pigs are registered. The term covers a fixed geographical location but can refer to more than one age group.

A Danish pig herd can house one or more of the following age groups: sows with piglets, weaners and fatteners. Each age group is housed in separate sections, which are often divided into several pens. Piglets are traditionally weaned at 4 weeks old, weighing around 7 kg. After weaning, the piglets are moved to a separate section. In this section, they are housed for about 8 weeks on average until they weigh 25 to 30 kg. During this period, they are referred to as weaners. The weaners are then moved to the finisher unit, where they stay until they reach the final slaughter weight.

Herd types relevant to this thesis are described in **Table 1**. In 2021, the number of herds with sows or farrow-to-finisher production represented 54% of the total number of Danish pig herds. Herds exclusively rearing weaners or finishers represented 46% (Danish Agriculture & Food Council, 2022).

Table 1*. Overview of common herd structures in Danish pig production relevant to this thesis.*

Herd type	Description
Farrow-to-finisher herds	Pigs from farrowing until slaughter. All age groups are kept in one geographical location (CHR number). Some weaners can be sold to finisher herds in Denmark or exported at approx. 30 kilos.
Farrow-to-weaner herds	Pigs from farrowing until the pigs reach approx. 30 kilos. Sows and weaners are kept in one geographical location. The weaners are either sold to finisher herds in Denmark or exported.
Weaner-to-finisher herds	Weaners and finishers are kept at the same geographical location. Newly weaned pigs are moved to this location and are kept until slaughter.
Weaner-only herds	After weaning, the pigs are moved to this location until they reach 25-30 kg. The weaners are either sold to finisher herds in Denmark or exported.
Finisher-only herds	When pigs reach 25-30 kg, they are moved to a separate location and reared until they reach slaughter weight.

2.1.2. Monitoring of pen places and movements of pigs

The Central Husbandry Register (CHR) holds detailed information on all holdings with livestock, fish, fur animals and horses (Ministry of Food, 2022b). Each herd in Denmark is assigned a CHR number that serves as a unique herd identification code. In the CHR, information on all Danish herds can be obtained. This information includes the specific herd identification code (CHR number), name and address of the owner and user, address and geographical coordinates of the holding, production type and animal species, animal movement agreements and supply partnerships and whether the herd is under veterinary official supervision (Ministry of Food, 2022b)

The register also includes information on age groups within all Danish pig herds and the number of pen places. The number of pen places specifies the capacity of the farm buildings but often reflects the number of pigs housed on the premises at any given time (Ministry of Food, 2022b).

The CHR database is owned and managed by the DVFA and was established for effective national disease detection and surveillance (Danish Food and Veterinary Administration, 2024a). Pig producers are required by law to ensure that the number of pen places is accurate and must conduct twice-a-year updates if the number of pen places is more than 300 sows, 3000 finishers, or 6000 weaners (Ministry of Food, 2022b).

All information from the CHR database is publicly available on the internet (Danish Food and Veterinary Administration, 2021).

The Danish Pig Movement Database holds information on all movements of pigs between premises. This includes movements of pigs to properties with different CHR numbers, abattoirs, and exports. Dead pigs rendered for carcass disposal are also recorded in the Pig Movement Database. Each movement is registered with information on the CHR number on the holding where the pigs originate, the CHR number of the receiving herd, the number of pigs moved, the age groups, the license plate or identification number of the vehicle, and if pigs are exported, an official trading identification code (TRACES number) (Ministry of Food, 2022b). The database is publicly accessible, and data extractions are available at the herd level. The database is owned and managed by the DVFA, and the information is publicly available on the internet (The Danish Food and Veterinary Administration, 2024b).

2.1.3. The Specific Pathogen-Free System

The Danish Specific Pathogen Free (SPF) system is a health declaration system in Danish pig production offering stringent rules on biosecurity, health and transportation. SPF Health manages the SPF system and is part of the Danish Agriculture and Food Council (SPF Health, 2021).

In the SPF system, Danish pig herds are categorised into three safety levels: Red, blue and green. Each safety level specifies mandatory biosecurity measures. A red safety level denotes the highest biosecurity level applicable to breeding herds. A blue safety level applies to production herds raising weaners and finishers for sale, export and slaughter. The green safety level applies to herds aspiring to achieve the blue safety level. Herds not enrolled in the SPF system are classified as “conventional” (SPF Health, 2021).

In addition to the safety levels, SPF health monitors specified SPF diseases, indicated by abbreviations (denoted Appendix in the SPF system). The SPF diseases cover enzootic pneumonia, porcine pleuropneumonia, porcine reproductive and respiratory syndrome, swine dysentery, atrophic rhinitis, mange and lice (Ministry of Food, 2023b; SPF Health, 2021). SPF Health monitors health status by regular blood sampling. Knowing the health status of a herd enables veterinary practitioners and other professionals who visit multiple herds in a day to plan their itinerary based on herd-level health status (SPF Health, 2021).

In Denmark, there is a high level of transparency in livestock production, and the SPF system operates as a publicly accessible registry where information on health declaration and safety levels are available on the internet (SPF Health, 2021).

2.1.4. Monitoring of Porcine Reproductive and Respiratory Syndrome

Starting October 2023, Danish pig producers must obtain an official Porcine Reproductive and Respiratory Syndrome (PRRS) health status for each CHR number and update this status annually (Ministry of Food, 2023b). Veterinary practitioners, SPF, and the DVFA collaborate to determine and maintain this status, following the SPF health declaration system. Herds are classified as PRRS-negative, PRRS-positive, pending, or under partial eradication. To attain a PRRS-negative status, producers and veterinary practitioners must carry out eradication measures, either partial or complete, and have them endorsed by the SPF and the DVFA (Ministry of Food, 2023b).

Total eradication involves removing all pigs, keeping stables empty for at least 7 days, cleaning and disinfecting, and reintroducing PRRS-negative pigs (Ministry of Food, 2023b). Partial eradication entails a selective removal of PRRS-positive pigs, often in conjunction with blood testing, improved biosecurity measures, strictly sectioned housing, age segregation, and overall management (Ministry of Food, 2023b). The strategy allows producers to gradually reduce the prevalence of PRRS within their herds without fully emptying the herd. The health status of each herd is publicly available through SPF-Sund (SPF Health, 2021), and the movement of pigs is monitored through the Pig Movement database. This allows veterinary practitioners, producers, and authorities to ensure that pigs are only moved based on their health status and thus prevents any compromise to the health status of the receiving herd. Additionally, veterinary practitioners and regulatory authorities must now consider the PRRS status when planning herd visits.

2.1.5. Productivity control systems

In Danish pig production, data-driven productivity control systems monitor and analyse herd-level productivity. Producers can collect productivity data daily and input the data into software systems like AgroVision and CloudFarms (SEGES, 2020). This allows producers to track detailed metrics, including piglet weight at birth, litter size, number of live and stillbirths per litter, weaned per litter and lactation days, weight at weaning or weight at entry, daily weight gain, feed days and waste feed days, feed consumption and feed loss days, weight at departure, mortality rates, and average meat percentage (SEGES, 2020; SEGES, 2023). The software systems generate performance indicators, compiled into productivity reports, allowing the producers to monitor detailed herd performance characteristics and understand the factors contributing to overall productivity. The reports can be customised for specific periods, such as monthly, quarterly, or annually, enabling producers to conduct detailed analyses of productivity over time and insight into areas where improvements can be made, such as feed conversion rates, mortality reduction, or enhancing growth performance in specific age groups (SEGES, 2020; SEGES, 2023). Producers can submit productivity data to the Danish Agriculture & Food Council, which publishes an annual report detailing the national average productivity in Danish pig production. This allows producers to benchmark their results against industry-wide standards (SEGES, 2023). Furthermore, the national average is used in industry-wide assessments to calculate the environmental impact of pig production and economic trends and productivity changes across the sector (SEGES, 2020).

2.1.6. Antimicrobial use

Since introducing the Yellow Card scheme in 2010, AMU in Danish pig production has decreased significantly from 100.5 tonnes of active compound in 2010 to 71.3 tonnes in 2022 (DANMAP, 2011; DANMAP, 2023). However, this reduction varies across age groups. In 2022, AMU for sows and finishers amounted to 17,083 kg active compound and 15,249.5 kg active compound, respectively. For sows, this marks a 43% reduction from the 30,150 kg active compound sold in 2010, while sales of antimicrobials for use in finishers were reduced by 52% from the 31,552 kg active compound in 2010. In contrast, sales of antimicrobials for use in weaners increased from 38,597 kg of active compound in 2010 to 39,023.4 kg in 2022 (DANMAP, 2011; DANMAP, 2023).

In 2017, colistin, as part of the Yellow Card scheme, was given a multiplication factor of 10. This means that fluoroquinolones, 3rd and 4th generation cephalosporins, and colistin are weighted highest overall, while tetracycline has a multiplication factor of 1.5 (DANMAP, 2022). In addition to the phase-out of critically important antimicrobials, this weighting has led to an overall reduction in broad-spectrum antimicrobials in Danish pig production (DANMAP, 2023).

Between 2010 and 2022, the use of tetracyclines decreased from 32,319 kg of active compound in 2010 to 10,807.7 kg in 2022. In contrast, the sale of macrolides and aminoglycosides increased from 12,978 kg and 4,950 kg in 2010 to 11,596.1 kg and 15,159 kg in 2022, respectively (DANMAP, 2011; DANMAP, 2023). This shift reflects changes in treatment procedures, particularly in the peroral treatment of gastrointestinal disorders in pig production (DANMAP, 2023).

When antimicrobial sales data are linked to production outcomes, which in Denmark include both meat production and a substantial export of weaners for rearing and breeding purposes (Danish Agriculture & Food Council, 2023c), then the sales of antimicrobials for use in pig production amounted to 64.46 mg per kg of pig produced in 2022 (**Figure A2** in Appendix A). This estimate represents the total amount of antimicrobials sold for use in pig production, covering an annual production of 18 million pigs for slaughter and 13.8 million weaners exported for rearing and breeding purposes. The estimate covers antimicrobials sold for use in sows, weaners and finishers, live pigs still in production, and those that have died or been culled during the production stages. In 2014, the estimate was 76.56 mg per kg of pig produced (**Figure A1** in Appendix A).

2.1.7. Antimicrobial resistance

Between 2010 and 2022, the resistance level of several zoonotic bacteria changed markedly. For *Salmonella typhimurium* (*S. typhimurium*) from Danish pigs, 53 % of the isolates tested in 2010 were resistant to one or more of the antimicrobial types tested (DANMAP, 2011). Of these, 47% were resistant to tetracyclines and 49% to ampicillin, while resistance to neomycin and colistin was 3% and 0%, respectively (DANMAP, 2011). In the following 10 years, only 3 - 15 % of the tested isolates were susceptible in the routine pigs and fresh pork samples from Danish abattoirs (DANMAP, 2022; DANMAP, 2023). In Danish pork, resistance was 67% and 79% for tetracycline and ampicillin, respectively. Resistance to colistin was unchanged at 0% in the tested isolates for both live pigs and pork (DANMAP, 2022; DANMAP, 2023). In 2010, AMR was identified for *Escherichia coli* (*E. coli*) in 33% of the tested isolates from pigs (DANMAP, 2011). Of these, resistance to tetracycline and ampicillin was found in 37% and 23% of the isolates, respectively. In 2022, resistance to tetracycline was reduced to 28%, but resistance to ampicillin increased to 35% (DANMAP, 2023).

In 2010, 1% of the tested isolates showed resistance to colistin. In 2022, no resistance to colistin and ciprofloxacin was found in the tested isolates (DANMAP, 2011; DANMAP, 2023). However, a significant increase in resistance to neomycin, one of the first-choice antimicrobials for post-weaning diarrhoea (PWD) in piglets, was observed in E. coli isolates, increasing from 6.9% in 2016 to 43.2% in 2022 (DANMAP, 2023).

In 2022, resistance to antimicrobials critical for human medicine remained low among the most frequently occurring swine-specific pathogens, including *Actinobacillus pleuropneumonia*, *Bordetella bronchiseptica*, *Clostridium perfringens*, *Erysipelotrix rhusiopathiae*, *Klebsiella pneumoniae*, *Staphylococcus hyicus*, and *Streptococcus suis* (DANMAP, 2023). Methicillin-resistant Staphylococcus Aureus (MRSA), especially the livestock-associated serotype CC398, has become a significant concern over the past decade. In 2009, MRSA CC398 was detected in 16% of Danish pig herds (DANMAP, 2011). By 2018, the prevalence had increased to 89% (DANMAP, 2019).

2.1.8. Biosecurity

Biosecurity plays an important role in preventing diseases in pig production by limiting the introduction and spread of infectious agents within and between farms. It involves external and internal measures to control the spread of pathogens (Dhaka et al., 2023). Biosecurity practices are typically divided into external and internal biosecurity. External biosecurity involves measures aimed at preventing the introduction of pathogens onto the farm buildings. These measures include restricted movement of personnel, animals, and vehicles, disinfection protocols for equipment, and quarantine procedures for the introduction of live animals (Dhaka et al., 2023; Laanen et al., 2013). Internal biosecurity involves limiting the spread of pathogens within a farm, especially between different age groups. These measures include the separation of pigs according to health and age group, cleaning and disinfection routines, handling of manure, and ventilation and temperature control (Dhaka et al., 2023).

2.1.9. Meat inspection

All pigs delivered to Danish abattoirs are required to undergo thorough ante- and post-mortem inspections to ensure high standards of food safety, animal welfare, and animal health. These inspections are conducted by official veterinarians employed by the DVFA or trained technicians, who apply a standardised coding system across all Danish abattoirs (Ministry of Food, 2022a). This system, which comprises over 70 individual codes, is designed to capture a wide range of abnormalities, including organ lesions, signs of respiratory or gastrointestinal disease, and other health indicators that may affect the safety and quality of the meat (Alban et al., 2022; Ministry of Food, 2022a).

The results from these inspections are recorded in a national database managed by the Danish slaughterhouses, providing a comprehensive and centralised repository of health data at the herd level (Alban et al., 2022). This database allows pig producers and veterinary practitioners to monitor disease trends and identify health issues. The main purpose of these inspections is to guarantee that only meat deemed safe for human consumption reaches the food chain, and any abnormalities detected can lead to the condemnation of parts of, or the entire, carcass based on strict criteria established by food safety standards (Vieira-Pinto et al., 2022).

Beyond ensuring food safety, meat inspection data is a valuable surveillance tool that can provide early warnings of disease outbreaks or other health concerns. Additionally, there is growing recognition of its potential to contribute to animal welfare monitoring. Studies suggest that integrating meat inspection data with other animal welfare indicators could offer a broader assessment of welfare conditions, helping to identify systemic issues in production practices (Stärk et al., 2014).

2.2. Danish companion animals

2.2.1. Population size

In 2021, approximately 33% of Danish households owned one or more companion animals. Most had either a dog (20%) or a cat (14%). Other forms of companion animals (horses, exotics, reptiles and amphibians) were kept less frequently (< 3% of households in Denmark) (Lund & Sandøe, 2021). In Denmark, no official statistics are available on the number of companion animals. However, a 2021 survey estimated that Danish families keep 808,519 dogs and 730,432 cats as companion animals (Lund & Sandøe, 2021).

2.2.2. Antimicrobial use

AMU in Danish companion animals decreased between 2010 and 2022. The total amount of antimicrobials sold for use in companion animals was reduced from 3084 kg of active compound in 2010 to 2180.4 kg in 2022 (DANMAP 2011; DANMAP, 2023). While there is considerable inaccuracy in AMU data for companion animals, the overall use of fluoroquinolones is attributed to this group (DANMAP 2023). In 2010, the sales of fluoroquinolones amounted to 14 kg of active compound, compared to 12.5 kg in 2022 (DANMAP, 2011; DANMAP, 2023).

2.2.3. Antimicrobial resistance

The most commonly reported pathogens among Danish dogs and cats are *Staphylococcus pseudintermedius* (*S. pseudintermedius*) and *E. coli*. *S. pseudintermedius* is mainly isolated from skin, wounds, and ears, while *E. coli* is typically found in the urinary tract (DANMAP 2023). Since 2016, DANMAP has monitored AMR in isolates from these pathogens. In dogs and cats, resistance to ampicillin in *E. coli* and *S. pseudintermedius* isolates increased from 14% and 59% in 2016 to 22% and 65% in 2022, respectively. For other antimicrobials included in the surveillance, resistance levels remained stable from 2016 to 2022, with AMR of 0-8% observed for amoxicillin/clavulanic acid, enrofloxacin, gentamicin, and marbofloxacin. Resistance to chloramphenicol, clindamycin, doxycycline, and erythromycin ranged from 16-33% in *S. pseudintermedius* isolates, complicating the treatment of skin infections in clinical practice (DANMAP 2023).

2.3. Quantification of antimicrobial use

2.3.1. Monitoring systems

Many EU MS have set up systems to monitor veterinary antimicrobial sales and usage data. These systems vary in data types, organisational structures, coverage, and measurement units. Generally, the systems can be categorised into three main types: sample survey systems, partial sector coverage systems, and full sector coverage systems (AACTING, 2021; Sanders et al., 2020).

Sample survey systems collect antimicrobial sales or usage data from a representative subset of animal populations, aiming to estimate trends across different species or production systems (AACTING, 2021; Sanders et al., 2020). Examples include the French INAPORC and GVET systems covering parts of the French pig sector and the Italian Classyfarm system covering a convenience sample of Italian pig farms (AACTING, 2021).

Partial sector coverage systems monitor AMU within specific animal populations or production systems, providing data within targeted areas but not encompassing the entire animal population (AACTING, 2021). Examples include the Belgian Sanitel-Med system, managed by the Belgian Federal Agency for Medicines and Health Products (FAMHPS), which requires veterinary practitioners to register antimicrobial prescription, use, and sales data. The system applies EMA-recommended standard weights and data on farm capacity from official databases to calculate AMU as the Belgian "defined daily dose for animals" (DDDA-bel) and "treatment days per 100 days" (BD100). The system benchmarks herds and veterinary practitioners (AACTING, 2021). In Germany, the HIT system calculates AMU as “treatment frequency” (TF). Pig producers are benchmarked against national standards twice a year. However, herds with fewer than 250 weaners or finishers are excluded (AACTING, 2021). The eMB-pigs system in the United Kingdom covers 94% of pig production and measures AMU as mg/kg based on EMA-proposed standard weights (AACTING, 2021; Sanders et al., 2020). In Ireland, the National AMU database for pigs requires producers who slaughter more than 200 finishers per year to register AMU quarterly. The AMU is calculated as mg/kg using the EMA-proposed standard weights (AACTING, 2021).

Full-sector systems monitor all animals or farms within selected species or cover the full veterinary sector. (AACTING, 2021; Sanders et al., 2020). In the Netherlands, the Veterinary Medicines Institute (SDa) monitors antimicrobial sales and usage data, calculating AMU as "defined daily doses" (DDDA) and daily doses per year, with benchmarking of farms and veterinary practitioners (AACTING, 2021). In Sweden, the 'Djursjukdata DAWA' system, managed by the Swedish Board of Agriculture, collects AMU data from veterinary practitioners and pharmacies, using kg active compound and mg/PCU for livestock (AACTING, 2021). In Norway, the VetReg system, managed by the Norwegian Food Safety Authority, calculates AMU as kg active compound (AACTING, 2021). In Finland, the SIKAVA system, covering 97% of pig production, collects AMU data from producers and veterinary practitioners (AACTING, 2021). In Denmark, the VetStat system, managed by the DVFA, collects AMU data from veterinary practitioners and pharmacies, calculating AMU as "Animal Daily Doses (ADD) per 100 animals per day" (ADD/100 animals/day). In Spain, the National Database of Veterinary Antibiotic Prescriptions (PRESVET) collects prescription data for livestock, calculating AMU as mg/PCU (AACTING, 2021).

Despite efforts across the EU MS to establish and improve AMU data collection systems, no official monitoring systems are in place in Poland, a country with a pig population of 10.2 million (Danish Agriculture & Food Council, 2023; European medicines Agency, 2023b).

2.3.2. The Danish Veterinary Statistics Database

All sales of veterinary medicinal products are recorded in the VetStat database (Stege et al., 2003). VetStat is owned and managed by the DVFA. The database is a relational database, and the DVFA regularly updates product-specific information in the database environment (Dupont & Stege, 2014). The information includes product trade name, active ingredient, product strength, package amount, preparation, Anatomical Therapeutic Classification (ATC-vet) code and ATCvet codes (Stege et al., 2003).

Information on sales of antimicrobials for veterinary use is entered into the VetStat database from three primary sources: pharmacies, veterinary practitioners, and feed mills (Stege et al., 2003). Antimicrobials are mainly dispensed through pharmacies. At the pharmacy, antimicrobials are purchased by veterinary practitioners, veterinary clinics, livestock producers and pet owners. Here, each purchase is transferred to VetStat with information on the prescribing veterinarian, the reporting pharmacy, date of purchase and product information (identification code and amount), and animal species codes (Stege et al., 2003). Antimicrobials sold for use in pig production also contain a herd identification code (CHR number), the animal age group and disease code indicating the target organ system (Stege et al., 2003). A replacement code is available if no animal species is specified on the veterinary prescription or if sales are made directly to veterinary clinics (Dupont and Stege, 2014). For specific animal species codes, age groups and target organ systems used in VetStat for pigs, dogs and cats, see **Table 2**.

Veterinary practitioners treating livestock are legally obliged to report AMU data to VetStat (Ministry of Food, 2023a). Data is often transferred to VetStat from the electronic billing systems within the medical journal software. The usage data is linked to information on the herd identification code (CHR number), animal species code, age group and target organ system (Ministry of Food, 2023a). Veterinary practitioners treating companion animals are not subject to the same legal requirements (Ministry of Food, 2023a). As a result, VetStat does not provide antimicrobial use data from small animal veterinary clinics.

Table 2*. Animal species codes, age groups, target organ systems and replacement codes used in the VetStat database for pigs, dogs, and cats. Standard weights in kilograms are shown (in parenthesis)*

Species	Age group (standard weight)	Prescription group and target organ system
15 - Pigs	55 – Sows[a] (200) 56 – Weaners (15) 57 – Finishers[b] (50)	10 – Reproduction, urogenital system 11 – Udder 12 – Gastrointestinal disorders 13 – Respiratory tract disorders 14 – Joints, limbs, hooved, CNS, skin 15 – Metabolism, digestion, circulation
90 – Companion animals		
0 – Replacement code		0 Replacement code

[a]Including piglets, gilts and boars, [b]Including non-gestating gilts. (Source: VetStat)

2.3.3. Estimating antimicrobial use in Danish pig production

AMU in Danish pig production is calculated and presented separately for each age group using standardised measurement units from the DVFA and population data from the CHR database (Dupont et al., 2016). In VetStat, sales data is used as a proxy for AMU and calculated as the dose-based measurement of Animal Daily Doses (ADD), indicating the average maintenance dose in a defined animal species (Jensen et al., 2004). For calculation of Animal Daily Doses, see **Eq. 1**; for standard weights, see **Table 2**.

$$ADD\ sold = \frac{active\ compound\ sold\ for\ a\ given\ age\ group}{standard\ dosage\ per\ kilo * standard\ weight} \quad (1)$$

In Denmark, AMU in pig production is often related to the population at risk and calculated as Animal Daily Doses per 100 animals per day (ADD/100 animals/day, see **Eq. 2**), indicating the percentage of animals treated per day in each age group (Dupont et al., 2016). The number of pen places is transferred from the CHR database and included in the denominator to indicate the number of animals in the herd "at risk" of antimicrobial treatment.

$$ADD\ per\ 100\ animals\ per\ day\ = \frac{ADD\ sold}{Number\ of\ pen\ places * days} * 100 \quad (2)$$

2.3.4. The Yellow Card scheme

In the Yellow Card scheme, ADD/100 animals/day is specified as monthly calculations using a rolling 9-month average and allows the DVFA to monitor and identify herds that exceed the permitted limit values (Ministry of Food, 2018a). Initially, the permitted limit values were set to twice the mean for each age group (Alban et al., 2013). The limit values have been lowered over time, with the latest reduction in 2019 (Ministry of Food, 2018b), for changes in permitted limit values, see **Figures 2**, **3** and **4**. The Yellow Card scheme targets Danish pig herds with an AMU above the permitted limit value for at least one month, indicating an AMU higher than 75% of the average AMU per age group (**Fig. 2**, **3** and **4**). Each month, the DVFA identifies herds with an AMU above the permitted limit values in one of the three age groups. The producer is heard, and if a Yellow Card is upheld, he or she must reduce the AMU level to below the permitted limit values in the specific age group within 9 months (Ministry of Food, 2018a). During this period, the DVFA may carry out unannounced herd inspections at the cost of the producer. In addition, producers are no longer allowed to keep and refill previously prescribed antimicrobials for peroral administration (Ministry of Food, 2018a). In 2015, a second action plan was introduced to reduce MRSA CC398, aiming for a 15% reduction in AMU in pig production between 2015 and 2018 (DANMAP, 2019). This led to the differentiated Yellow Card introduced in 2016 with a dynamic weighing of critically important antimicrobials. Currently, colistin, 3rd and 4th generation cephalosporins and fluoroquinolones have an additive factor of 10, while tetracyclines have a factor of 1.5 (Ministry of Food, 2018b). Since introducing the Yellow Card scheme, national sales of antimicrobials for use in pig production have been reduced (**Figure 1**).

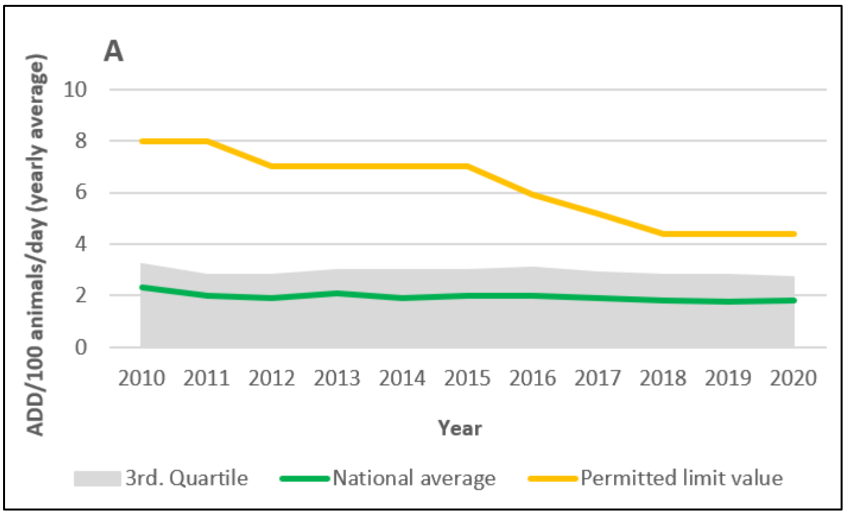


***Figure 2**. Timeline of the changes in permitted limit values for AMU in the Yellow Card scheme for Danish finishers (A), the national average for AMU in all Danish finishers and the third quartile indicating 75% of AMU in finishers. (Source: DVFA and VetStat)*

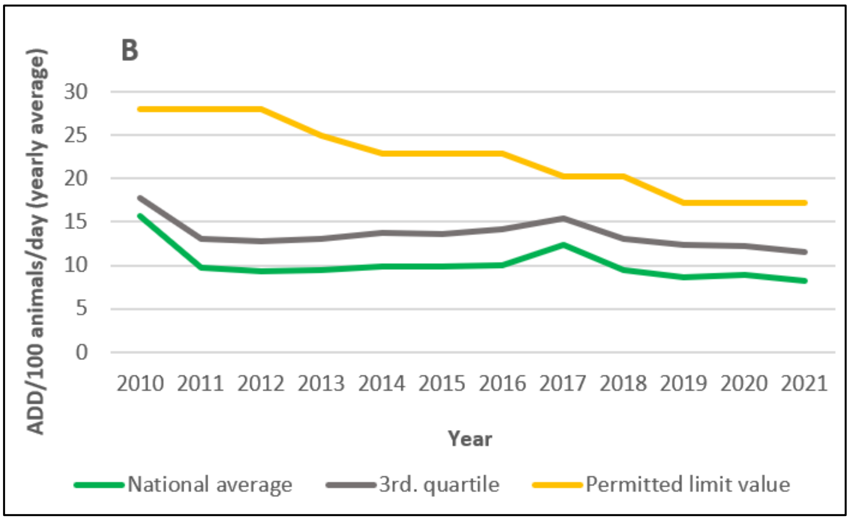


***Figure 3**. Timeline of the changes in permitted limit values for AMU in the Yellow Card scheme for Danish weaners (B), the national average for AMU in all Danish weaners and the third quartile indicating 75% of AMU in weaners. (Source: DVFA and VetStat)*

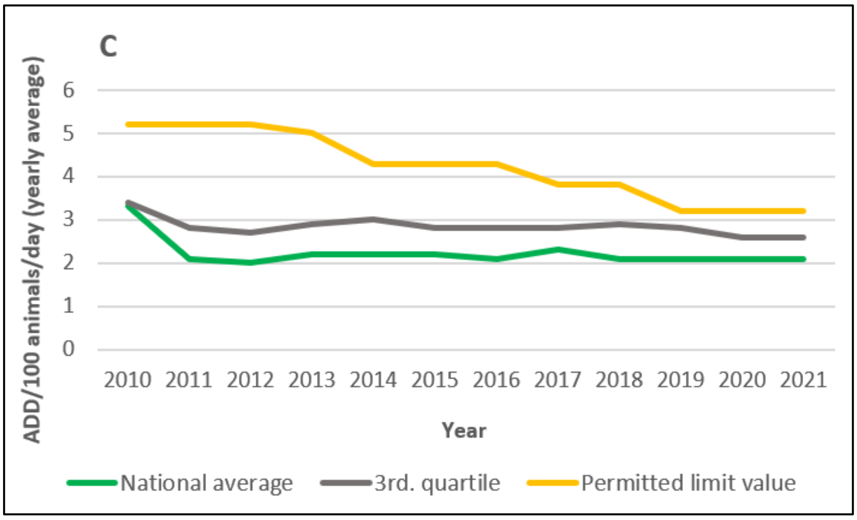


***Figure 4**. Timeline of the changes in permitted limit values for AMU in the Yellow Card scheme for Danish sows incl. piglets (C), the national average for AMU in all Danish sows incl. piglets and the third quartile indicating 75% of AMU in sows. (Source: DVFA and VetStat)*

2.3.5. Estimating antimicrobial use in Danish companion animals

Sales of antimicrobials for use in Danish companion animals are reported annually by DANMAP. The estimates are based on sales data from VetStat (DANMAP, 2023). The estimates are calculated from antimicrobial sales recorded on the specific companion animal group code (primarily sales of antimicrobials from pharmacies to pet owners) and antimicrobials recorded on the replacement code (tablets and preparations licensed exclusively to companion animals). For the specific codes, see **Table 2**. Sales of antimicrobials to companion animals are presented as the weight-based unit "kilo active compound" (DANMAP, 2023). The use of critically important and broad-spectrum antimicrobials is relatively common in companion animals, and most fluoroquinolones and cephalosporins registered in VetStat are sold for use in companion animals (DANMAP, 2023).

2.4. Antimicrobial use guidelines

In 2018, the DVFA published antimicrobial guidelines for veterinary practitioners working in pig production (Danish Food and Veterinary Administration, 2018). The guidelines aim to reduce the use of antimicrobials critical for human medicine to mitigate the risk of AMR in pigs and serve as a practical resource for pig practitioners. Antimicrobials licensed for use in pig production are divided into three categories. Category 1 comprises products considered the first choice for treating infections in pigs, while products in Category 2 should only be chosen in case of resistance to AMs in Category 1. Category 3 products are the ones that the DVFA considers unnecessary in pig production (Danish Food and Veterinary Administration, 2018). The guidelines are available as online spreadsheets and assess prudent AMU for each combination of AM, pig disease or pathogens. The guidelines serve as dynamic lists and contain all currently licensed AMs for use in pig production, and suggested dosages and treatment intervals are listed for each disease in drop-down menus. The spreadsheet is colour-coded, indicating the most appropriate options (Danish Food and Veterinary Administration, 2018).

In 2012, the Danish Veterinary Association (DVA) published antimicrobial use guidelines for veterinary practitioners working in small animal medicine. The guidelines provide organ-specific recommendations and diagnostic descriptions (Jessen et al., 2019). The guideline classifies antimicrobial products approved for small animal practice into five categories. These categories are intended to guide veterinary practitioners in their selection based on the importance of the product for both human and veterinary medicine. DVA ranks the products from top to bottom as follows: 1) products restricted to critical or life-threatening infections, 2) products should only be used following prior susceptibility testing, 3) products should only be used if resistance to products at the bottom of the pyramid has been found, 4) products can be used only if the infection is believed to be fully cured, 5) products can be used in case of treatment with alternatives to antimicrobials is deemed ineffective (Jessen et al., 2019).

3. Materials and methods

3.1. Data sources and data management

In this thesis, data were collected from official and private databases and from a questionnaire survey. **Table 3** presents the methods and data sources used for each objective.

3.1.1. Information on assigned Yellow Cards

A list of Yellow Cards assigned to Danish pig herds from 2015 to 2020 was obtained by request to the DVFA in 2020 and 2021. The following information was included: date of enforcement of the Yellow Card, CHR numbers, and name and address of the pig producer. Herd-level AMU (in ADD/100 animals/ day in a rolling 9-month average) were extracted from the VetStat interface (www.vetstat.fvst.dk) in 2020 and 2021. The herds were grouped according to the age groups with an AMU above the permitted limit values (weaners, finishers and sows). Herds with invalid data (no records of either antimicrobials or pen places) were further examined by consulting the DVFA to verify the Yellow Cards.
Herds with a Yellow Card in weaners between 2016 and 2020 were included in the eligible study population for Manuscript I. Herds with a Yellow Card in finishers between 2016 and 2020 were included in the study population in Manuscript II.

3.1.2. CHR data

CHR data were used to extract and merge information on health status from SPF Health, production type and herd demographics from CHR, antimicrobial sales data from VetStat and meat inspection lesions to herds with a Yellow Card. For objective 1, CHR data were used to group and extract herds with a low AMU in weaners and to merge information on health status, production type and herd demographics. For objective 2, CHR data were used to group and extract herds with a medium and low AMU in finishers and to merge information on production type, herd demographics and meat inspection lesions.

Information on the number of pen places recorded in CHR for each herd between 2015 and 2018 was extracted from the VetStat interface (www.vetstat.fvst.dk). The DVFA provided information on the number of pen places between 2018 and 2020.

3.1.3. VetStat data

Information on AMU in Danish pigs was obtained in three separate ways. First, a dataset covering monthly ADD/100 animals/day in a 9-month rolling average between 2016 and 2020 was obtained from the VetStat interface (www.vetstat.fvst.dk) in 2020 and 2021. The dataset was used to extract herds with a low AMU in weaners to answer objective 1. Next, a dataset covering monthly ADD/100 animals/day in a 9-month rolling average between 2016 and 2020 was obtained from the DVFA in 2021. The dataset was used to extract herds with a medium and a low AMU in finishers to answer objective 2. Lastly, antimicrobial sales data was calculated as kg active compound from raw VetStat data obtained from the DVFA in 2019 and 2021, covering 2015 to 2020. The data was used to calculate antimicrobial sales data in herds with a Yellow Card before and after the Yellow Card to support the findings in Manuscripts I and II. The data was also used to examine and calculate antimicrobial sales in Danish dogs and cats to answer objective 3.

For objective 1, monthly ADD/100 animals/day in weaners were used to extract control herds for the case-control study in Manuscript I. Herds were considered eligible controls if AMU in weaners were below the national average (10.7 – 8.8 ADD/100 animals/day) throughout 12 consecutive months.

For objective 2, monthly ADD/100 animals/day in finishers were used to extract herds with a low and medium AMU for Manuscript II. Herds that had not previously received a Yellow Card were assigned a random date between 2016 and 2020, and AMU data covering 9 months before this assigned date was extracted from the VetStat data. The herds were then grouped into herds with a low or medium AMU. An AMU above 2.5 ADD/100 animals/day in at least one month out of nine consecutive months between 2016 and 2020 was categorised as a medium AMU. An AMU equal to or lower than 2.5 ADD/100 animals/day in nine consecutive months between 2016 and 2020 was categorised as a low AMU.

3.1.4. Meat inspection data

Data on meat inspection lesions was included in Manuscript II. The meat inspection data covered monthly registrations from 9 large Danish abattoirs and were provided and merged with monthly VetStat data in an anonymised form by the Danish Agriculture and Food Council in the spring of 2023 and in January 2024. The data covered recordings from June 2015 to March 2021. For each herd, the following information was provided: lesions recorded during meat inspection, abattoir identification code and number of pigs delivered to the abattoir. Lesions related to infectious diseases were selected for Manuscript II. The number of pigs delivered to the abattoir was used to create a new variable indicating herd size. This was done because monthly data extracts from the CHR database were unavailable for use in this study. For each herd included in Manuscript II, meat inspection data was extracted covering 6 months before and 3 months after the randomly assigned data for herds with a low and medium AMU. For herds with a Yellow Card in finishers, meat inspection data covered 6 months before and 3 months after they were assigned a Yellow Card. This was done to align the meat inspection data with the general production routines as Danish finishers generally reach final slaughter weight within 10-12 weeks after entering the fattening unit (See **Figure A1** in Manuscript II).

3.1.5. Productivity in Danish pig herds

The number of pigs produced per pen place was calculated for each herd from the number of pigs produced in one year and the number of pen places registered in CHR (see **Eq. 3**). The number of pigs produced per pen place was calculated for all 66 herds with a Yellow Card in weaners between 2015 and 2020 and for the 28 herds with a low AMU enrolled in the case-control study.

$$Pigs\ produced\ per\ pen\ place = \frac{Pigs\ produced\ in\ 12\ months}{Number\ of\ pen\ places} \tag{3}$$

Data from the Pig Movement Database was used to estimate the number of pigs produced in one year. For each herd, the following data was extracted: the number of weaners delivered from one herd (first unique CHR number) to another Danish pig herd for fattening (second unique CHR number) or for export. The number of pigs delivered to the abattoir was included for herds with weaners and finishers on the same holding (one unique CHR number). Data on the number of pigs produced was manually extracted from the Pig Movement Database.

The study did not include movements of newly weaned piglets (moved at 7 kg). Antimicrobial treatments in piglets would have been registered for sows and not weaners. These types of movements were identified as single loads of 800-1000 animals on one truck. Weaned pigs weighing 25 - 30 kilos are moved on trucks at a maximum of 500-600 at a time due to the legal requirements for loading density in the European Transport Regulation (European Union, 2005).

3.1.6. Antimicrobial use in companion animals

Sales of antimicrobials for use in Danish dogs and cats were calculated as kilo active compound from raw VetStat data extracted in 2019 by the DVFA. Sales of antimicrobials recorded in VetStat under the animal species code for companion animals (code 90) and under the replacement code indicating no recorded animal species (code 0) were used. Products registered with the replacement code 0 were included to examine all sales of antimicrobials sold from pharmacies directly to veterinary clinics. For each product, approved animal species were determined by cross-referencing product names against registers from the Veterinary Industry Nordic (ViNordic) and Danish Medicines Agency (Danish Medicines Agency, 2020; ViNordic, 2020). A detailed description of the data examination is presented in Manuscript III.

3.2. Questionnaire survey

Information on herd factors in Manuscript I was obtained by telephone interviews with Danish pig producers. The interviews were conducted using a questionnaire developed in the spring and summer of 2020. The questionnaire was designed according to guidelines defined by Stone (1993). The scope of the questionnaire was defined based on what has been previously investigated in studies on herd factors in weaners and finishers (Amezcua et al., 2002; Laine et al., 2008), information routinely monitored by the producers as part of the productivity control system (SEGES, 2020) or documented by the consulting veterinary practitioner in their health advisory services (Ministry of Food, 2021), and biosecurity measures specific to Danish conditions (SPF Health, 2021). The questionnaire was presented and discussed with pig producers, veterinarians, and researchers. Additional topics highlighted as relevant by producers and veterinarians were added to the questionnaire. Next, two researchers with experience working with questionnaires and pig production discussed and reviewed the questionnaire. The questions and wording were revised to minimise the chances of misunderstandings and ambiguities.

3.2.1. Questions and scope of the questionnaire

The first part of the questionnaire consists of 41 closed-ended questions. The introductory section of the questionnaire describes the demographic range and herd structure with questions related to age, years of experience, number of employees, and purchase and transportation of newly weaned pigs. The following sections cover the most frequently treated target organ systems for which a herd diagnosis is available, antimicrobial management, weaning, water and feed, housing conditions, cleaning and disinfection, and biosecurity. The results from the first part of the questionnaire are included in Manuscript I.

The last part of the questionnaire consists of open-ended questions allowing the pig producers to express opinions and views more freely. Producers in the case group were asked to describe why the herd received a Yellow Card and the approaches taken to reduce the AMU effectively. Producers in the control group were asked to describe the challenges in maintaining a low AMU and strategies to overcome them.

The author of this thesis designed the questionnaire. The questionnaire was prepared in Danish. For the English version, see Appendix B.

3.2.2. Herd enrolment

In Manuscript I, the target population was Danish pig producers with weaners for sale, fattening or export. Contact information was obtained from public registers. Producers were contacted by telephone in the spring/summer of 2022 and fall/winter of 2022/2023. Each producer was contacted three times, with attempts made on various days and at different times to ensure a comprehensive outreach. However, several producers could not be reached due to outdated contact information or inaccessibility by phone. Moreover, a subset of producers opted not to partake in the study. The enrolment process is presented as a flowchart in **Fig. 5**

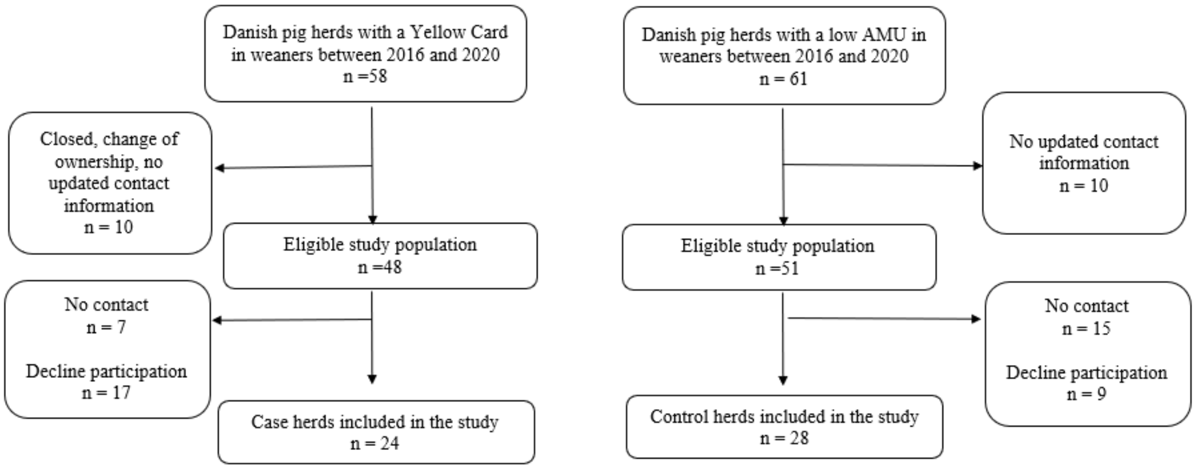


Figure 5. *Flowchart of the enrolment process of the case and control herds in Manuscript I.*

3.2.3. Data collection

Prior to the interviews, all producers in the case group were instructed to answer the questions based on the routines that were in place before receiving a Yellow Card. The producers in the control group were instructed to answer questions based on the 12 consecutive months when the herd had a very low AMU. After each interview, the CHR number was replaced with a herd identification code to anonymise the data. The author of this thesis conducted all telephone interviews. Prior to the work in this thesis, the author conducted similar interviews (via telephone and herd visits) in a study on biosecurity in Danish farrow-to-finish herds (Ramvad et al., 2017).

3.3. Statistical analysis

The association between the herd characteristics and AMU levels in herds with weaners in Manuscript I was analysed using univariable and multivariable logistic regression. Univariable associations between the outcome and predictor variables were examined using a chi-square test. In the case of variables with expected cell frequency below 5, Fisher's exact test was used. Variables that were in complete agreement between the cases and controls were excluded from the analysis. The multivariable logistic regression model included only predictor variables with a significance level of $P < 0.1$. A stepwise backward elimination process was used to retain only variables with a significance level of $P < 0.05$ in the final model. The final model was tested for multicollinearity, and the effect of being an SPF herd was tested on the significant covariates.

The association between meat inspection lesions in finishers and AMU levels (high, medium and low AMU) in Manuscript II was analysed using descriptive statistics and mixed-effects logistic regression models. The statistical significance between the individual meat inspection lesions in finishers and the AMU classes was tested using chi-square tests, while Fisher's exact test was used for lesions with very low prevalence. Only lesions with a prevalence > 0.2% and P-value < 0.1 were included in the mixed-effects logistic regression models. First, pairwise biological associations between the meat inspection lesions were assessed at the herd level. Odds ratio (OR) > 3 or < 0.33 and a P-value < 0.001 indicated strong positive or negative associations. Next, based on previous studies on meat inspection data in Danish pig production by Alban et al. (2013), mixed-effects logistic regression models were used to test the potential associations between the AMU levels and the meat inspection lesions. For each lesion, the models were fitted with AMU level, region, production type, and herd size as covariates. Herd and abattoir were included as random effects in all models. Only covariates with a significance level of P < 0.01 were retained using a stepwise backward elimination process.

R (version 4.0.3 of 2020 – The R Foundation for Statistical Computing) was used for statistical analysis and data management

***Table 3**. Overview of the hypotheses, data sources, study designs and materials and methods included in this thesis.*

Hypo-theses	Study description	Included data sources	Analysis	Results
1	Association between herd factors and AMU level in Danish pig herds with weaner production. Difference in herd characteristics and management in herds with a Yellow Card and herds with a low AMU in weaners between 2016 and 2020	Combination of register data and data from a questionnaire from telephone interviews. Data on SPF status from SPF Health. Number of pen places from CHR/VetStat. 52 pig herds with weaners included in the case-control study (24 cases with a Yellow Card and 28 controls with a low AMU)	Case-control study Univariable and multivariable logistic regression model. Dichotomous outcome variable. Variable selection using stepwise backward selection (5% significance levels).	Details are presented in Manuscript I
	Producer perceptions on reasons for a Yellow Card and actions taken towards reducing AMU in herds with a Yellow Card in weaners between 2016 and 2020. Producer perceptions on precautions for maintaining a low AMU in herds with a low AMU in weaners between 2016 and 2020.	Questionnaire data from telephone interviews (from the case-control study in Manuscript I). 52 producers included in the case-control study (24 producers with a Yellow Card in weaners and 28 producers with a low AMU in weaners)	Descriptive statistics	Details are presented in sections 4.4.2 and 4.4.3.
	The number of pigs produced per pen place in herds with a Yellow Card in weaners and in herds with a low AMU in weaners between 2016 and 2020	Combined register data. Number of pigs produced from the Danish Pig Movement Database. The number of pen places from CHR and VetStat. 92 pig herds included (66 with a Yellow Card and 28 with a low AMU)	Descriptive statistics	Details are presented in section 4.4.
2	Prevalence of meat inspection findings in herds with a Yellow Card in finishers (i.e. a high AMU) and herds with a medium or low AMU in finishers between 2016 and 2020.	Combined register data. Antimicrobial sales data from VetStat. Production types and regions from CHR and VetStat	Biological association (odds ratio) between the lesions. Univariable and mixed-methods logistic regression models for each lesion. Herd and abattoir are included as fixed variables.	Details are presented in Manuscript II

	Association between meat inspection findings and AMU levels, herd size, regional location within Denmark and production type	Meat inspection lesions, abattoir identification and number of pigs delivered to the abattoir. 10.5 million finishers delivered to 9 Danish abattoirs between 2016 and 2020. • 348,124 from herds with a high AMU • 5,976,589 from herds with a medium AMU • 4,186,343 from herds with a low AMU	The outcome variable consisted of the number of finishers with the specific lesion (y) divided by the number of finishers delivered from each herd. Variable selection using stepwise backward selection (5% significance levels)	
	Prevalence of meat inspection findings in herds with a Yellow Card in finishers (i.e. a high AMU) and herds with a medium or low AMU in finishers between 2016 and 2020 according to production type (indoor versus outdoor production types).	Combined register data. Antimicrobial sales data from VetStat. Production types from CHR. Meat inspection lesions, abattoir identification and number of pigs delivered to the abattoir. 10.5 million finishers delivered to 9 Danish abattoirs between 2016 and 2020.	Descriptive statistics Variables included AMU levels and production type.	Details and results are presented in section 4.2.
1 and 2	Changes in AMU in Danish herds with a Yellow Card in weaners, finishers and sows between 2015 and 2020.	Antimicrobial sales data from VetStat. List of Danish herds with a Yellow Card between 2015 and 2020 from the DVFA. 350 herds with a Yellow Card.	Descriptive statistics	Details are presented in sections 4.3.1., 4.3.2. and 4.3.3.
3	Total sales of antimicrobials for use in Danish dogs and cats	Combined register data. Antimicrobial sales data from VetStat and product information from Danish ViNordic and DMA in 2018.	Descriptive statistics	Details are presented in Manuscript III

4. Results

This chapter summarises key findings from Manuscripts I, II, and III and presents additional unpublished data on AMU, management, and productivity in Danish pig herds.

4.1. Assigned Yellow Cards

Between 2015 and 2020, the DVFA assigned and upheld Yellow Cards in 350 Danish pig herds. The number of Yellow Cards between 2015 and 2020 represents less than 4% of the total number of Danish pig herds. The number of Yellow Cards increased substantially in 2019 and 2020 (**Table 4**), the same period as the latest reduction in the permitted limit value (Ministry of Food, 2018b).

Table 4. *Yearly distribution of assigned Yellow Cards between 2015 and 2020 in Danish pig herds and the number of Danish holdings with pigs, including the proportion (in parenthesis) of the number of Yellow Cards to the total number of Danish pig herds.*

	Year					
	2015	2016	2017	2018	2019	2020
Number of herds in Denmark	3769	3294	3226	3125	2890	2921
Number of assigned Yellow Cards[a] (% of total herds)	61 (1.6)	30 (0.9)	32 (1)	38 (1.2)	98 (3.4)	91 (3.1)

[a]Including 68 herds with invalid data in VetStat. (Source: DVFA and Statistics Denmark)

The distribution of age groups with an AMU above the permitted limit value in herds with a Yellow Card between 2015 and 2020 are: 1) finishers (including non-gestating gilts), 2) sows (including piglets, boars and gilts) and 3) weaners (**Fig. 6**). Between 2015 and 2020, 66 herds had invalid data in VetStat. Although the DVFA was consulted, the age group with an AMU above the permitted limit value could not be determined.

The regional distribution of herds with a Yellow Card reflects the general distribution of Danish pig herds. In Denmark, the highest pig densities are found in the Northern, Central and Southern parts of Jutland and on Funen (**Fig. 7**). Many of the Yellow Cards were assigned to herds located in Southern Denmark and Central Jutland (**Fig. 8**).

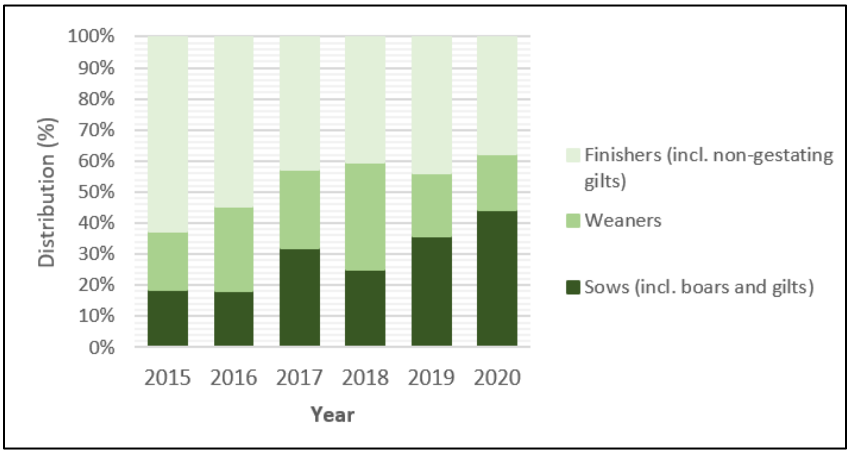


Figure 6. *Distribution of Yellow Cards between 2015 and 2020 according to age group. The 66 herds with invalid data are not included. (Source: DVFA and VetStat)*

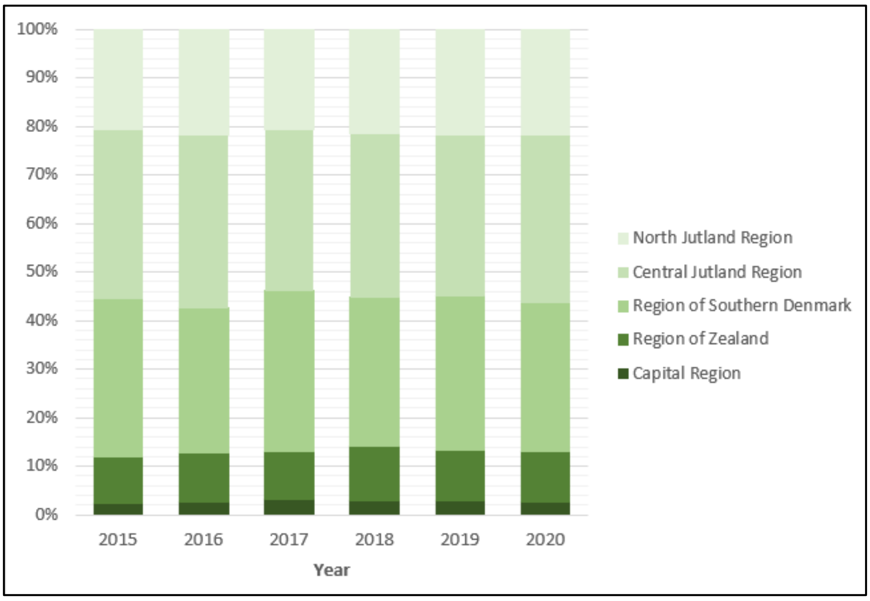


Figure 7. *Regional distribution of Danish pig herds between 2015 and 2020. Southern Denmark includes southern Jutland and Funen. (Source: Statistics Denmark)*

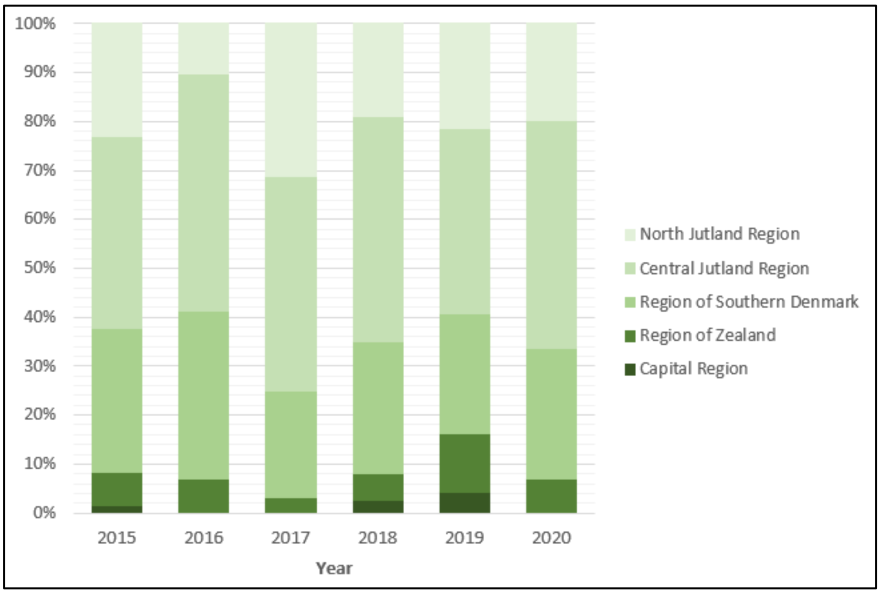


Figure 8. *Regional distribution of Danish pig herds assigned a Yellow Card between 2015 and 2020. Southern Denmark includes southern Jutland and Funen. (Source: DVFA and VetStat)*

4.1.1. Herds assigned a Yellow Card in weaners

A total of 119 herds (66 cases and 53 controls) were eligible for the study population in the case-control study for objective 1. However, only 52 Danish pig producers participated in and finalised the questionnaire surveys (24 case herds and 28 control herds) in the case-control study (Manuscript I). A flowchart of the enrolment process is presented in **Fig. 5.**
The participation rate was 36% in the case group and 53% in the control group. The herds that opted out or were not reachable by phone had a median herd size of 2000 pen places (min: 150; max: 12061), and 70 % were enrolled in the SPF system. In the herds enrolled in the case-control study (both cases and controls), the median herd size was 1800 pen places (min: 400; max: 9500), and 58% were enrolled in the SPF system.

Both the case and control herds were characterised by having mainly farrow-to-finish and weaner-to-finish productions. Only two herds in the case group and two in the control group had sows and weaners at the premises. None of the participating herds had only weaners at the property (**Table 5**).

Table 5*. Age groups at the CHR level in the 52 Danish pig herds (24 case herds and 28 control herds) included in the case-control study on herd factors associated with a Yellow Card (Manuscript I).*

Herd type	Participating herds	
	Cases[a] (n = 24)	Controls[b] (n = 28)
Farrow – to – finisher herds	10	8
Farrow – to – weaner herds	4	2
Weaner – only herds	-	-
Weaner – to – finisher herds	10	18

[a]Herds with a Yellow Card in weaners between 2016 and 2020. [b]Herds with an AMU below the national average (10.7 – 8.8 ADD/100 animals/day) in weaners for 12 consecutive months between 2016 and 2020.

Selected results from the univariable analysis in Manuscript I of the association between herd characteristics and AMU levels are presented in **Table 6**. Here, variables with a statistically significant association with the outcome (i.e. being a case or control herd) are listed. All results from the univariable analysis are presented in **Table 1** in Manuscript I.
The results of the multivariable regression model in Manuscript I showed that herds assigned a Yellow Card in weaners between 2016 and 2020 were less likely (OR = 0.04) to have strict routines working from youngest to oldest, tending to sick pigs last. Herds with a Yellow Card were also less likely (OR = 0.08) to have sufficient room for all newly weaned pigs to eat than those with very low AMU in weaners (**Table 4** in Manuscript I).

In Manuscript I, herds with a Yellow Card in weaners and those with a low AMU were characterised according to the most commonly treated herd diagnoses and treatment protocols related to the use of flock medication instead of single animal treatment. The results of the univariable models showed that herds with a Yellow Card in weaners were more likely to treat gastrointestinal diseases (OR = 4.8) than those with a low AMU. This difference was also reflected in the use of product preparations. Here, herds with a Yellow Card in weaners were more likely to use flock medication (oral preparations) (OR = 10.5) than those with a low AMU (**Tables 1** and **3** in Manuscript I).

Table 6*. Selected results of the univariable analysis of explanatory variables tested for association with AMU levels on 52 Danish pig herds from 2016 to 2020. (Full table is presented in Manuscript I).*

Variables	Categories	Distribution		P-value[a]
		Cases	Controls	
Herd health and antimicrobial treatment				
Gastrointestinal diseases are commonly treated in newly weaned pigs (< 3 weeks postweaning)	Yes No	19(79) 4 (21)	12(43) 16(57)	0.02
Neurological diseases are commonly treated in newly weaned pigs (< 3 weeks postweaning)	Yes No	1 (4) 23(96)	8 (29) 20(71)	0.02
Flock medication is commonly used to treat herd diagnoses	Yes No	23(96) 1 (4)	19(68) 9 (32)	0.01
Only using single-animal treatment	Yes No	1 (4) 23(96)	9 (32) 19(68)	0.01
Feed				
There is sufficient room for all pigs to eat simultaneously < 4 weeks postweaning	Yes No	6 (25) 18(75)	19(68) 9 (32)	0.005
Hygiene and biosecurity				
Use of heat sources to dry the pens between batches	Yes No	18 (75) 6 (25)	27(96) 1 (4)	0.04
Cleaning of hallways after moving pigs	Yes No	20 (83) 4 (17)	27 (96) 1 (4)	0.2
Working routines being from young to old and healthy to sick	Yes No	15(62) 9 (38)	27(96) 1 (4)	0.003

[a]P-value for association (Chi-square)

4.1.2. Reasons for a Yellow Card in weaners

The main reasons reported by the producers for exceeding the permitted limit values and receiving a Yellow Card in weaners are presented in **Fig 9**. Eleven of the 24 producers interviewed in the case-control study reported that errors and an increase in pre-existing herd health diseases were important reasons why they received a Yellow Card. The errors mainly covered registration errors in VetStat (wrong age group, incorrect amount).

The main approaches to reducing AMU following a Yellow Card are presented in **Fig. 10**. Twelve of the 24 producers with a Yellow Card in weaners reported that they increased the awareness of AMU and, when possible, treated fewer pigs or even delayed treatment. Six of the 24 producers reported an increased use of vaccines.

4.1.3. Measures to maintain a low antimicrobial use

Of the 28 producers with a low AMU in weaners, 19 out of 28 reported that one of the most important reasons to how they maintained a low AMU was overall management routines (**Fig. 11**). More than half of the producers reported that focus on feed and water, hygiene and biosecurity and a general focus on AMU was important. However, several producers reported that it was difficult to pinpoint only one or a few reasons. Instead, they considered the overall management routines focusing on AMU to be the most important. In addition, several felt that they generally accepted having to produce fewer pigs per pen place to make more room in the barn (i.e. time between batches). Several producers stated that a low AMU was not without consequences. Instead, they encountered problems with umbilical hernias, just as some accepted a higher level of disease.

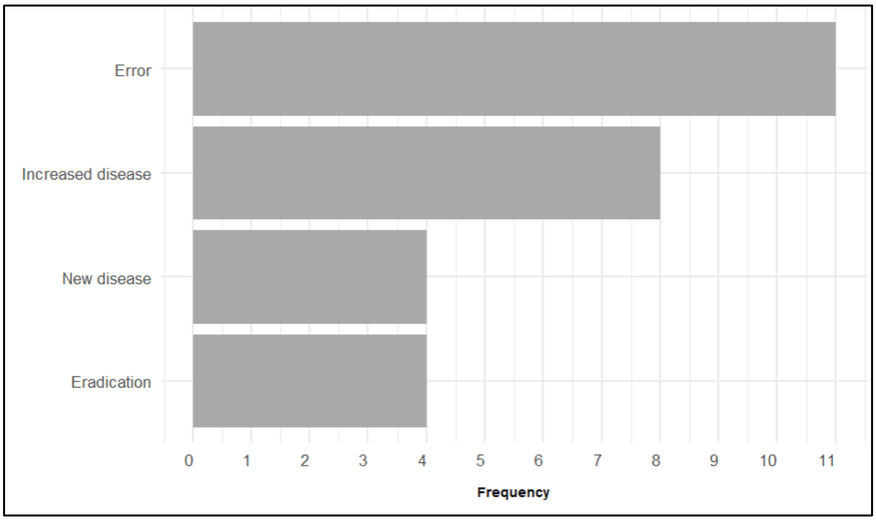


Figure 9. *Results from the qualitative interview in the case-control study with 24 producers with a Yellow Card in weaners on producer perspectives of important reasons for an AMU above the permitted limit values in weaners between 2016 and 2020.*

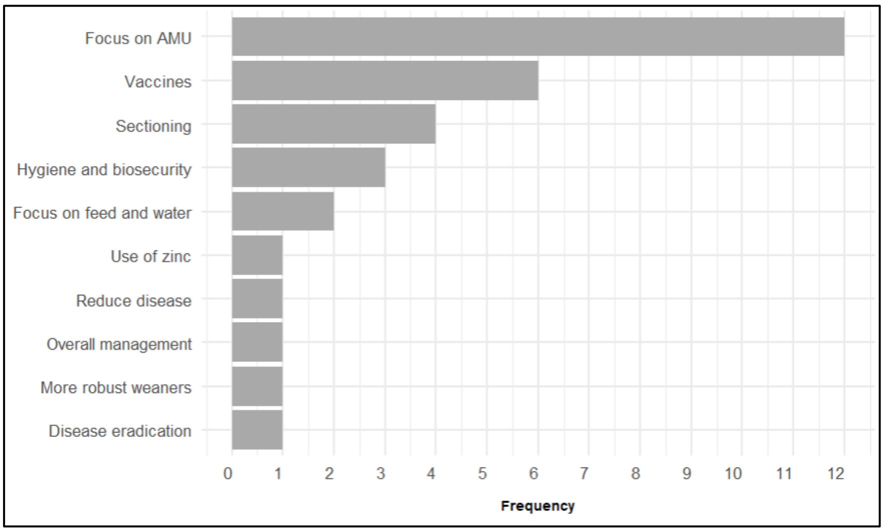


Figure 10. *Results from the qualitative interview in the case-control study with 24 producers with a Yellow Card in weaners on important measures to reduce AMU following a Yellow Card.*

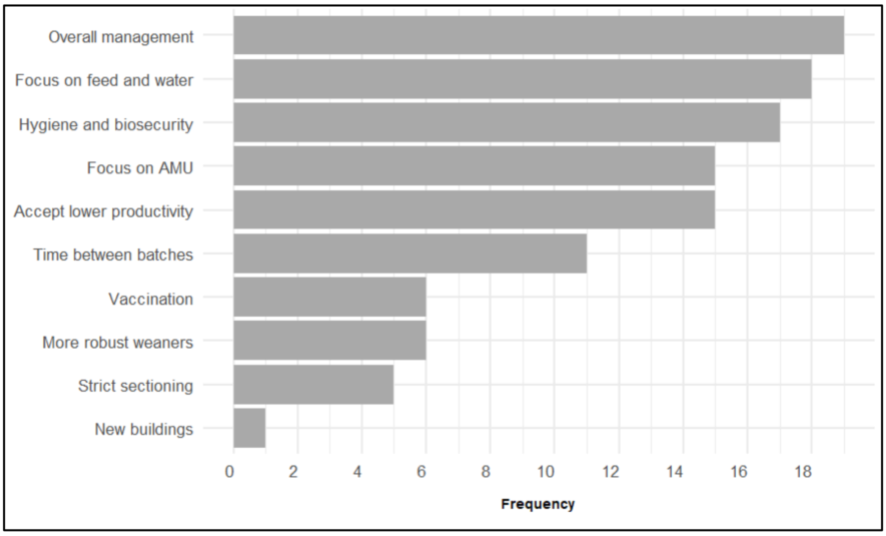


Figure 11. *Results from the qualitative interview in the case-control study with 28 producers with a low AMU in weaners on producer perspectives of important reasons for maintaining a low AMU.*

4.2. Association between antimicrobial use and finisher pig health

A total of 10.5 million pigs delivered to 9 Danish abattoirs between 2016 and 2020 were analysed, including 348,124 pigs from herds with a high AMU, 5,976,589 pigs from herds with a medium AMU and 4,186,343 pigs from herds with a low AMU.

The lesion prevalence differed by less than 0.5% between the three AMU levels. Lung lesions were the most common lesion type (16-17%) in all AMU levels. The other lesions were less frequent (< 3 %). Lesion prevalence for each AMU level is presented in detail in **Table 2** in Manuscript II.

The differences in lesion prevalence between the three AMU levels are most apparent when the herds are divided according to production type (outdoor versus conventional indoor) (**Table 7**). Here, a higher lesion prevalence is seen in herds with outdoor production types at all three AMU levels. The differences are especially pronounced for herds with high or medium AMU.

The results in Manuscript II indicate that production type, herd size and the location of the herd affect the likelihood of delivering pigs with several of the lesions included in the study.

The result of the multivariable models showed that herds with a medium AMU were associated with the lowest prevalence of 5 out of the 8 meat inspection lesions included in the study (peritonitis, abscess in the trunk, abscesses in the extremities, tail lesions and arthritis). Large herds were associated with a lower prevalence of 7 out of the 8 lesions than the small herds (pericarditis, peritonitis, abscess in the trunk, abscesses in the extremities, tail lesions, osteomyelitis and arthritis). Outdoor production types were associated with a higher prevalence of osteomyelitis, arthritis and tail lesions. However, AMU level interacted with the effect of herd type. Herds located in the western regions of Denmark were associated with a higher prevalence of lung and tail lesions than those in the eastern regions.

Overall, the fixed effects (abattoir and animals delivered from the same herd) were important in capturing the variability in the data. Detailed results of the mixed-effects logistic regression models for each lesion are presented in **Table 3** in Manuscript II.

Table 7. *Prevalence of lesions recorded during meat inspection in finishers from herds with a high, medium and low AMU during 9-month periods from 2016 to 2020 from 10 Danish abattoirs, sorted by production type.*

Lesions[c]	Lesion prevalence[a]					
	High[b]		Medium[b]		Low[b]	
	Indoor n = 328,818	Outdoor n = 19,306	Indoor n = 5,947,285	Outdoor n = 27,940	Indoor n = 4,108,422	Outdoor n = 84,328
Pyemia	<0.1	0.1	<0.1	0.1	<0.1	<0.1
Pericarditis	1.2	2.4	1.2	0.7	1.4	0.8
Lung lesions	16.5	17.3	16.0	32.5	16.4	17.9
Peritonitis	0.5	3.4	0.6	1.7	0.7	1.3
Hernia	1.1	1.5	1.1	0.8	1.4	0.9
Osteomyelitis	0.1	0.9	0.1	0.5	0.2	0.4
Arthritis	0.3	2.8	0.2	1.7	0.3	1.1

Table 7 continued

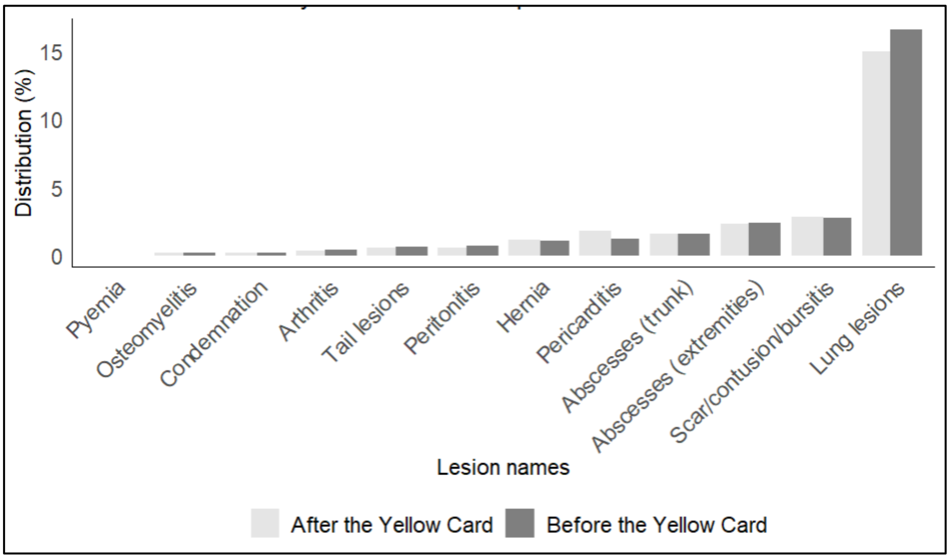
Abscesses, trunk	1.5	3.0	1.4	2.3	1.7	1.8
Abscesses, extremities	2.4	2.5	2.1	2.5	2.3	1.9
Tail lesions	0.5	3.7	0.5	2.3	0.6	1.6
Scar/bursitis	2.9	1.2	2.8	2.5	2.8	1.1
Lesion prevalence	27.2	38.9	26.2	47.9	27.9	28.9
Condemnation	0.2	0.4	0.2	0.4	0.2	0.2

[a]The prevalence for each antimicrobial use level was calculated from the number of lesions divided by the total number of finishers delivered to the abattoir during the 9 months. [b]Total number of finishers slaughtered in the study period: high AMU = 348,124 heads, medium AMU = 5,975,225 heads, low AMU = 4,192,804 heads. [c]More than one lesion per animal can be recorded.

4.2.1. Changes in meat inspection lesions in herds assigned a Yellow Card

In the 9 months following a Yellow Card, there were only minimal changes in lesion prevalence amongst the herds with a Yellow Card in finishers (**Fig. 12**). There were marginal increases in lesion prevalence for arthritis, abscesses in the extremities and lung lesions, and only slight decrease in lesion prevalence for pericarditis.

Figure 12*. Prevalence of meat inspection lesions in herds with a Yellow Card in finishers between 2016 and 2020 during the 9 months before and 9 months after a Yellow Card.*



4.3. Antimicrobial use in Danish pig herds assigned a Yellow Card

4.3.1. Changes in antimicrobial use

Danish pig herds assigned a Yellow Card between 2015 and 2020 were compliant with the regulatory restrictions imposed by the Yellow Card scheme when considered as a whole. AMU levels were reduced for all three age groups during the nine months that followed a Yellow Card. The median ADD/100 animals/day was reduced across all age groups (**Fig. 13**).

In weaners, the median monthly AMU was reduced from 18 ADD/100 animals/day before the Yellow Card to 13 ADD/100 animals/day after the Yellow Card. The median AMU was reduced for finishers from 4.5 ADD/100 animals/day to 3 ADD/100 animals/day. For sows, the median AMU was reduced from 3.4 ADD/100 animals/day to 2.7 ADD/100 animals/day.

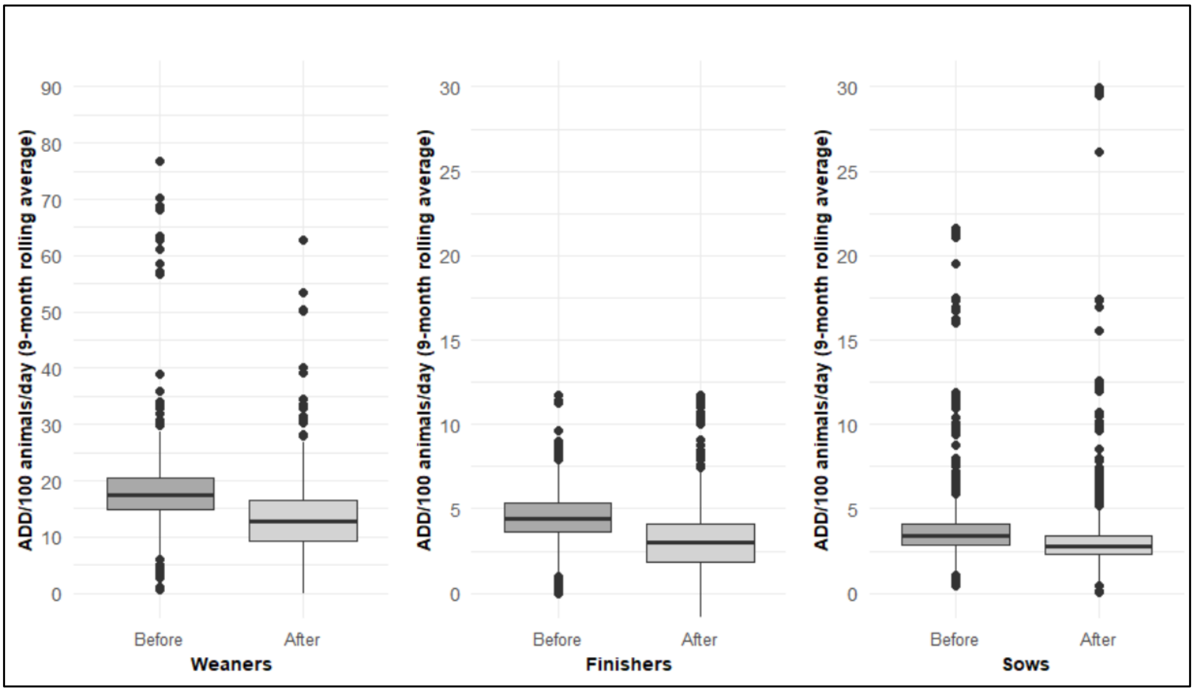


***Figure 13**. Changes in AMU (ADD/100 animals/day in a 9-month rolling average) for each age group following a Yellow Card. A: changes in AMU in herds with a Yellow Card in weaners. B: Changes in AMU in herds with a Yellow Card in finishers. C: Changes in AMU in herds with a Yellow Card in sows. Note the difference in Y-axes for Figure A and Figures B and C.*

4.3.2. Changes in product preparations and target organ systems

The amount of antimicrobials purchased in herds with a Yellow Card was statistically significantly lower ($P < 0.05$) after the Yellow Card compared to before the Yellow Card (**Table 8**). This reduction involved both parenteral and peroral preparations (**Table 9**).

Changes in prescription patterns across the target organ systems were also evident (**Table 10**). Herds with an AMU above the permitted limit values in sows reduced treatments of reproductive, urogenital, and respiratory disorders. Herds with an AMU above the permitted limit values in weaners and finishers reduced treatments of gastrointestinal disorders.

Table 8. *Sales of antimicrobials (kg active compound) in Danish pig herds with a Yellow Card between 2015 and 2020 in sows, weaners or finishers, 9 months before and 9 months after a Yellow Card*

Age groups	Total AMU		P-value[c]
	Before[a]	After[b] (% change)	
Sows (n = 90)[d]	1959.8[e]	1135.9[f] (- 42)	<0.001
Weaners (n = 66)	1233.2[g]	798.2[h] (-35)	0.05
Finishers (n = 126)[i]	1135.3	698[j] (-39)	0.01

[a]Total prescribed AMs 9 months before the Yellow Card for sows, weaners and finishers. [b]Total prescribed AMs 9 months after the Yellow Card for sows, weaners and finishers. [c]P-value for comparison (t-test). [d]Including piglets, gilts and boars. [e] Includes 4.2 kg active compound of topical preparations. [f] Includes 3.5 kg active compound of topical preparations. [g] Includes 0.3 kg active compound of topical preparations. [h] Includes 0.2 kg active compound of topical preparations. [i]Including non-pregnant gilts. [j]Includes 0.4 kg active compound of topical preparations. (Source: VetStat)

Table 9. *Sales of antimicrobials (kg active compound) in Danish pig herds with a Yellow Card between 2015 and 2020 in sows, weaners and finishers for 9 months before and 9 months after a Yellow Card sorted by product preparation.*

	Preparations			
	Before		After (% change)	
	Peroral	Parenteral	Peroral	Parenteral
Sows[a]	703.3	1252.3	155.7 (-78)	976.8 (-22)
Weaners	1089.9	143.6	670.2 (-40)	128.1 (-11)
Finishers[b]	741.8	393.5	365.9 (-51)	331.7 (-16)

[a]Including piglets, gilts and boars. [b]Including non-gestating gilts. (Source: VetStat)

***Table 10**. Sales of antimicrobials (kg active compound) in Danish pig herds 9 months before and 9 months after a Yellow Card sorted by target organ systems and age groups.*

Target organ system	Before a Yellow Card					After a Yellow Card				
	10	11	12	13	14	10	11	12	13	14
Sows[a]	441.3	165.5	226.6	448.1	671.8	224.7	129.3	137.6	110	532.5
Weaners	0.9	-	734.4	242.2	252.7	1.3	-	545.6	81.4	167.6
Finishers[b]	13.3	-	575.1	187.4	358.5	13.4	-	281.2	123.7	278.8

[a]Including piglets, gilts and boars. [b]Including non-pregnant gilts. 10: Reproduction and urogenital. 11: Udder. 12: Gastrointestinal disorders. 13: Respiratory tract disorders. 14: Joints, limbs, hooves, central nervous system, skin. (Source: VetStat)

4.3.3. Changes in antimicrobial classes

Antimicrobial reduction measures following a Yellow Card resulted in reductions across all the antimicrobial classes used for weaners, sows, and finishers compared to the use levels before a Yellow Card. There were no significant changes in the type of antimicrobial classes for any of the age groups (**Fig. 14**).

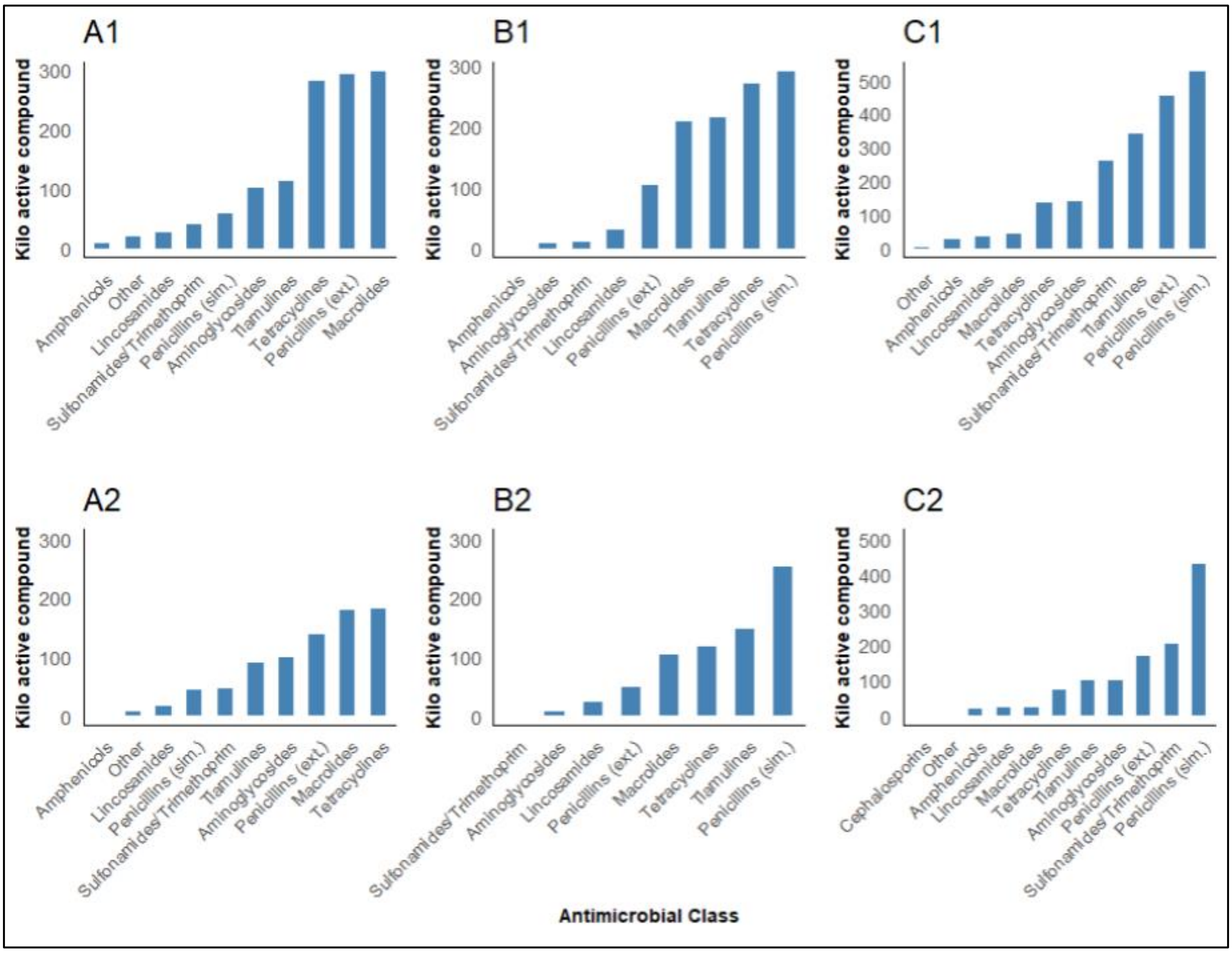


Figure 14. *Antimicrobial use (kg active compound) in Danish pig herds 9 months before and 9 months after a Yellow Card sorted by antimicrobial classes. A: Herds with a Yellow Card in weaners 9 months (1) before and 9 months (2) after the Yellow Card. B: Herds with a Yellow Card in finishers 9 months (1) before and 9 months (2) after the Yellow Card. C: Herds with a Yellow Card in sows 9 months (1) before and 9 months (2) after the Yellow Card. Note the difference in Y-axes for Figure A and Figures B and C.*

4.4. Productivity in selected Danish pig herds

The median number of pigs produced per pen place in the 66 herds with a Yellow Card in weaners between 2015 and 2020 was 6.4 (mean = 9.1) (**Fig. 15**). The number of pigs produced per pen place varied considerably among the 66 herds (SD = 8.2). It took an average of 7.9 weeks for the 66 herds with a Yellow Card in weaners to produce a pig from 7 to 30 kg (**Fig. 16**). Herds producing more than 6.4 pigs per pen place used between 1.1 and 8 weeks to produce a pig from 7 to 30 kg. As it is not biologically possible to produce a pig from 7 to 30 kg in just 1.1 weeks, this is considered an outlier, potentially due to erroneous registrations in the CHR register. Herds producing less than 6.4 pigs per pen place used between 8 and 17 weeks (**Fig. 16**).

The 28 herds with a low AMU produced fewer pigs per pen place than those with a Yellow Card (Fig. 15). They produced an average of 6 pigs per pen place (median = 5.8, SD = 2.4). It took an average of 10.3 weeks to produce a pig from 7 to 30 kg (**Fig. 15**).

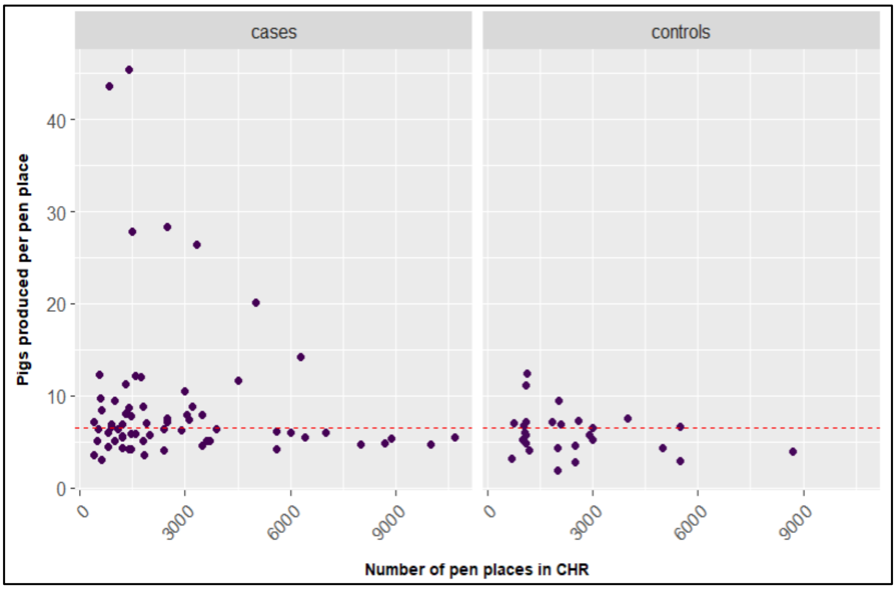


Figure 15*. Number of pigs produced per pen place for 66 herds assigned a Yellow Card between 2015 and 2020 (cases) and 28 herds with a low AMU in weaners (controls). The dotted line indicates the average number of pigs produced per pen place in a standard production where the weaner unit is emptied after 8 weeks.*

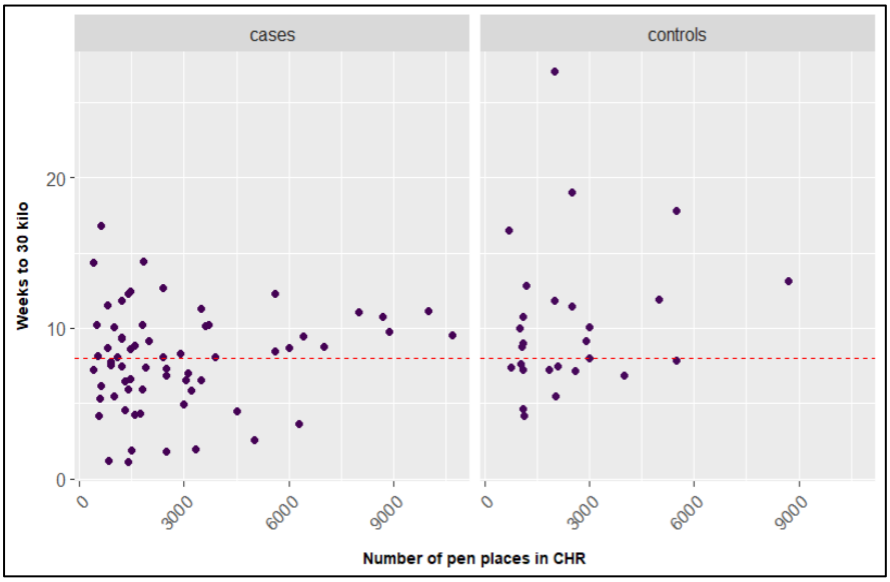


Figure 16. *Number of weeks it takes to produce a pig from 7 to 30 kg for 66 herds assigned a Yellow Card between 2015 and 2020 (cases) and 28 herds with a low AMU in weaners (controls). The dotted line indicates the average of 8 weeks to 30 kg in standard productions*.

4.5. Antimicrobial use in companion animals

To estimate AMU in Danish dogs and cats in 2018, sales of antimicrobials were extracted from VetStat from animal species code 90 and the replacement code 0.
In 2018, sales of antimicrobials recorded on animal group code 90 amounted to 515 kg active compound. However, 53% were products licensed solely for use in livestock or horses. Antimicrobials licensed solely for dogs and cats on animal group code 0 covered 706 kg active compound (the product preparations covered tablets, ointment, ear- and eye drops). The aggregated dataset (described in detail in Manuscript III) on antimicrobial sales in dogs and cats (AMU_{calc}) was unsuitable as a proxy for AMU in Danish dogs and cats.

While veterinary recordings readily provide data on products sold from pharmacies to veterinary practitioners and used for treating livestock, veterinary practitioners treating companion animals are not obliged to transfer data to VetStat. Therefore, VetStat does not contain any treatment data for Danish companion animals. Suppose sales data were extracted from only animal group code 90. In that case, the dataset would almost exclusively consist of pharmacy recordings of products sold to pet owners (i.e. veterinary prescriptions). In contrast, peroral and topical preparations sold or used by veterinary practitioners in small animal clinics would be unaccounted for.

5. Discussion

The overall aim of this PhD thesis was to strengthen the evidence-based use of VetStat data in research and to extend its application across different animal species by improving the knowledge of the relationship between AMU, herd characteristics and animal health in pig production and the usability of VetStat data in companion animals. The close collaboration between Danish research institutions and Danish authorities means that a better understanding of VetStat data, including its limitations, will provide important insights for authorities when using the data to create regulatory restrictions, guidelines, and monitoring in the veterinary sector.

Data on meat inspection findings, SPF status, productivity and sales of antimicrobials in pigs were collected from Danish registers, and information on herd characteristics was collected through interviews. The data were explored and analysed using multivariable and mixed model regression analyses. Antimicrobial sales data were collected from VetStat. Data was investigated to determine the applicability of allocating sales data to cats and dogs.

The research presented in this thesis also demonstrates that both high and low levels of AMU were associated with differences in herd characteristics and health challenges in finisher pigs. Despite efforts to estimate accurate AMU levels in companion animals, challenges remain due to the structure of the VetStat database.

5.1. General discussion

VetStat data was used to address all three hypotheses outlined in this thesis. However, the methods used in Manuscripts I and II were considerably different than in Manuscript III due to the difference in quality and usability of the VetStat data in pigs compared to dogs and cats. Antimicrobial sales data for pigs is very detailed and covers the entire pig population due to the thorough monitoring of AMU in pig production and the potential repercussions for pig producers under the Yellow Cards scheme. The data can thus be readily retrieved from VetStat and linked directly to other data sources which improves the usability of VetStat data for research purposes. It allows for studies to explore the relationship between AMU and biosecurity (Kruse et al., 2017; Kruse et al., 2019), vaccination (Kruse et al., 2017), animal health (Alban et al., 2013; Dupont et al., 2017a) and retrospective analysis of AMU (Dupont et al., 2016; Fertner et al., 2015; Kruse et al., 2017; Kruse et al., 2019; Kruse et al., 2020). The VetStat database thus provides a more comprehensive analysis of AMU in Danish pig production compared to data collected by private entities or producers.

Given the extensive data coverage in VetStat (Stege et al., 2003), antimicrobial sales data were used to select herds according to AMU in weaners for objective 1 and AMU in finishers for objective 2. However, the high usability of VetStat data was only the case for antimicrobial sales data in pigs. The challenges of using VetStat data to estimate AMU and usage patterns in companion animals are presented in detail in **Table 2** in Manuscript III. This study serves as a starting point for how future research on AMU in companion animals can be conducted to avoid structural errors. A detailed discussion outlining the steps required to obtain an overall estimate of AMU in Danish dogs and cats in 2018 is presented in detail in Manuscript III.

If antimicrobial sales data from VetStat is to be used for research on AMU in companion animals, researchers must understand how to extract the data from within the VetStat environment. Such measures were previously discussed by Dupont et al. (2017b) in the context of VetStat data for pigs, but it is now highly relevant when using VetStat data for companion animals.

5.1.1. Antimicrobial use trends

In Manuscript I, producers with a low AMU reported more frequent use of single animal treatment than those assigned a Yellow Card, who relied more on flock medication (**Table 3** in Manuscript I). In Danish pig production, more than 80% of antimicrobials sold for use in weaners are peroral preparations (Moura et al., 2023). This suggests that, not only in the herds assigned a Yellow Card in weaners in Manuscript I, but also throughout Danish pig production, single-animal treatment is less common than flock medication. The extensive use of peroral antimicrobials, especially in weaners, is not favourable to a prudent AMU, as this method necessitates treating many pigs at once. It is also concerning in light of increasing AMR in zoonotic pathogens, including resistance to tetracycline in *Salmonella* spp., and particularly the increase in neomycin resistance.

Contrary to Denmark, in other Scandinavian and Nordic countries, single-animal treatment is far more commonly used in pig production.

In Sweden, approximately 90% of the veterinary antimicrobials sold were for single-animal treatment, leaving less than 10% for group treatment (Swedres-Svarm 2024). Similarly, in Norway, most products sold for use in livestock were for single-animal treatment, with only 2.7% for group treatment (NORM-VET 2023). Finland presents a similar trend, with 74% of veterinary antimicrobials sold in 2022 being for single-animal treatments (FINRES-Vet 2023). However, direct comparisons with Danish pig production, which operates on a significantly larger scale, are complicated by the sheer difference in pig populations. The Danish pig population amounts to 13.5 million pigs compared to 1.4 million pigs in Sweden and 1.1 million pigs in Finland (Danish Agriculture & Food Council, 2023c).

Like Denmark, in countries with large pig production, such as Belgium, antimicrobials sold for use in pig production account for a large part of total sales of veterinary antimicrobials. In 2022, the Belgian pig population amounted to 6 million heads (Danish Agriculture & Food Council, 2023c), and in 2022, 28.2% (34.5 tonnes of active compound) of the overall sales of veterinary antimicrobials were authorised for use in pigs, 29.1% for both pigs and cattle, and 17.5% for cattle, pigs, and poultry (BelVet, 2023). Data from Sanitel-Med show that 60.1 tonnes of active compounds were used in pig production in 2022 (BelVet, 2023). Unlike Denmark, where AMU is highest in weaners, Belgium recorded the highest AMU in fatteners, with 33.1 tonnes used compared to 24 tonnes for weaners (Belvet, 2023). This discrepancy may be explained by the large export of weaners in Denmark, which complicates direct comparisons and highlights the importance of considering different production structures and market dynamics when evaluating AMU data across countries. The high AMU in pig production in both Denmark and Belgium highlights the need for targeted policies, such as the Yellow Card scheme in Denmark, to limit AMU.

In the Netherlands, targeted policies have led to a significant decrease in sales of veterinary antimicrobials by more than 70% since 2009. Sales across all animal species were reduced from approximately 500 tonnes of active compound in 2009 to just under 100 tonnes in 2022 (SDa, 2023), highlighting the effectiveness of policy interventions in addressing AMU at the national level. In Dutch pig production, with a pig population of 10.9 million in 2022, AMU was reduced from around 120 tonnes in 2009 to less than half that amount in 2022 (Danish Agriculture & Food Council, 2023c; SDa, 2023). Belgium has also set ambitious targets through an agreement between the Antimicrobial Consumption and Resistance in Animals (AMCRA) organisation and national authorities, aiming for a 75% reduction in AMU by 2024 compared to 2011 levels (BelVet, 2023). In Denmark, a similar approach was taken, with the 2018 target of a 15% reduction in AMU in pig production being part of the national MRSA action plan. While this target was met in 2019, the subsequent 2% reduction by 2022 was not achieved, leading to a revised target in 2024, aiming for an 8% reduction by 2027 (DANMAP, 2023). Past reductions in permitted limit values in the Yellow Card scheme suggest that Denmark could see further reductions in AMU limits.

Regulations of colistin in pig production offer another perspective on how countries are managing critically important antimicrobials. In 2018, colistin was added to the World Health Organisation’s list of critically important antimicrobials for human medicine (the WHO CIA List) (World Health Organization, 2019). In the Netherlands, colistin use within the veterinary sector amounted to 806 kg of active compounds in 2022, with 683 kg used in pig production (SDa, 2023). Despite marked reductions in colistin use in Danish pig production, overall AMU in weaners in Denmark has increased. Between 2021 and 2023, sales of antimicrobials for use in weaners increased from 35,571.6 kg of active compound to 42,065.6 kg (DANMAP, 2022; DANMAP, 2023). This trend is likely linked to the weighting of colistin in 2017, followed by the ban on medicinal zinc oxide in June 2022, which has led to a notable increase in neomycin use, as it remains one of the few viable options for treating PWD in weaners (DANMAP, 2023). While significant progress has been made in reducing AMU in countries like the Netherlands and Belgium, the increase in AMU observed in Danish weaners highlights the difficulty of balancing regulatory measures with practical treatment protocols in pig production. This is further emphasised by the findings in Manuscript I, which show that most producers who received a Yellow Card were primarily treating newly weaned pigs for gastrointestinal disorders.

5.1.2. Changes in antimicrobial resistance

The restrictions on tetracyclines, 3rd and 4th generation cephalosporins, and fluoroquinolones under the Yellow Card scheme reflect a strategy to reduce and maintain low levels of AMR in pathogens affecting animal and human health. In pig production, *E coli*, *Enterococcus faecalis*, and *Salmonella* spp. are commonly monitored due to their potential to transfer resistance through the food chain to humans (DANMAP, 2023). However, despite the ongoing reduction in sales of tetracycline in Danish pig production, increased AMR in *S. typhimurium* isolates between 2010 and 2022 highlights the importance of the restrictions imposed by the Yellow Card scheme, and thus the weighting factor of 1.5 on tetracyclines.

Neomycin resistance has increased in *E. coli* isolates from 6.9% in 2016 to 43.2% in 2022, while resistance to colistin and ciprofloxacin remains under 1% (DANMAP, 2023). Before the phase-out of colistin in Danish pig production, the low resistance to colistin was significant, given that the WHO has classified it as a critically important antimicrobial since 2018. However, the phase-out of colistin has led to an increased reliance on neomycin in treating PWD, increasing neomycin sales and AMR (DANMAP, 2023). This is concerning, as the use of peroral antimicrobials for treating gastrointestinal disorders remains very high in Denmark (Moura et al., 2024) and was confirmed to be widespread in herds with a Yellow Card in weaners in Manuscript I.
The restrictions on colistin also emphasise the potential challenges following the ban on medicinal zinc oxide in pig production. There is a growing risk that, as effective antimicrobials against PWD become less available, animal welfare could be significantly impacted. This situation calls for producers to adopt alternative, non-medical strategies to improve the health and resilience of newly weaned pigs.

5.2. The Yellow Card scheme

5.2.1. Change in antimicrobial use for herds assigned a Yellow Card

Prior to a Yellow Card, gastrointestinal disorders and peroral preparations accounted for the highest use in weaners and finishers (**Tables 9** and **10**). This is in accordance with the results presented in Manuscript I. Here, herds with a Yellow Card in weaners were more likely to treat gastrointestinal disorders in newly weaned pigs and to use peroral preparations (flock medication) (**Table 3** in Manuscript I). Sows were mainly treated with parenteral preparations, prescribed for joints, limbs, hooves, and CNS (**Tables 9** and **10**). The overall AMU patterns in herds with a Yellow Card are also consistent with what Moura et al. (2023) described for Danish pig herds in 2020.

When herds assigned a Yellow Card between 2015 and 2020 are considered as a group, similar to how national AMU data is reported, compliance with the restrictions imposed by the Yellow Card is evident. However, the study does not account for changes at the herd level, such as the proportion of herds closed following a Yellow Card, changes in age groups, or other significant changes following a Yellow Card. As a result, it is impossible to determine whether all herds assigned a Yellow Card have successfully reduced herd level AMU. Nevertheless, the overall findings (**Tables 8** and **9** and **Fig. 14**) suggest that a Yellow Card changes antimicrobial treatment patterns in the following 9 months.

The largest reduction was observed for peroral preparations (**Table 9**). Following a Yellow Card, peroral preparations were reduced by 78% for sows, 40% for weaners, and 51% for finishers. However, there was no trend towards increased use of parenteral preparations but rather a general reduction in overall AMU. An overall reduction is also observed for the individual antimicrobial classes (**Fig. 14**).
For all three age groups, there was a statistically significant difference in mean AMU following a Yellow Card (**Table 8**). The changes presented in **Figure 14** indicate that during the 9 months following a Yellow Card, antimicrobial reduction is mainly achieved by reducing overall AMU. This indicates an overall trend of a reduction in AMU during the nine months immediately following a Yellow Card, when the herds assigned a Yellow Card are considered as a whole. Pig producers may prioritise a marked decrease in AMU immediately upon receiving a Yellow Card, allowing treatment behaviour to shift once they are no longer affected by the restrictions. The results in **Tables 9** and **10** suggest that when all herds that have received a Yellow Card in weaners are viewed as a whole, similar AMU patterns are observed in this group as those described by Moura et al. (2023) for the general Danish pig production. This indicates that in Danish pig production, regardless of whether a Yellow Card has been assigned, continued focus must be directed towards reducing gastrointestinal disorders in weaners as part of the ongoing efforts to reduce AMU.

There was a significant increase in the number of assigned Yellow Cards in 2019 and 2020 compared to previous years (**Table 4**). This coincides with the latest lowering of the permitted limit values in 2019 (Ministry of Food, 2018b), bringing the limit value closer to the national average for all three age groups (**Figures 2**, **3** and **4**). Consequently, the Yellow Card scheme may affect a significant proportion of Danish pig herds in the years to come.

5.2.2. Characteristics of herds assigned a Yellow Card in weaners

The results from the multivariable regression model in Manuscript I indicate that herds assigned a Yellow Card in weaners differ from those with a low AMU regarding internal biosecurity measures and feeding routines. The results are presented in detail in **Table 4** in Manuscript I.

Herds with a Yellow Card were less likely to have sufficient room for newly weaned pigs to eat at the same time and to have strict routines working from youngest to oldest and handling sick pigs last. The producers also regularly treated newly weaned pigs for gastrointestinal disorders and used group medication (peroral preparations) more often than those with a low AMU (**Table 3** in Manuscript I). However, there was only minimal variation between the case and control herds across most of the predictor variables included in the study. These covered the origin of the pigs, treatment routines, feed regiments, weaning, housing, overall hygiene, and internal biosecurity. Specific prevalences for each predictor variable and associated P-values are listed in Manuscript I (**Table 1**).

Danish pig herds generally maintain a high level of external biosecurity (Kruse, 2016; Kruse et al., 2020), and producers in both the case and control groups all reported daily washing of footwear upon completion of the workday and entering the herd through a designated entry room (**Table 1** in Manuscript I). In Denmark, there are a number of requirements for the layout of these types of entry rooms to prevent pathogens from being carried in or out of the herd via clothing, equipment, footwear or personnel. The consulting veterinarian ensures that herds comply with these requirements during the regular advisory visits (Ministry of Food, 2021). In addition, the SPF system provides a comprehensive regulatory framework for external biosecurity measures (SPF Health, 2021). This is likely the reason why these rules are adhered to in all herds enrolled in the case-control study.

High internal biosecurity is also associated with lower treatment incidence in farrow-to-finish herds in several European countries (Laanen et al., 2013; Postma et al., 2015). Surprisingly, most of the internal biosecurity measures in Manuscript I were not associated with the AMU level. Unlike internal biosecurity, most external biosecurity measures are not very time-consuming to adhere to and do not take time away from other, more pressing tasks. It is also possible that these routines are so ingrained in Danish pig production that it would take a lot to change them. Although the herds represented very high and very low AMU, only one parameter on internal biosecurity differed between the herds, and that was working routines throughout the herd in terms of age groups and disease. This parameter may reflect several underlying routines that were not addressed in the study. However, the case and control herds were very similar regarding all in/all out, mixing of age groups, equipment solely for weaners, and cleaning routines. Previous studies in Danish farrow-to-finish herds also found no significant association between biosecurity measures and AMU (Kruse et al., 2020). It is possible that the findings in Manuscript I are due to a low number of herds enrolled in the study. However, as discussed by Kruse et al. (2020), Denmark has a generally high level of biosecurity and general adherence to the SPF regulations (Filippitzi et al., 2018), which can also have a spill-over effect on the routines inside the herds.

Although there was no significant association between hygiene measures and AMU levels, case herds were more likely to treat gastrointestinal disorders in newly weaned pigs compared to the control herds (**Table 3** in Manuscript I). E. coli is often part of the infectious load in (PWD (Rhouma et al., 2017). Important measures to reduce the infectious load on the pen level are cleaning, disinfection, and drying (Mannion et al., 2007), as infectious pathogens like rotavirus and E. coli can survive for months in biological material (Amass and Clark, 1999). It is plausible that differences between case and control herds were present if an on-farm evaluation of the cleaning and drying routines had been conducted instead of telephone interviews, as on-farm routines and conditions may differ from those reported by the producers (Ramvad et al., 2017).

In Manuscript I, there was no statistically significant difference in age and weight at weaning between herds assigned a Yellow Card in weaners and those with very low AMU. Both groups generally weaned or received piglets at approximately 7 kg and 4 weeks of age (**Table 2** in Manuscript I). Weaning age varied considerably across the case and control groups (SD = 3.2 in the case group and SD = 4.1 in the control group), suggesting that weaning practices differ substantially. However, the weight at weaning in the herds included in Manuscript I is higher than the average weight at weaning in the general Danish pig production, which in 2023 was 6.3 kg, the lowest in almost a decade. In 2014, the average weight at weaning was 6.8 kg (SEGES, 2023). This decline is concerning, given the negative consequences associated with lower weaning weights. For herds with lower weaning weight, management during weaning should be a key area of focus, as low weaning weight and age are recognised as risk factors for PWD and reduced pig performance (Bogere et al., 2019; Madec et al., 1998; McLamb et al., 2013; Melin et al., 2004; Rhouma et al., 2017; Skirrow et al., 1997; Svensmark et al., 1989). It is possible that the herds with low AMU in this study had better housing conditions to support early weaned pigs. However, Manuscript I do not provide enough information to conclude whether the weaning age and weight may have contributed to higher AMU in herds with a Yellow Card, potentially in combination with other management, hygiene, or biosecurity challenges.

Even though 18 out of 28 producers with a low AMU reported that they considered feed and water to be important in maintaining a low AMU (**Fig. 11**), only feeder spaces were associated with AMU level (**Table 4** in Manuscript I). Herds with a Yellow Card were less likely to have sufficient room for newly weaned pigs to eat compared to herds with a low AMU (**Table 4** in Manuscript I). This difference may reflect that 54% of producers with a low AMU accepted lower productivity to maintain a low AMU (**Fig. 11**). Lower productivity allows for fewer pigs in the pens and more eating space for each pig. Even without infectious exposure, reduced feed intake or even weaning anorexia impairs intestinal villus height, and this alone would negatively affect intestinal health (Dong and Pluske, 2007; McCracken et al., 1999; Spreeuwenberg et al., 2001).

The results presented in Manuscript I support, to some extent, the hypothesis that a Yellow Card is associated with management-related herd characteristics such as biosecurity, feed regimens and antimicrobial treatment routines. However, the results in Manuscript I may also reflect additional characteristics not included in the study. Gender may influence AMU with a higher treatment incidence in female producers or employees (Backhans et al., 2016). The gender and education level of farm personnel were only briefly discussed in the interviews for Manuscript I and not included in the final analyses.

Vaccination against common endemic diseases in pig production is often considered an important strategy to reduce AMU (Postma et al., 2017). However, multiple studies reported positive associations between vaccination and PWD in sows and nursery pigs (Amezcua et al., 2002), between vaccination and AMU in farrow-to-finish herds (Postma et al., 2016), between vaccination and AMU in Danish weaners (Temtem et al., 2016), and no clear effect of vaccination and AMU (Kruse et al., 2017). In the case-control study, only 6 out of 24 producers with a Yellow Card reported that vaccination was important in reducing AMU (**Fig 10**). In some cases, preventive strategies may only be implemented once there is increased disease pressure. For those with a low AMU, 6 out of 28 producers reported that vaccination was important to maintain a low AMU (**Fig 11**).

5.2.3. Meat inspection findings in herds assigned a Yellow Card

Results from Manuscript II show that although the meat inspection lesions were of infectious origin, lesion prevalence in Danish finishers varied by less than 0.5% across the high, medium and low AMU levels. Lesion prevalence was highest for lung lesions in all three AMU levels. This is in accordance with a previous study on Danish finishers (Alban et al., 2013).

The results from the mixed methods regression models showed that herds in the high AMU level were more likely to deliver pigs with arthritis or abscesses in the extremities. In contrast, herds in the low AMU level were more likely to deliver pigs with abscesses in the trunk or tail lesions (**Table 3** in Manuscript II). In all three AMU levels, lesion prevalence for osteomyelitis, arthritis, and tail lesions was higher in outdoor production types (**Table 7**). This is in accordance with other Danish studies on meat inspection lesions in outdoor production types (Alban et al., 2015; Kongsted and Sørensen, 2017).

It is, however, important to consider that antimicrobial treatment may be initiated for several underlying reasons. It is possible that lesion prevalence at the high AMU level would have been even higher if fewer pigs had been treated. Conversely, the small difference in lesion prevalence between the low AMU level and those with medium and high AMU levels suggests that there could also be health challenges associated with maintaining a very low AMU.
In Denmark, the tendency towards a non-use of antimicrobials is more pronounced in special label productions (such as organic and free-range) than in conventional indoor production types (Nielsen et al., 2021). If the low AMU levels reflect a strategy of delaying, reducing, or avoiding treatment to ensure that AMU does not exceed the permitted limit value, then the aim of a very low AMU could potentially compromise animal health. The similarity in lesion prevalence between the medium AMU level and the high and low AMU levels indicates that this group may be quite diverse. The medium AMU level may represent herds with a well-managed disease burden but also herds withholding AMU to stay below the permitted limit value.

A large herd size was associated with a lower prevalence of pericarditis, peritonitis, osteomyelitis, arthritis, tail lesions and abscesses in the trunk and extremities (**Table 3** in Manuscript II). This study did not examine biosecurity measures, but higher biosecurity has been associated with a larger herd size in other studies (Boklund et al., 2004; Laanen et al., 2013; Raasch et al., 2018).
The fixed effects (abattoir and animals delivered from the same herd) were important in capturing the variability in the data. This may indicate that the location of the individual herds influences the variability observed in the data, as location can determine which abattoir the

pigs are delivered to. A standardised set of codes is used at all abattoirs to record findings during post-mortem inspection (Ministry of Food, 2022b).
However, this study has not examined whether there are significant differences in lesion prevalence across individual abattoirs.

There is a strong positive biological association between many of the lesions (**Fig. 1** in Manuscript II), indicating that the lesions occur in herds as disease complexes or sequelae. The positive biological association between the lesions is relatively similar for each of the three AMU levels (See **Appendix C**). This indicates that other aspects of the production system besides AMU must be studied further to improve finisher health. This is further supported by the fact that the difference between lesion prevalence in finisher pig herds before and after a Yellow Card was relatively modest (**Fig. 12**). During the 9 months following a Yellow Card, there was a slight increase in the prevalence of arthritis, abscesses in the extremities and lung lesions, and a slight reduction in pericarditis, which is not entirely consistent with Alban et al. (2013), who investigated changes in meat inspection lesions after the introduction of the Yellow Card. It is possible that Alban et al. (2013) found more significant differences as the study was conducted just after the Yellow Card was introduced, when, in general, there was a substantial reduction in AMU in Danish pig production. For herds with a Yellow Card between 2016 and 2020, the permitted limit values have already been reduced several times, and as shown in **Table 2** in Manuscript II, lesion prevalence remains relatively consistent regardless of AMU level.

Analysis of meat inspection lesions in herds with a high, medium and low AMU accepts the hypothesis that a Yellow Card is associated with poor animal health. However, the results presented in Manuscript II indicate that neither a high nor a low AMU allowed for optimal finisher pig health. This means that it is not exclusively herds with a Yellow Card that face challenges with finisher pig health. The results in Manuscript II also indicate that it is necessary to consider other external factors like production type, herd size and the location of the herd when considering the impact of AMU levels on the likelihood of delivering pigs with several of the lesions included in the study.

We cannot, however, conclude that the absence of lesions equals good health. Nor can we conclude from the data alone that the lesions found in meat inspection translate into reduced welfare in those herds included in the study. However, a recent study in Germany found that meat inspection lesions can, to some extent, indicate on-farm welfare in live animals four weeks prior to slaughter, especially lung lesions and arthritis (Witt et al., 2024). Several indicators are suggested within the context of welfare assessments (Keeling et al., 2013), including the absence of disease. Here, meat inspection data is suitable. The results in Manuscript II imply that it is important to continuously monitor meat inspection lesions, regardless of whether herds have a high, medium or low AMU.

5.2.4. Producer perceptions on reasons for a Yellow Card

In the case-control study, the producers with a Yellow Card in weaners reported that apart from relying more on vaccines, AMU was mainly reduced by being more aware of how the prescriptions were written (checking age group, product and amount), treating fewer animals or awaiting treatment for longer than they usually would (**Fig. 10**). From a welfare perspective, treating fewer animals and delaying treatment is concerning. This implies that, at least in the short term, a change in the treatment threshold and disease acceptance is needed to reduce AMU. To maintain a low AMU, most producers with a very low AMU in weaners reported that they perceived overall management routines, focus on feed and water, hygiene and biosecurity and a general focus on AMU as most important. This is in accordance with previous studies in Denmark (Fertner et al., 2015). The active choice of whether or not to initiate antimicrobial treatment described by the producers enrolled in the case-control study suggests the findings presented in Manuscripts I and II could, to some extent, be influenced by producer heterogeneity.

In Danish pig herds characterised by high productivity and low AMU in weaners, Fertner et al. (2015) identified large differences among the producers on what they perceived as most important for their success. In Swedish pig herds, Backhans et al. (2016) reported that producer characteristics, rather than biosecurity measures, had a notable influence on AMU levels. Here, herds where the producers had lower treatment thresholds had a higher AMU. Sweden has a very low AMU compared to other EU MS with a large pig production (Sjölund et al., 2016), including Denmark. However, Danish pig production is characterised by high productivity, where fluctuations in AMU in just one month can result in a Yellow Card. The risk alone of a Yellow Card may be an important criterion for Danish producers in deciding when, how or even if they should initiate antimicrobial treatment. This means that future studies on the relationship between AMU levels and herd characteristics in Danish pig production should also attempt to determine whether different treatment thresholds among producers influence herd-level AMU.

5.3. Challenges in estimating antimicrobial use

5.3.1. Estimating population at risk in pig production

In the Yellow Card scheme, sales of antimicrobials are converted using standardised measurement units and used as a proxy for AMU (Dupont and Stege, 2014). However, the calculation methods should be continuously evaluated because the ADD/100 animals/day is a statistical measure and not an expression of true antimicrobial exposure at the herd level (Jensen et al., 2004). This is especially important since it is used as a basis for legal repercussions. The results from this thesis show that there are several challenges related to the current calculation method.

The Danish ADD value is calculated using the amount of antimicrobials sold and the standard weight of the animals (See **Eq. 1**) (Dupont et al., 2016). A standard weight of 15 kg in weaners, as applied by the DVFA (Dupont & Stege, 2014), is reasonable in a 7-30 kg production setting. However, the results in Manuscript I suggest that antimicrobials are mainly used when the pigs weigh well below or well above 15 kg. Over 80% of the herds enrolled in the case-control study treated weaners within 3 weeks post-weaning. Here, the median weight was 7.5 kg (case herds) and 8 kg (control herds). More than 60% of the herds treated weaners after 4 weeks post-weaning. The median weight was 19.5 kg (case herds) and 23.8 kg (control herds). The results from Manuscript I suggest that the standard weight applied by the DVFA for use in the denominator does not accurately reflect true antimicrobial exposure at the herds.

Another challenge arises when estimating sales data in relation to production outcomes, which in Denmark includes meat production and export of weaners for rearing and breeding purposes (Danish Agriculture & Food Council, 2021). **Tables A1** and **A2** in Appendix A show that significantly more antimicrobials are used per kg of pig reared from 7 – 30 kg compared to rearing pigs from 30 kg to slaughter weight (188.52 mg/kg in weaners for export versus 16.94 mg/kg in finisher pigs for slaughter).

A large part of Danish pig production is centred on producing weaners for export. As a result, trends in AMU within Danish pig production may not be directly comparable to those of other countries that primarily import weaners for rearing until they reach slaughter weight. To some extent, these countries do not necessarily use the same amount of antimicrobials to produce weaners and manage sows. Therefore, it is essential to evaluate trends in antimicrobial sales data not only by biomass or kilograms of meat produced but also by considering both the quantity of meat produced and the number of pigs exported for rearing and breeding.

Standard weights also pose a challenge when estimating sales data in relation to overall production outcomes. The standard weights used by the DVFA in Denmark differ from those suggested by the EMA (Veterinary Medicines Directorate, 2016), with the EMA weights being consistently higher across all age groups. Table A3 in Appendix A shows that this discrepancy can make AMU in Denmark appear higher compared to other countries, purely based on the choice of standard weights. Therefore, if national antimicrobial sales data are to be compared, it is essential to specify the standard weights used and the animals at risk.

Some herds may also incorrectly use antimicrobials prescribed for finishers in weaners instead (Fertner et al., 2015). More than 80% of the herds participating in the case-control study had both weaners and finishers on the property (**Table 5**). However, the study did not examine whether using fewer antimicrobials in weaners is linked to the presence of finishers in the herd. Further research is needed to explore this relationship. Similar limitations of the standardised measures applied in VetStat have been discussed by Dupont et al. (2016). Here, it was demonstrated that different methods of calculating AMU within national bodies resulted in different AMU levels, depending on the applied ADD and population measure.

A very important limitation in estimates of national AMU is that it does not account for the efficiency of pig production. Simply assessing AMU based on the number of pen places or the number of animals produced does not consider whether pigs are reared from piglets to finishers or how many pigs are produced per pen place. The amount of antimicrobials purchased by the producer each month is indicated in the nominator. However, using the number of pen places from CHR as a proxy for the herd size in the denominator does not consider the rate whereby a herd is able to produce a 30 kg pig, thereby failing to consider how many weaners are actually "at risk" of treatment each month.

On average, herds are expected to produce 6.5 weaners per pen place (52 weeks per year/8 weeks to reach 30 kg) (Temtem et al., 2016). A median productivity of 6.4 pigs per pen place in the 66 herds with a Yellow Card in weaners is very close to what is expected in an average Danish pig production. However, the spread was large (SD = 8.2), which may be because some herds had very high productivity. It seems biologically unlikely to have a productivity of over 20 pigs per pen place, which was the case for some of the herds with a Yellow Card (**Fig 15**). This may be due to errors in either the Pig Movement Database or CHR. All records in the Pig Movement database were cross-checked against the number of pigs entering the herd, and there were no obvious errors in this part of the data. It is possible that the number of pigs in the CHR database could be significantly underestimated. Even if these herds are excluded, there is still a large heterogeneity in productivity among the herds with a Yellow Card. Some herds produced more than 6.5 pigs per pen place and may purchase more antimicrobials than those producing less than 6.5 pigs per pen place. Here, low productivity may result from reduced growth or increased mortality due to disease.

However, many of the 28 herds with a low AMU enrolled in the case-control study also had low productivity. It took, on average, 10.3 weeks to produce a pig to 30 kg. According to several producers, they actively favoured low productivity to ensure a low AMU.

The number of pigs produced per pen place may have contributed to a high AMU for herds producing more than 6.5 pigs per pen place. These herds have more batches of pigs in one standard production cycle than herds with lower productivity and thus more often have pigs "at risk" of treatment. Therefore, high productivity could be a confounding factor and should be included in future studies examining the association between herd factors and AMU levels. Additionally, further research should be conducted on how productivity can be included in the denominator when calculating AMU in Danish pig production.

5.3.2. Companion animals

The third objective of this thesis was to assess the usability of VetStat data in estimating the national sales of antimicrobials for use in Danish dogs and cats. The findings presented in Manuscript III highlight an important issue regarding how peroral antimicrobials licensed for use in dogs and cats are recorded in the VetStat database. A significantly larger amount of peroral preparations were registered under the error code 0 rather than under the animal species code 90. This was likely because these products were sold to veterinary clinics.

Secondary databases, such as the Danish VetStat database, are crucial for automated, digitised, and comprehensive data collection, and VetStat is an indispensable tool for monitoring antimicrobial sales data in Denmark. However, the results from Manuscript III emphasise the need for users of VetStat data to have a thorough understanding of the VetStat data structure, advocating for strict data checks before applying data for research purposes (Emanuelson and Egenvall, 2014). Gaining a comprehensive understanding of any secondary database and data structure is paramount to ensure that the use of secondary data is not hampered by errors or misunderstandings (Birkegård et al., 2018; Dupont et al., 2017b).

Since animal species code 90 is used to register antimicrobial sales data for dogs and cats, it may be natural for users without prior knowledge of VetStat to estimate AMU based on the sales of antimicrobials recorded under this animal species code in VetStat. However, relying solely on data from animal group code 90 would significantly underestimate the true antimicrobial exposure in dogs and cats.

The results are consistent with previous research by Dupont et al. (2017b) on the use of VetStat data in Danish pig production, which highlighted the need for careful data checking and validation.

Despite an overall aim in Denmark to achieve a prudent AMU in the veterinary sector, there are no regulatory requirements for AMU in Danish companion animals, which results in significantly lower data quality compared to the quality of VetStat data in pigs.

Although the Danish VetStat database is well-developed and data are registered with a high level of granularity, the database structure does not enable Denmark to report antimicrobial sales data for companion animals to the same extent as required for the mandatory collection on the ASU platform, neither as a weight-based nor as a dose-based estimate.

A numerator measuring sales of antimicrobials to Danish dogs and cats would be highly inaccurate since the numerator would not include antimicrobials used in small animal veterinary clinics. The denominator presents even greater challenges since there are no official statistics on the number of Danish dogs and cats. Additionally, antimicrobial sales data for cats and dogs cannot be calculated separately for each species since data are recorded under the same animal species code in the VetStat database. A more accurate estimate could potentially be obtained through larger studies incorporating data from veterinary clinics. However, these estimates would still be generalised if they follow the same methodology used for livestock, where there are relatively few standard weights for each species. Therefore, it would be necessary to have more detailed animal species codes for companion animals to distinguish between the antimicrobials sold for dogs and those for cats. The standard weights proposed by DANMAP are 20 kg for dogs and 4 kg for cats. Given the significant variation in weight among dogs, these standard weights may need to be refined by breed, with one standard weight for small breeds and another for large breeds.

The results from Manuscript III support the hypothesis that VetStat data, for now, is not a suitable proxy for estimating AMU in companion animals. Significant structural changes, including the transfer of antimicrobial use data from veterinary practitioners in small animal veterinary clinics, are required before VetStat data can accurately serve as a proxy for AMU in Danish dogs and cats.

The antimicrobial use guidelines from the DVA enable Danish veterinary practitioners in small animal clinical practice to decide on treatment plans from an evidence-based approach (Jessen et al., 2019). These guidelines do have some mitigating effects on AMU (Jessen et al., 2017; Weese, 2006). However, the prescription behaviour of small animal veterinary practitioners can deviate from national antimicrobial use guidelines (Hopman et al., 2019; Lutz et al., 2020; Van Cleven et al., 2018). Without options for robust antimicrobial monitoring in Danish dogs and cats, it is impossible to assess whether this is also the case in Denmark.

5.4. Alternative measures to reduce antimicrobial use

If the permitted limit values under the Yellow Card scheme continue to be lowered, it will become increasingly important for Danish pig producers to transition from flock medication to individual treatments and to concentrate on implementing more effective disease-prevention strategies. To ensure Danish pig producers continue to move towards prudent AMU, producers must adopt additional non-medical management strategies to improve overall herd health. This section discusses further methods that may support antimicrobial reduction efforts

5.4.1. Targeted counselling

In Denmark, pig producers that fail to reduce AMU following a Yellow Card are provided with external advice from a veterinary practitioner approved by the DVFA (Ministry of Food, 2018a). In a similar approach to addressing high AMU, an intervention study in the Netherlands involved 45 pig herds with persistently high AMU (Prinsen et al., 2024). The intervention included targeted coaching in two years with farm-specific guidance on antimicrobial reduction strategies, and feedback on antimicrobial practices. The study by Prinsen et al. (2024) found that coaching, increased farmer awareness, and improved management practices led to a reduction in AMU. The Danish VASC agreements provide a legal framework for managing AMU in pig production through regular visits from veterinary practitioners, who monitor herd health, prescribe antimicrobials, and offer advice on reducing AMU (Ministry of Food, 2021). In the study by Prinsen et al. (2024), the herds were visited 4–6 times during the study period, which is fewer visits than those made by veterinary practitioners under the VASC agreements (Ministry of Food, 2021). The success of coaching varied depending on individual farmer engagement, suggesting that the effectiveness of coaching was partly influenced by farmer motivation and the willingness to implement changes (Prinsen et al., 2024). However, the role of the veterinary practitioner in Danish herds with a Yellow Card could benefit from incorporating elements of the coaching strategies described by Prinsen et al. (2024), where coaches worked alongside producers, adjusting farm management based on ongoing observations and specific herd needs. This highlights the potential for some herd visits to focus more on counselling than antimicrobial prescription.

5.4.2. Disease eradication measures

The Porcine Reproductive and Respiratory Syndrome virus (PRRSV) weakens the immune system, making pigs more susceptible to secondary infections like *Mycoplasma hyopneumoniae* and porcine circovirus. These co-infections make pigs more susceptible to secondary bacterial infections, increase mortality rates and production losses, and negatively affect herd health and productivity (Zimmerman et al., 2008).

A compulsory approach to disease surveillance and eradication, now incorporated into the Danish PRRS surveillance may prove necessary in Danish pig production. With the permitted limit values in the Yellow Card scheme already being lowered several times (Ministry of Food, 2018b), pig producers must adopt additional strategies to reduce AMU. While vaccination and

biosecurity are essential aspects of disease control (Dhaka et al., 2023), these measures may not meet the growing regulatory demands. In this context, disease eradication measures, which have been part of managing herd-level PRRS status in Denmark since October 2023 (Ministry of Food, 2023b), could serve as effective tools to improve herd health and further reduce AMU, especially in herds assigned a Yellow Card or herds with an AMU close to the permitted limit values.
The advantage of compulsory PRRS control programs, as emphasised by Magalhães et al. (2021), lies in their ability to ensure a consistent implementation of action plans and ensure full compliance among participants, including producers and veterinary practitioners. Contrary to voluntary initiatives, mandatory schemes ensure the adoption of measures such as vaccination, biosecurity, and real-time monitoring, leading to more effective disease management (Magalhães et al., 2021).

Considering the results of Manuscript I (**Tables 3** and **4**), partial or total eradication measures in herds with a very high AMU may be necessary to target and reduce the infectious load in a broader term, not limited only to PRRS. Such voluntary control measures can be carried out in close collaboration with the veterinary practitioner as part of the VASC agreement. Successful control of PRRS may reduce disease outbreaks, reduce production losses, and enhance sustainability, ultimately ensuring improved animal health (Zimmerman et al., 2008; Magalhães et al., 2021). Specific eradication measures targeting production-related diseases may become necessary in the context of national strategies to reduce AMU, and thus also the Yellow Card scheme.

The results in the multivariable models in Manuscript II suggest that herds in Western Denmark are more likely to deliver pigs with lung lesions. This could be linked to the higher pig density in Western regions of Denmark compared to the Eastern (Danish Agriculture & Food council, 2023c). The close proximity between premises can negatively impact herd health, as PRRSV can spread between neighbouring herds through aerosol transmission and transport vehicles (Mortensen et al., 2002). Therefore, controlling the airborne transmission of PRRSV between herds is important in disease management (Magalhães et al., 2021). A reduction in PRRSV prevalence in Denmark may contribute to fewer lesions at the time of slaughter. Additionally, results of the multivariable models in Manuscript II indicate that larger herds were generally less likely to deliver pigs with most of the meat inspection lesions studied, except for lung lesions (**Table 3** in Manuscript II).

In a previous study on vaccination strategies and biosecurity measures in Danish pig production, Kruse et al. (2020) found an association between herd size and the age of farm buildings, suggesting that larger herds are typically housed in newer facilities. Although Manuscript I did not find a significant correlation between AMU levels in weaners and the age of farm buildings, it is reasonable to assume that newer buildings support better biosecurity through improved workflows, temperature control, ventilation, and separation of age groups. This association between biosecurity level and herd size is further supported by Laanen et al. (2013), who found a correlation between internal biosecurity and the age of farm buildings in Belgian pig production. However, upgrading older farm buildings to meet these standards may not always be financially viable.

5.4.3. Weaning and breeding strategy

A shift in breeding and weaning practices may be necessary to improve piglet health and thus reduce the reliance on antimicrobials. In Denmark, pig producers are permitted to wean piglets at four weeks of age (Ministry of Food, 2020). However, later weaning at five weeks can help improve piglet health, support higher growth rates, and result in more robust piglets (Sørensen et al., 2023). In Danish pig production, pre-weaning mortality is at 14.5% (SEGES, 2023). Focusing on the period from birth to weaning is, therefore, also crucial. The average litter size in Denmark is 20.1 piglets, with 18.2 live-born and 1.9 stillborn per litter. This large litter size means that sows often cannot nurse all piglets, making cross-fostering a common practice to create more uniform litter and provide extra care to smaller piglets (SEGES, 2023).

Combining later weaning with a reduction in the number of times that piglets are moved during the lactation period has been associated with lower post-weaning mortality (Sørensen et al., 2023). Implementing these changes, however, will require adjustments to the breeding programs used by Danish breeding companies.

Historically, breeding programs have focused on piglet survival by day five (LG5), without distinguishing whether survival was due to genetic improvements in piglet survivability or decreased litter size. In 2022, the breeding programme was revised to separate piglet survival as a distinct trait based either on the piglet's own genetic resilience, the sow's genetics, or litter size measured as the number of piglets born (Danish Agriculture & Food Council, 2023d).

Selecting genetics that favour fewer but stronger piglets per litter could improve animal welfare by enabling the sow to nurse all piglets in her litter (Sørensen et al., 2023). Combining improved management practices on weaning with targeted breeding goals could improve piglet health and reduce antimicrobial reliance.

5.5. Limitations

5.5.1. Limitations due to methods and study design

To examine the relationship between AMU and herd factors (Hypothesis 1), the association between AMU levels and herd characteristics such as biosecurity, hygiene, feeding regimens, and antimicrobial treatment routines in Danish pig herds with weaners was examined in a case-control study. However, in Manuscript I, the study design of the case-control study introduced limitations regarding the ability of the multivariable model to detect associations between herd factors and a Yellow Card (i.e., high AMU). The small sample size of only 52 participating herds, with 24 cases and 28 controls, meant that the control-to-case ratio was close to 1:1. A 1:1 ratio can reduce study power and increase the risk of Type II error, leading to true, but more subtle, associations being undetected (Dohoo et al., 2009). While it is not uncommon to use a 1:1 ratio, increasing the number up to 3-4 controls per case, especially in studies with a small number of cases, is suggested (Dohoo et al., 2009). The multivariable model in Manuscript I identified strong associations between two herd factors (feeding spaces and routines) and AMU level. However, the small sample size may limit the model to detecting only very large (or small) odds ratios. Future studies with larger sample sizes and a higher control-to-case ratio are needed to provide more reliable results regarding the effects of herd factors on the risk of a Yellow Card.

This is further emphasised by the frequent occurrence of expected values of 5 or lower in the contingency tables, indicating insufficient sample size and sparse data (Dohoo et al., 2009). Initial sample size calculations, assuming an OR > 2 with 80% power and a 0.05 significance level, suggested that 72 cases and 72 controls would be needed for the case-control study in Manuscript I.

However, a random sample of this size was not possible due to the number of eligible case herds (herds with a Yellow Card in weaners) during 2016–2020 being lower than 72. Consequently, the final study population included 24 case herds and 28 control herds. Despite the small sample size, power calculations for the two herd factors in the multivariable model in manuscript I (sufficient room for all pigs to eat <4 weeks post-weaning and working routines from young to old and healthy to sick) suggest high power (99% and 98.5%, respectively) for detecting the observed effect sizes, with odds ratios of 0.08 and 0.04 indicating strong association. While these estimates appear very high given the small sample size, large effect sizes generally require smaller samples to reach statistical significance, indicating that these associations are strong enough to be detected even in a very small sample.

To examine if a Yellow Card is associated with poor animal health in Danish pig herds (Hypothesis 2), the study assessed the relationship between meat inspection findings in data from Danish abattoirs and AMU levels in herds with high, medium, and low AMU in finishers. Unlike the small sample size in Manuscript I, the study design in Manuscript II resulted in an imbalance in group sizes, described by Zhou et al. (2018) as a control-to-case ratio of 10:1. The study included 84 herds with high AMU in finishers compared to 1,332 herds with medium AMU and 1,305 herds with low AMU. Due to the large dataset, especially for those with a medium and a low AMU, a significance level of $P < 0.01$ instead of $P < 0.05$ was used. A common challenge in Manuscripts I and II was that the number of herds assigned a Yellow Card from 2016 to 2020 was consistently below 98 herds (**Table 4** in this thesis). This is encouraging from the perspective of pig production and AMR, but from a study perspective, it limits the size of the eligible study population.

In both Manuscripts I and II, it would have improved the study design to focus less on whether herds received a Yellow Card. Instead, it would be better to establish a threshold that is higher than the national average but lower than the permitted limit values in the Yellow Card scheme. This threshold could serve as a proxy for high AMU in the two studies and ensure a larger eligible study population.

For several herds included in Manuscript I, a very high AMU in one or a few months led to them being assigned a Yellow Card. However, since AMU in the Yellow Card scheme is calculated as a rolling 9-month average, it is a prerequisite that the AMU for the specific age group during that period is already increasing. Despite this, herds assigned a Yellow Card may not maintain a high AMU throughout or beyond the 9 months.

This limitation also applies to Manuscript II. Enrolling herds with a consistently high AMU in finishers would likely have improved the study design by allowing for a more robust assessment of whether a high AMU affects the prevalence of meat inspection findings at the time of slaughter. Thus, defining a threshold for a high AMU instead of relying solely on assigned Yellow Cards would have improved both Manuscripts I and II.

Regardless of whether herds were selected based on assigned Yellow Cards or a defined threshold indicating high AMU, a prerequisite is that the permitted limit values in Danish pig production have been continuously reduced. As the permitted limit value decreases, the number of assigned Yellow Cards has increased, making it likely that differences in management routines, and even meat inspection lesions, exist between herds in the study populations due to the varying limit values they have been subject to. However, this is a general condition for all Danish pig herds. To avoid this uncertainty, selecting a limit value indicating high AMU that is set at a level ensuring a sufficiently large study population would be necessary.

By defining a high AMU threshold, the study populations in Manuscripts I and II could be selected based on herds that have maintained a high AMU for an extended period, ideally 12 to 24 months. This approach would ensure that high AMU is not merely the result of a disease outbreak or other temporary fluctuations but rather reflects prolonged biosecurity measures and management practices. In Manuscript I, this strategy could have increased the number of case herds, and in Manuscript II, it would have allowed for a more robust assessment of the long-term impacts of a high AMU.

The need for a larger study population is further highlighted because 11 of the 24 producers who received a Yellow Card in Manuscript I indicated that data errors significantly contributed to the AMU exceeding the permitted limit value. Before assigning a Yellow Card, the DVFA reviews VetStat data and considers information provided by the producer or veterinary practitioner during the hearing process. Thus, irrespective of individual circumstances, these herds were eligible and thus assigned a Yellow Card by the DVFA. As both Manuscript I and

II focused on herds with a Yellow Card, evaluating the justification of each Yellow Card fell outside the scope of these studies. However, focusing future studies on herds with high AMU, rather than exclusively on those issued a Yellow Card, could improve results that are more applicable to pig producers and veterinary practitioners, enhancing the relevance and acceptance of the findings.

To examine if VetStat data is a suitable proxy for estimating AMU in companion animals (Hypothesis 3), total national sales of antimicrobials in 2018 were assessed to calculate sales of antimicrobials for use in Danish dogs and cats. The study aimed to explore the potential of the VetStat database for calculating the total sales of antimicrobials for use in Danish dogs and cats. VetStat data provides comprehensive and robust data on antimicrobial sales and prescription patterns in the veterinary sector. However, a significant limitation of the methodology applied in Manuscript III was that VetStat data primarily focuses on livestock production and includes much less detail regarding companion animals.
This led to challenges in data categorisation and, consequently, reduced precision in interpreting prescribing patterns.

As VetStat data for companion animals does not include information on disease codes or target organ systems, the outcome of the descriptive study in Manuscript III could have provided more useful information if it had been compared with, for example, usage data from larger Danish small animal clinics or hospitals during the same period covered by the study. This comparison would have offered insights into how antimicrobials were used during the study. Additionally, the parenteral products with multi-species approval might have been purchased by large animal veterinarians. Had there been a more thorough examination of the veterinary clinics that have purchased these products, the clinics treating only companion animals could have been deduced from this amount to reach a more useful conclusion on the amount of parenteral antimicrobials that might have been used to treat dogs and cats.

5.5.2. Volunteer and non-response bias

Questionnaire surveys entail a risk of volunteer bias (Sedgwick, 2013). In the case-control study (Manuscript I), the response rate was 36% in the case group and 53% in the control group, leaving very high non-response rates. The non-response rate is significantly higher than in other Danish questionnaire-based studies on Danish pig production (Dupont et al., 2017a; Kruse, 2016), increasing the risk of volunteer bias. It might be due to the study focusing on the Yellow Card, which, for some producers, can be a sensitive topic. Herd owners who feel unfairly treated by the restrictions imposed by the DVFA following a Yellow Card or disagree with the decision made by the DVFA may be more prone to participate in the study. Herd owners for which the Yellow Card has had far-reaching consequences may even decline to participate, leading to significant non-response bias and distorting the results of the case group. In the control group, producers with great commitment (and success) in reducing AMU may be more prone to participate in the study.

The difference in median herd size between the participating herds and those who opted out was only minimal. However, slightly more herds that opted out were enrolled in the SPF system (70%) compared to the participating herds (58%). It is possible that the results could have been different in terms of biosecurity and hygiene measures had the participation rate been higher.

5.5.3. Response and recall bias in self-reported data

Using a questionnaire to obtain information may result in response bias (Coughlin, 1990) due to poorly phrased questions, lack of useful answers or misunderstanding. A previous study by the author of this thesis (Ramvad et al., 2017) found disagreement between answers to an online questionnaire on biosecurity in Danish pig production and answers obtained at herd visits. The differences were mainly due to misunderstanding. This can be minimised by answering the questionnaire through telephone interviews. This way, the questions can be elaborated or explained. However, it increases the risk of influence from the interviewer.

In Manuscript I, all interviews were conducted by the author of this thesis who is experienced in interviewing pig producers and doing herd and welfare control in Danish pig production as an official veterinarian in the DVFA. The consistency in interviewing style, phrasing of questions, and interpretation of responses with only one interviewer reduces variability that might occur with multiple interviewers. However, interviewer bias might occur if the interviewer unintentionally prompts or interprets responses in a way that could influence the answers. Although structured questions help to standardise the interview process, subtle variations in tone or emphasis could impact how questions are perceived. This could lead to social desirability bias, where participants provide answers they believe are expected or favourable rather than strictly accurate. Future studies might consider incorporating multiple interviewers with inter-interviewer calibration or post-interview validation measures.

In Manuscript I, recall bias is also particularly relevant due to the reliance on self-reported data from producers via the questionnaire survey. Herd owners who have received a Yellow Card may be prone to recollect past events in more detail than herd owners who have not been exposed to the same events. Although the VetStat database provides reliable data on AMU, the emphasis on management routines, biosecurity protocols, and herd health introduces an inherent risk of recall bias because producers in 2022 and 2023 were asked to recollect specific routines or practices extending back to 2016 - 2020, prompting results to possibly depend on faulty or partial recollections (Coughlin, 1990).

To minimise the risk of recall bias, many of the routines referenced in the questionnaire were based on the SPF system. Specific data was derived from information that the producer is already monitoring and documenting, including efficiency reports, visits from the herd veterinarian, and mandatory medication logs. During the interviews, producers showed a strong understanding of the standard practices and were aware of when and why they might deviate from the established guidelines. However, the producers in the case group may underreport practices they perceive as suboptimal, while producers with a low AMU may unintentionally overstate their adherence to biosecurity measures. The misreporting of key variables could influence the study results, potentially underestimating or overestimating the association between management and biosecurity measures and herd-level AMU in Manuscript I. A general problem with using questionnaires that are not done in combination with herd visits is construct validity (Motheral and Fairman, 1997), which entails the risk that the questionnaire may not necessarily capture what is intended. For the case-control study in Manuscript I, construct validity was improved by reviewing the questionnaire with veterinarians and professionals with experience in pig production, but future studies should seek to validate questionnaire responses through observational data or other objective measures where possible. However, while on-farm observations provide a more objective way to assess herd management practices and biosecurity measures, they come with certain limitations.

An important consideration is the potential for producers to be overly "prepared" for the visit or make efforts to improve biosecurity and management practices temporarily. This behaviour reflects the Hawthorne effect, where individuals alter their behaviour simply because they know they are being observed (McCambridge et al., 2014). This can lead to an overestimation of day-to-day practices and give an improper or favourable depiction of routines in the herd (McCambridge et al., 2014). A more accurate depiction of on-farm routines may be achieved by combining unannounced visits with routine on-site observations, which aligns more closely with the methods used by the DVFA when conducting welfare inspections (Danish Food and Veterinary Administration, 2024c).

6. Conclusions

This PhD thesis provides new knowledge on the practical use of VetStat data in research on Danish pig production, exploring the relationship between AMU levels, herd characteristics in Danish pig herds with weaned pigs, and animal health in Danish finisher pigs. The thesis also examines the potential use and limitations of VetStat data as a proxy for AMU in Danish dogs and cats.

Given the central role of the VetStat database in the Danish veterinary sector, particularly in the legislative framework governing antimicrobial use in pig production, continued work to improve the evidence-based use of VetStat data in research is important. The collaboration between Danish research institutions and authorities supports the idea that improved knowledge can potentially guide effective regulations, guidelines, and monitoring practices for both authorities and veterinary practitioners.

The Yellow Card scheme is important to how veterinarians and pig producers manage antimicrobial treatment plans. The number of Yellow Cards assigned in 2019 and 2020 increased significantly compared to previous years, aligned with national action plans for a continued reduction in antimicrobial use in the livestock sector, especially in pig production. This increase also coincides with the 2019 reduction in the permitted limit values for the maximum allowed antimicrobial use across all age groups, bringing them closer to the national average.

The studies presented in this thesis presented several limitations and constraints related to the study design and study population. Notably, the very small sample size reduces statistical power, which may result in true associations going undetected. Additionally, the lengthy study period and variations in inclusion criteria due to changes in the limit values of the Yellow Card scheme further introduce potential bias. These factors affect the reliability and broader applicability of the findings in Manuscripts I and II and limit the ability to draw strong, direct inferences from the results.

However, the studies indicate that as the permitted limit values in the Yellow Card scheme continue to decrease, it becomes increasingly important for Danish pig producers to transition from flock medication in weaners to single-animal treatments to ensure a prudent AMU.

This thesis identified some differences between herds with a Yellow Card in weaners and those with very low AMU. Herds with a Yellow Card were more likely to treat gastrointestinal diseases in newly weaned pigs and to use flock medication as the first choice. Herds with a Yellow Card were less likely to have sufficient room for all weaners to eat and to focus on working from the youngest to oldest animals and handling sick pigs last. A potential explanation for some of the differences found in this study could be attributed to variations in disease and treatment strategies and producer heterogeneity.

The findings further suggest that, despite differences in AMU among Danish herds with finishers, lesion prevalence at meat inspection showed only minor variation across herds with high, medium, or low AMU. Lesion prevalence was also associated with region, herd size and production type. Herds with a medium AMU were associated with a lower risk of delivering pigs with peritonitis, abscesses in the trunk, abscesses in the extremities, tail lesions and arthritis. Large herds were associated with a lower risk of delivering pigs with pericarditis, peritonitis, abscesses in the trunk, abscesses in the extremities, tail lesions, osteomyelitis and arthritis. Outdoor production types were associated with a higher prevalence of delivering pigs with osteomyelitis, arthritis and tail lesions.

To adjust current antimicrobial treatment strategies, Danish pig production may need to focus even more on disease-preventive measures. Effective breeding strategies and weaning procedures should aim to ensure robust pigs at the time of weaning, while a general approach to disease management should aim toward a reduction in overall disease pressure through disease eradication initiatives and enhanced advisory support. Achieving these goals will require coordinated efforts from multiple stakeholders, including veterinary authorities, to ensure a unified and proactive approach.

Lastly, this thesis found that VetStat data was not applicable as a proxy for AMU in dogs and cats. With the current structure of the database, a calculated AMU from VetStat data will only be an approximation. The true antimicrobial exposure may be significantly higher than what is currently calculated from the available data because the majority of parenteral preparations used by veterinary practitioners in small animal clinics are not possible to quantify. Estimating national AMU in dogs and cats is not possible until antimicrobial treatment data from veterinary practitioners in small or mixed practices is transferred to VetStat via the billing system. The VetStat database only allows monitoring of overall changes, but it is not feasible

7. Implications and perspectives

The results in this PhD thesis emphasise that even though companion animals will be included in international antimicrobial monitoring systems within the next few years, the use of databases primarily developed to monitor AMU in livestock presents several structural challenges. This knowledge is relevant for other EU MS that are in the process of establishing national antimicrobial monitoring systems.

For now, the structural challenges in VetStat give rise to a paradox. While there are comprehensive legislative measures in place to govern antimicrobial use in Danish pig production, there are no such regulations for companion animals like dogs and cats, making the process of identifying what is used, how much is used and how antimicrobials are applied very difficult.

The opposite is true for Danish pig production. Here, the work of the veterinary practitioner and the producer is governed by numerous regulations. The findings of this thesis suggest that although there are still differences among Danish pig producers, future antimicrobial reduction measures could benefit from incorporating measures that are already in place in Danish pig production, especially the herd veterinarian.

Postma et al. (2017) described that "*Guided interventions as a team effort of farmer and herd veterinarian/other advisors have shown to be a promising method in the reduction of AMU in pig production*". In Denmark, the veterinary practitioner already visits the herd regularly due to the VASC agreement. As part of working towards a prudent AMU, greater use of this agreement could benefit by, for example, considering action plans for antimicrobial use and animal health, without it having to be because of a Yellow Card.

If the limit values in the Yellow Card scheme are to be lowered again as part of a prudent AMU, additional studies should first and foremost be carried out to meet the uncertainties presented in this thesis:

1) How much will the AMU in companion animals be altered if the antimicrobial use data in small animal veterinary clinics is transferred to VetStat in the same way as in livestock production?
2) How much is the AMU in Danish pig herds dependent on producer-perceived treatment thresholds?
3) How will AMU in Danish pig herds change by including the number of pigs produced per pen place in the calculation?

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9. Manuscripts

Manuscript I

Anne-Sofie Glavind, Amanda Brinch Kruse and Helle Stege (2024).
A case-control study on herd factors associated with a Yellow Card in Danish weaners
Ready for submission

Manuscript II

Anne-Sofie Glavind, Amanda Brinch Kruse, Helle Stege and Lis Alban (2024).
Association between antimicrobial use levels and meat inspection lesions in Danish finishers
Under revision at Preventive Veterinary Medicine

Manuscript III

Anne-Sofie Glavind, Amanda Brinch Kruse, Liza Rosenbaum Nielsen and Helle Stege (2022). Monitoring antimicrobial usage in companion animals: exploring the use of the Danish VetStat database. Acta. Vet. Scand. 64, 11-27
https://doi.org/10.1186/s13028-022-00647-w

9.1. Manuscript I

Anne-Sofie Glavind, Amanda Brinch Kruse and Helle Stege (2024).
A case-control study on herd factors associated with a Yellow Card in Danish weaners
Ready for submission

1

A case-control study on herd factors associated with a Yellow Card in Danish weaners

Abstract

Increased awareness of prudent antimicrobial use (AMU) in livestock has led to implementation of national antimicrobial surveillance systems. In Denmark, the Veterinary Statistics Database (VetStat) covers sales of all veterinary medicinal products. VetStat data forms the basis for the Yellow Card scheme, a penalty imposed by the Danish Veterinary and Food Administration to reduce AMU in Danish pig production. Denmark has an intensive pig farming system. The challenges faced by the individual herd may vary greatly and depending on factors such as disease burden, work routines, and management. This case-control study aimed to improve the knowledge of the association between herd characteristics and AMU level in Danish pig herds. The objective was to examine overall disease challenges and antimicrobial treatment strategies in herds with a high AMU in weaners and to examine the differences in herd-level characteristics among herds with a high AMU compared to those with a low AMU. Data was collected through telephone interviews with 24 pig producers assigned a Yellow Card in weaners between 2016 and 2020 and 28 herds with a low AMU in weaners.

Herds assigned a Yellow Card in weaners were more likely to treat gastrointestinal diseases (OR = 4.8) and to use flock medication (peroral preparations) (OR = 10.5). Herds with a Yellow Card were less likely (OR = 0.04) to have strict routines working from youngest to oldest, tend to sick pigs last and have sufficient room for all newly weaned piglets to eat (OR = 0.08).

This knowledge can benefit future antimicrobial reduction schemes. Instead of solely relying on legislative restrictions, a targeted counselling approach could be adopted to offer advice to producers who experience increased AMU.

Keywords: Antimicrobial use, Pig production, VetStat, Yellow Card

Introduction

According to the World Health Organization (WHO), antimicrobial resistance (AMR) is one of the most significant threats to public health in recent times (World Health Organization, 2024). Antimicrobial use (AMU) patterns in humans and animals play a crucial role, with use and misuse as a driving force (Velazquez-Meza et al., 2022). The rapidly evolving AMR could lead to simple infections becoming difficult to treat and jeopardise the advancement of modern medicine. It contributes to greater inequality, particularly in low- and middle-income countries and affects animal welfare, as certain products may need to be reserved exclusively for human use (World Health Organization, 2024). This critical balance between using antimicrobials (AMs) to safeguard animal health and welfare and preventing AMR is highlighted in Regulation (EU) 2016/429 on transmissible animal diseases, often referred to as the Animal Health Law (AHL). The AHL emphasises the importance of effective disease control and stresses the need for surveillance and monitoring programs to track AMR to protect animal health and welfare (European Parliament and Council, 2016).

Detailed and valid AMU data is essential for monitoring and regulating AMU. Since 2011, the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) has been responsible for collecting and publishing data on AMU from mainly food-producing animals in the European Union/European Economic Area (EU/EEA) (European Medicines Agency, 2022). Over recent decades, many European Union/European Economic Area Member States (EU/EEA MS) have developed various systems to monitor AMU, broadly classified into three types (Sanders et al., 2020). The first type, partial coverage systems, collects data from a representative subset of the animal populations to estimate overall trends in AMU. The second type, partial sector coverage systems, monitors AMU within specific livestock sectors or farm systems. The third type, full sector coverage systems, encompasses all animals and farms across the entire veterinary sector.

The Danish Veterinary Statistics (VetStat) database, a full sector coverage system, collects antimicrobial sales data across the whole veterinary sector. Furthermore, Denmark is one of the few countries with farm-level benchmarking in place (AACTING, 2021; Sanders et al., 2020). The Danish VetStat database was established in 2000. The database is managed by the Danish Food and Veterinary Administration (DVFA) and covers sales of all veterinary medicinal products (Stege et al., 2003). In Denmark, antimicrobials (AMs) are prescription-only, and pharmacies are responsible for dispensing AMs to veterinary practitioners, veterinary clinics, livestock producers, and pet owners (Ministry of Food, 2023a; Stege et al., 2003). All transactions are recorded in VetStat with additional information on the dispensing pharmacy, the prescribing veterinarian, the receiving herd, animal species, animal age group, amount and disease code (Stege et al., 2003). VetStat is a relational database, and the database environment includes product-specific tables that link each purchase with information on active ingredients, ATC codes, ATCvet codes, strength, package size and route of administration (Dupont and Stege, 2014b). Additional information on standardised weight for each age group and standardised doses for each product allows for the calculation of AMU in Animal Daily Dosages (ADD).

Information on the population size at the herd level is transferred to VetStat from the Central Husbandry Register (CHR). The CHR is an official database owned and managed by DVFA, listing the geographical location, production type, animal species (including age groups and number of animals), ownership and delivery agreements for each Danish pig herd (Ministry of Food, 2022). The number of pen places recorded in CHR is used as a proxy for the number of animals. This number is applied as the denominator to calculate the ADD per 100 animals per day (ADD/100 animals/day), which translates into the percentage of animals treated per day (Dupont et al., 2016). This measure forms the basis for the Yellow Card scheme, a legislative penalty introduced by the DVFA in 2010. The purpose of the penalty was to reduce AMU in Danish pig production (Ministry of Food, 2018a).

In the Yellow Card scheme, the ADD/100 animals/day is presented as a 9-month rolling average for the individual herd and age group, the national average and fixed limit values defining the maximum allowed AMU for each age group. The limit values have been reduced several times, and since 2019, the maximum allowed AMU in weaners is 17.2 ADD/100 animals/day (Ministry of Food, 2018b). Herds exceeding the permitted limit values are assigned a Yellow

Card (Ministry of Food, 2018a; Ministry of Food, 2018b). The producer is required to reduce herd level AMU within nine months. Official veterinarians will make unannounced visits to the herd, and options to refill prescriptions of orally administered AMs are limited (Ministry of Food, 2018a).

The CHR and VetStat databases are publicly accessible. The same applies to the Danish Specific Pathogen Free system (SPF). The SPF system is part of The Danish Agriculture and Food Council, a sector organisation representing the Danish agricultural and food industries. It was designed as a production and health system in 1971 (SPF Health, 2021). The SPF system monitors the health status of individual herds through regular blood tests aimed at specific SPF diseases, including enzootic pneumonia, porcine pleuropneumonia, swine dysentery, atrophic rhinitis, lice, scabies, and PRRS. The SPF system currently covers 78% of pigs for rearing and 100% of pigs for breeding purposes. The SPF health status is publicly declared to help producers trade animals of the same or higher health status (SPF Health, 2021). In addition to regular health surveillance, herds enrolled in the SPF system must strictly comply with SPF regulations. These regulations encompass biosecurity measures at the farm level, covering personnel entry and exit, feed, bedding, and equipment requirements, and correctly entering materials and live animals into the farm premises. Additionally, the SPF system has set rules for loading and transporting pigs between herds (SPF Health, 2021).

In addition to the national monitoring of AMU at the herd level, all medicinal products used on Danish pig farms are prescribed by veterinary practitioners. Veterinary Advisory Service Contracts (VASCs) signed between the veterinary practitioner and pig producer obligate the veterinary practitioner to conduct regular herd visits (Ministry of Food, 2021). During these visits, the veterinary practitioner documents recurring disease problems, defined as herd health diagnoses, and performs diagnostic testing allowing the veterinarian to prescribe AMs for up to 65 days for use in weaners. These advisory contracts are a prerequisite for the producer to be allowed to initiate antimicrobial treatment independently without consulting a veterinarian (Ministry of Food, 2021). The continuous health monitoring included in the VASC agreements is important in Danish pig production to continue to reduce herd level AMU. In Denmark, AMU in weaners is equal to that for sows, gilts, and finishers combined (DANMAP, 2023). In weaners, a large proportion of AMs are prescribed for peroral treatment of gastrointestinal disorders (Moura et al., 2023).

Denmark has an intensive pig farming system and Danish law permits weaning of piglets after 28 days of age, although as early as 21 days if the piglets are moved to specialised housing facilities (Ministry of Food, 2020). Consequently, piglets are exposed at an early age to environmental changes. Post-weaning diarrhoea (PWD) challenges Danish pig production as it reduces animal welfare and causes financial losses. PWD occurs in the first two weeks after weaning and is often associated with low daily weight gain, dehydration and growth retardation (Fairbrother et al., 2005; Madec et al., 1998).

Weaning is a considerable stressor that negatively affects piglet intestinal health (Campbell et al., 2013). This means that management routines in the weaner unit may be crucial for weaner pig health and productivity. In the early stages, this involves factors such as the age and weight

at weaning, the number of feeder spaces, and feed intake (Amezcua et al., 2002; Le Dividich and Sève, 2000; Main et al., 2004). It also includes housing-related factors like maintaining appropriate temperature, ensuring biosecurity, and maintaining pen-level hygiene (Gleeson et al., 2015; Madec et al., 1998).
In the Yellow Card scheme, Danish pig herds are subject to the same restrictions regarding permitted AMU levels. However, the challenges faced by each individual herd may vary greatly depending on disease burden, work routines, and overall animal health. To better understand the obstacles faced by Danish pig producers, it is necessary to consider the production-related challenges. By doing so, producers and consulting veterinarians can provide targeted planning aimed at reducing AMU while also maintaining weaner health, welfare and productivity.

The aim of this study was to improve the knowledge of the association between specific herd factors and the AMU level in Danish pig herds. Using a Yellow Card as a proxy for a high AMU, the first objective was to explore overall disease challenges and antimicrobial treatment strategies in herds with a high AMU in weaners compared to those with a low AMU in weaners. The second objective was to examine the differences in herd-level characteristics and routines related to management, housing, biosecurity, weaning, and animal health in herds with a high AMU in weaners compared to those with a low AMU in weaners.

Materials and Methods

Herd selection

The DVFA provided data on monthly AMU for Danish pig herds and records of herds assigned a Yellow Card between 2016 to 2020. The AMU data comprised ADD/100 animals/day, CHR numbers and age groups. The DVFA calculates the ADD/100 animals/day from the sales of antimicrobials recorded in the VetStat database, the standard dose per kg live weight, the standard weight for the given age group, the number of pen places registered in CHR and the number of days in a month (**Eq 1 and 2**) (Dupont et al., 2014a).

Eligible case herds comprised all herds assigned a Yellow Card in weaners between 2016 and 2020. Control herds were selected from the AMU data provided by the DVFA.

First, the average monthly AMU between 2016 and 2020 was calculated for all Danish pig herds and included as a variable in the dataset. The national average in weaners declined between 2016 and 2020 from 10.7 ADD/100 animals/day in 2016 to 8.8 ADD/100 animals/day in 2020. This means that the criterion for the control herds differed each month between 2016 and 2020.

During the same time period, the permitted limit values in the Yellow Card scheme were reduced several times, from 22.9 ADD/100 animals/day in 2016 to 17.2 ADD/100 animals/day in 2020. Next, herds with an AMU below the national average in weaners for at least one year were selected to ensure that herds in the control group maintained a low AMU throughout the seasonal changes. All herds assigned a Yellow Card were removed.

$$ADD = \frac{quantity\ of\ antimicrobials\ purchased}{dosage\ per\ kg\ live\ weight\ *\ standard\ weight}$$

Equation 1. Calculation of Animal Defined Daily Doses (ADDs) using antimicrobial sales data, standard dosages and species-specific standard weights.

$$ADD/100animals/day = \frac{Purchased\ ADDs\ in\ a\ defined\ time\ period}{number\ of\ pigs\ registered\ in\ the\ CHR\ *\ days\ in\ the\ time\ period}$$

Equation 2. Calculation of Animal Daily Dosages (ADDs) per 100 animals per day using the amount of ADDs purchased in a defined time period, the number of animals in a specific age group and the days in the time period.

Survey data

Information on the health status of herds enrolled in the Danish Specific Pathogen Free (SPF) system was obtained from SPF Health (SPF Health, 2021). Data on production type, geographical location, age groups and number of weaners at the herd level was obtained from the CHR database and VetStat. Information on herd-level characteristics was obtained through a questionnaire survey designed according to guidelines defined by Stone (1993). The scope of the questionnaire was defined according to previous studies on risk factors for weaning diarrhoea and AMU in weaners and finishers (Amezcua et al., 2002; Laine et al., 2008), biosecurity measures specific to the Danish SPF system (SPF Health, 2021), and information routinely monitored by the producers as part of the productivity control (SEGES, 2020) or documented by the consulting veterinary practitioner in their health advisory services (Ministry of Food, 2021). The questionnaire survey was designed and conducted in Danish. The questionnaire comprised 41 closed-ended questions covering disease status in weaned pigs, antimicrobial management protocols, weaning procedures, water and feed regiments, housing conditions, cleaning routines and internal biosecurity. A translated version can be obtained from the first author upon request.

Herd enrolment

Contact information was obtained from public registers, and the producers were contacted by phone and asked to participate in the study. All producers agreeing to participate were enrolled in the study. Producers in the case group were asked to answer the questionnaire based on the specific routines in place throughout the nine months before they were assigned a Yellow Card in weaners. Producers in the control group were asked to answer the questionnaire based on the routines throughout the year, during which the producers maintained a low AMU in weaners. If the producer was not regularly present in the herd, the interview was conducted with the farm manager. Data were collected through telephone interviews with the producer or manager during the spring and winter of 2022/2023.

Statistical analysis

The outcome variable was dichotomous, relating to the AMU level in weaned pigs being either high (i.e. a Yellow Card) or low (i.e. lower than the national average). The predictor variables included in this study covered continuous and categorical variables and are listed in **Tables 1** and **2**. The overall distribution of case and control herds was described using cross-tabulation. Following data collection, all closed-ended questions were dichotomised.

A t-test was performed to compare the difference in mean between the continuous predictor variables in the case and control group. A chi-square test examined the associations between the outcome and the categorical predictor variables. Fisher´s exact was used for variables with cell frequency lower than 5. The analysis did not include variables with complete agreement between cases and controls. Predictor variables with a significance level of $P < 0.1$ were considered for further analysis. After the preliminary variable selection, a multivariable logistic regression model was built to test the associations between the predictor variables and the possibility of being either a case or control herd.

Only variables with a significance level of $P < 0.05$ were retained using a stepwise backward elimination process. The final model was tested for multicollinearity, and the effect of being an SPF herd was tested on the significant covariates.

Variables related to the most commonly treated herd health diagnoses and to AMU (use of flock medication or single animal treatment only) were used to describe overall group characteristics. Due to multicollinearity, these were not included in the multivariable logistic regression model. Statistical analysis and data management were performed in R (version 4.0.3 of 2020 – The R Foundation for Statistical Computing).

Results

Descriptive results

Participating herds

119 herds fulfilled the enrolment criteria. In the case group, 17% did not have valid contact information or had undergone a change of ownership, 12% did not answer, and 29% declined participation. In the control group, 31% did not answer or have valid contact information, and 25% declined participation. Consequently, 28 control herds and 24 case herds were enrolled in the study.

The mean monthly AMU level in the case group was 17.9 ADD/100 animals/day, whereas the mean AMU in the control group were 2.3 ADD/100 animals/day. The herd size ranged from 400 to 8676 weaners in the case group (median = 1675), and from 700 to 9500 weaners (median = 2000) in the control group. There was no significant difference in the median herd size between the case and control herds (P-value = 0.84). Fifty-eight per cent of the participating herds (54% of case herds and 61% of controls) were enrolled in the SPF system. For non-participants, 70% (74% of case herds and 67% of controls) were SPF herds.

Herd characteristics and univariable associations

Results from the univariable analysis of predictor variables are presented in **Table 1**. Continuous variables are presented in **Table 2**. Explanatory variables regarding herd health, antimicrobial treatment, and the associations with AMU level (i.e. being a case or control herd) are presented in **Table 3**.

There was no statistically significant association (P >0.05) between the outcome and the categorical predictor variables in the following categories; origin of the pigs, commonly treating herd health diagnoses, treating respiratory diseases, estimating treatment weight visually and having written instructions available, using homegrown feed sources, use of organic acids, pelleted and ad libitum feed regiments, graduate shift between feed mixes, sectioned all in/all out, cleaning routines including wash, dry and disinfection, change activity material, mixing of age groups and use of equipment solely for weaners (**Table 1**). All producers in the case and control groups reported daily washing of footwear upon completion of the workday, and all entered the herd through a designated entry room (**Table 1**). None of the control herds reported the introduction of new diseases during the study period.

Table 1: Results of the univariable analysis of qualitative explanatory variables tested for association[a] with AMU level on 52 Danish pig herds from 2016 to 2020.

Variables	Categories	Distribution (%)		P-value[b]
		Cases (n = 24)	Controls (n = 28)	
Origin of the pigs				
Newly weaned piglets are transported to the property	*Yes* *No*	15(62) 9(38)	16(57) 12(43)	0.91
Herd health and antimicrobial treatment				
Herd diagnoses are commonly treated in newly weaned pigs (< 4 weeks postweaning)	*Yes* *No*	21(88) 3(12)	23(82) 5(18)	0.71
Gastrointestinal diseases are commonly treated in newly weaned pigs (< 4 weeks postweaning)	*Yes* *No*	19(79) 5 (21)	12(43) 16(57)	0.02
Respiratory diseases are commonly treated in newly weaned pigs (< 4 weeks postweaning)	*Yes* *No*	5(21) 19(79)	2(07) 26(93)	0.23
Neurological diseases are commonly treated in newly weaned pigs (< 4 weeks postweaning)	*Yes* *No*	1(4) 23(96)	8(29) 20(71)	0.02

Table 1 (continued)

Herd diagnoses are commonly treated in older pigs (4-8 weeks postweaning)	*Yes* *No*	18(75) 6(25)	18(64) 10(36)	0.60
Disease outbreak of newly introduced disease (no herd diagnosis)	*Yes* *No*	6(25) 18(75)	0(0) 28(100)	NE[c]
Flock medication is commonly used to treat herd diagnoses	*Yes* *No*	23(96) 1(4)	19(68) 9(32)	0.01
Only using single-animal treatment	*Yes* *No*	1(4) 23(96)	9 (32) 19(68)	0.01
Treatment weight is estimated by weighing	*Yes* *No*	5(21) 19(79)	11(39) 17(61)	0.26
Written instructions on AMU are available in the medicine room	Yes No	19(79) 5(21)	19(68) 9(32)	0.53
Feed				
Use of mainly homegrown feed	*Yes* *No*	13(54) 11(46)	21(75) 7(25)	0.20
Use of dry feed (pelleted) 1-3 weeks postweaning	*Yes* *No*	22(92) 2(8)	27(96) 1(4)	0.59
Use of ad libitum feeding 1-3 weeks postweaning	*Yes* *No*	23(96) 1(4)	25(89) 3(11)	0.61
Feed mixes are phased in gradually	*Yes* *No*	21(88) 3(12)	23(82) 5(18)	0.71
There is sufficient room for all pigs to eat simultaneously < 4 weeks postweaning	Yes No	6(25) 18(75)	19(68) 9(32)	0.005
Use of acidification in feed/water	*Yes* *No*	19(79) 5(21)	16(57) 12(43)	0.16
Hygiene and biosecurity				
Use of sectioned all in/all out	*Yes* *No/sometimes*	18 (75) 6 (25)	26(93) 2(7)	0.12
Cleaning routines between batches include washing, disinfection and drying	*Yes* *No*	17(71) 7(29)	22(79) 6(21)	0.75
Use of heat sources to dry the pens between batches	*Yes* *No*	18 (75) 6 (25)	27(96) 1 (4)	0.04

Table 1 (continued)

Cleaning or change of activity material (including use of straw) between batches	*Yes* *No*	14(58) 10(42)	14(50) 14(50)	0.75
Cleaning of hallways after moving pigs	*Yes* *No*	20 (83) 4 (17)	27 (96) 1 (4)	0.2
Working routines being from young to old and healthy to sick	*Yes* *No*	15(62) 9(38)	27(96) 1(4)	0.003
Mixing of age groups	*Yes* *No*	2(8) 22(92)	1(4) 27(96)	0.89
Equipment used solely for weaners	*Yes* *No*	14 (58) 10 (42)	19 (70) 8 (30)	0.55
Wash of footwear upon completion of the workday	*Yes* *No*	20 (83) 4 (17)	28 (100) 0 (0)	NE[c]
Herd always entered through a designated entry room	*Yes* *No*	24 (100) 0 (0)	28 (100) 0 (0)	NE[c]

[a]Variables with a P-value < 0.1 were re-examined in a multivariable logistic regression model. [b] P-value for association (Chi-square or Fisher's exact test). [c]Not estimated. Variables contain zero-value levels

There were no statistically significant differences (P > 0.05) between case and control herds in the following quantitative variables: number of farm personnel, age of buildings, empty time between batches, weaning age and weight and age and weight at treatment. However, the variation of individual variables differed, especially regarding the age of buildings and the weight of newly weaned pigs and older weaners during treatment (**Table 2**).

Table 2: Distribution of quantitative explanatory variables from 52 Danish pig herds between 2016 and 2020.

Variable	Mean	SD[a]	P-value[b]
Number of farm personnel			0.16
Cases	1.9	1.5	
Controls	1.4	0.7	
Farm personnel experience (years)			0.44
Cases	16.6	8.3	
Controls	18.9	12.1	
Age of buildings			0.55
Cases	20	13.1	
Controls	22	8.7	
Empty time between batches (days)			0.13
Cases	3.6	2.3	
Controls	4.7	2.8	
Age at weaning (weeks)			0.75
Cases	29.6	3.2	
Controls	30	4.1	

Table 2 (continued)

Weight at weaning (kilo)			0.98
Cases	7.2	1.2	
Control	7.2	1.1	
Age at treatment < 4 weeks postweaning (weeks)			0.30
Cases	1.2	0.4	
Controls	1.3	0.5	
Weight at treatment < 4 weeks postweaning (kilo)			0.26
Cases	8.8	2.9	
Controls	7.9	1.1	
Age at treatment > 4 weeks postweaning (weeks)			0.93
Cases	4.8	1.8	
Controls	4.9	1	
Weight at treatment > 4 weeks postweaning (kilo)			0.15
Cases	17.9	3.8	
Controls	21.2	5.1	

[a]Standard deviation. [b] P-value for comparison (two sample t-test)

In newly weaned pigs (< 4 weeks postweaning), 88% of cases and 82% of control herds reported regularly treating disease with a herd health diagnosis (**Table 3**). For case herds, the odds of treating gastrointestinal diseases were higher (OR = 4.8) than control herds. The predominant approach to treating herd diagnoses differed between case and control herds. Here, case herds were more likely (OR = 10.5) to use flock medication as the first choice (**Table 3**).

Table 3. Univariable associations on AMU level, antimicrobial treatment and herd diagnoses in newly weaned piglets in 52 Danish pig herds.

Characteristics	Prevalence		Odds ratio	P-value[a]
	Case	Control		
Using flock medication	96	68	10.5	0.001
Gastrointestinal diseases are the most commonly treated herd diagnosis in weaned pigs < 4 weeks postweaning	79	43	4.8	0.02
Use of single animal treatment only	4	32	0.1	0.01
Neurological diseases are the most commonly treated herd diagnosis in weaned pigs < 4 weeks postweaning	4	29	0.1	0.03

[a]P-value for association (Chi-square test)

Analytical results

Multivariable associations

The odds of having sufficient room for all newly weaned pigs to eat simultaneously were significantly lower (OR = 0.08) in the cases compared to the controls. Similarly, the odds of having strict routines working from youngest to oldest and tending to sick pigs last were lower (OR = 0.04) for case herds (**Table 4**). Being an SPF herd was not statistically significantly associated with the variables or the outcome. There was no multicollinearity in the full model.

Table 4: Results of the multivariable logistic regression model of risk factors for the association with a high AMU in 52 Danish pig herds between 2016 and 2020

Variable	Odds ratio	95% CI[a]	P-value
Sufficient room for all pigs to eat simultaneously < 4 weeks postweaning	0.08	0.02 – 0.31	0.004
Working routines being from young to old and healthy to sick	0.04	0.002 – 0.44	0.008

[a] 95% confidence interval of the odds ratio

Discussion

The first objective of this study was to examine overall disease challenges and antimicrobial treatment strategies in Danish herds assigned a Yellow Card in weaners compared to those with a very low AMU. The results of the univariable analysis showed that herds assigned a Yellow Card reported treating gastrointestinal diseases in newly weaned pigs (< 4 weeks post-weaning) more frequently than those with a very low AMU in weaners, who reported more treatments for neurological diseases. These differences also manifested in antimicrobial treatment strategies. Herds with a Yellow Card often used flock medication as a first choice compared to those with low AMU, where single animal treatment was predominantly used.

In Denmark, most antimicrobials prescribed for weaners are for peroral treatment of gastrointestinal disorders (Jensen et al., 2011; Moura et al., 2023). Gastrointestinal disorders are generally present as post-weaning diarrhoea associated with *Escherichia coli* and outbreaks of diarrhoea caused by *Lawsonia intracellularis* and *Brachyspira pilosicoli* in weaners older than four weeks post-weaning (Aarestrup et al., 2008).

Administering antimicrobials through feed or water allows all pigs in a pen or a batch to be treated simultaneously (Larsen et al., 2016). This method acts directly on the gut and is particularly useful in large outbreaks where many pigs need treatment, reserving single animal treatment for pigs unable to ingest enough feed and water on their own (Moura et al., 2023). Reducing the use of flock medication in favour of single-animal treatment reduces selection for AMR (European Medicines Agency, 2019), allowing the producer to treat animals individually and ensure that only pigs with clinical signs of disease are treated (Larsen et al., 2016). The use of single animal treatment was reported by 32 % of producers with a low AMU compared to only 4 % with a Yellow Card, suggesting that single animal treatment could be important to incorporate in antimicrobial reduction measures. However, in large disease outbreaks or cases of recurrent gastrointestinal disease, it may not be feasible from a welfare perspective to administer numerous treatments. This could lead to additional stress for the individual animal, as well as being labour-intensive (Moura et al., 2023).

The second objective of the study was to examine differences related to management, housing, biosecurity, weaning routines and animal health in herds with a Yellow Card in weaners compared to those with a very low AMU.

The results of the multivariable model suggested differences in working routines and the number of feeding places for newly weaned pigs between the case and control herds. Here, control herds were 12.5 times more likely to have sufficient room for all weaners to eat at the same time, and 25 times more likely to have strict routines working from youngest to oldest, tending to sick pigs last. However, Amezcua et al. (2002) reported a higher number of feeder spaces in herds with PWD. It is possible that the conflicting findings could be attributed to either inadequate sample size in the present study or unaccounted confounding variables. Disease susceptibility or immunity differences among the pigs may also contribute. For example, Amezcua et al. (2002) reported a median weaning age of 19.1-19.8 days, while in this study, the median weaning age was 29 days.

Case herds were less likely to follow strict routines, such as working from youngest to oldest and handling sick pigs last. This could result from case herds experiencing periods of high disease pressure. Here, attending to sick animals may take precedence over strict work routines. Either because the routines become too time-consuming during periods with high disease pressure or because the routines have not had the desired effect. In herds experiencing prolonged disease pressure, strict internal biosecurity measures may seem less important. It could also be that the herds are dealing with inexperienced or insufficient numbers of farm personnel. While this study found no statistically significant difference between the case and control groups (**Table 2**), there was a large variance in years of experience, indicating that several herds could have farm personnel with minimal experience.

Humans are important sources of pathogen introduction (Alarcón et al., 2021), and farm personnel can serve as mechanical vectors, thus transferring infectious agents between sections and from infected to susceptible pigs (Alarcón et al., 2021; Amass et al., 2003). Suboptimal work routines on case herds could lead to adverse disease transmission, and herds with a Yellow Card may benefit from reviewing work routines to identify possible improvements.

Previous studies have linked increased biosecurity to reduced AMU (Raasch et al., 2018), and pen-level hygiene to postweaning pig performance (Madec et al., 1998). Standard methods to reduce the infectious load at the pen level are cleaning, disinfection, and drying (Mannion et al., 2007). The present study did not identify a significant association between the outcome and hygiene factors commonly associated with diseases in weaned pigs, which is consistent with previous studies by Laine et al. (2008) and Amezcua et al. (2002).

The Danish SPF system has facilitated a high level of hygiene and biosecurity in Danish pig production. In addition to the comprehensive regulations regarding biosecurity, the regular health monitoring within the SPF system, combined with the monthly visits from a consulting veterinarian as required by the VASC agreements (Ministry of Food, 2021), allows producers to be aware of each disease affecting the herd and adjust management practices accordingly. This might be why there is no clear association between hygiene levels in the Danish herds in the present study. Even though only 58% of the participating herds were enrolled in the SPF system, Danish pig herds, in general, tend to comply with the SPF standards (Kruse et al., 2020).

The findings align with previous studies on herds with country-specific high AMU in Belgium and the Netherlands (Caekebeke et al., 2020). The lower levels of internal biosecurity compared to external biosecurity highlight the need for improved internal biosecurity measures at the herd level, with water quality identified as a particular area of concern (Caekebeke et al., 2020). In the present study, 19 out of 24 herds with a Yellow Card reported adding organic acid to the drinking water, although it was not statistically significant in the univariable model (**Table 1**). The results of the multivariable model showing herds with a Yellow Card being less likely to follow strict routines on working from youngest to oldest and tending to sick pigs last may be due to increased disease pressure, making it difficult to uphold strict biosecurity protocols.
The potential for improving internal biosecurity as part of antimicrobial reduction strategies at the herd level has also been discussed by Raasch et al. (2018) for German farrow-to-finish herds, where particular emphasis is placed on cleaning and disinfection protocols. They found that a higher treatment incidence was positively correlated with internal biosecurity, possibly due to increased attention to biosecurity during periods of high disease pressure. The present study may exhibit a similar pattern, where herds assigned a Yellow Card potentially adopted additional biosecurity measures to reduce AMU. The critical role of biosecurity in reducing antimicrobial use is further emphasised by Dhaka et al. (2023), who recommend enhancing the role of stakeholders and veterinary practitioners in overseeing biosecurity protocols at the herd level. In Denmark, the VASC agreements have already established close collaboration between veterinary practitioners and pig producers in Danish pig production. These agreements mandate the consulting veterinarian to assess zoonotic biosecurity measures, evaluate existing health issues, and provide recommendations for their prevention during each visit (Ministry of Food, 2021).

There were no differences between case and control herds regarding weaning age and weight. Case and control herds generally weaned (or received) the piglets at 7 kg and 4 weeks of age (**Table 2**). Low weaning weight and age have been considered a risk factor for PWD and a significant factor in decreased pig performance (Bogere et al., 2019; Madec et al., 1998; McLamb et al., 2013a; Melin et al., 2004; Rhouma et al., 2017; Skirrow et al., 1997; Svensmark et al., 1989), especially early weaning stress can lead to early and more severe outbreaks of diarrhoea due to impaired intestinal function (McLamb et al., 2013b; Melin et al., 2004; Moeser et al., 2007; Smith et al., 2010).

In a study conducted in Denmark on pig herds with a low AMU, Fertner et al. (2015) reported that producers had varying opinions on the conditions necessary to achieve low AMU. Fertner et al. (2015) identified several explanations for achieving low AMU among individual producers, including feeding, building quality and management. It may be necessary to use similar qualitative methods to identify differences in management and attitudes towards management in herds with either a high or low AMU. Apart from differences in disease and treatment strategies, pronounced producer heterogeneity may be responsible for the differences described in the present study. It is plausible that producer heterogeneity may affect the results of this study significantly. If diseases are generally well managed by AMs, producers may be less motivated to adhere to internal biosecurity or feeding routines. Conversely, producers may practise internal biosecurity and feeding routines more rigorously to maintain low AMU levels. A low AMU may also be because the pigs are produced in specialised label productions demanding low AMU, such as organic or raised without antimicrobials (RWA), or it may reflect a general desire to use as little as possible. In such cases, preventive measures may be highly prioritised. However, producers in this study were not directly asked about whether they had special label productions, nor was there a strong focus on individual treatment thresholds. Therefore, the relationship between AMU levels and biosecurity does not allow for direct causal conclusions. In addition, external factors such as new buildings or animals with a high health status can minimise the need for AM treatment.

The relationship between AMU levels and producer attitude was investigated by Backhans et al. (2016), who reported that treatment thresholds, rather than producer attitude and biosecurity, significantly impacted AMU levels in Swedish pig herds. Here, lower treatment thresholds led to higher AMU. In countries like Sweden and Denmark, AMU levels are already very low (European Medicines Agency, 2022). When overall AMU levels are low, only a subtle change may be necessary for herd-level AMU to be considered high. However, in monitoring schemes such as the Yellow Card, with fixed permitted limit values, some producers may see this threshold as a minimum requirement to be met rather than an upper limit under which usage is to be kept well below.

Due to multifactorial aetiology and the potential role of producer differences, it is challenging to identify specific measures targeting a high AMU. Additional research on different types of production systems and producer characteristics is needed to identify other potential predisposing factors at the herd level. The results in the present study might also be affected by limitations inherent to the study design. Questionnaire surveys carry a risk of introducing volunteer bias to the study data (Sedgwick, 2013). The response rates of 36% for cases and 53% for controls are significantly lower than in other questionnaire-based studies in Danish pig production (Dupont et al., 2017; Kruse, 2016).

The subject matter alone may negatively impact participation rates, especially in the case group, as questions concerning the Yellow Card scheme can lessen the willingness of the producers to participate. In the control group, producers committed to reducing AMU might, on the contrary, be more inclined to participate. Additionally, the study relied entirely on self-reported data. Producers were asked to recall specific routines and practices from several years prior, which may result in incomplete or inaccurate recollections increasing the risk of recall bias (Coughlin, 1990). Producers in the case group may underreport practices they perceive as suboptimal, while producers with a low AMU may unintentionally overstate their adherence to biosecurity measures. However, many of the routines included in the questionnaire were anchored in the

SPF system, and during the interviews, producers demonstrated a clear understanding of the standard practices, being well aware of when and why they might deviate from established guidelines. Nevertheless, future studies should aim to validate questionnaire responses with observational data. Another challenge when using questionnaires is obtaining responses from producers that accurately convey the intended meaning of the questions (i.e., construct validity) (Motheral and Fairman, 1997). To improve construct validity, all questions were reviewed by veterinary practitioners and professionals experienced in pig production. Another approach involved requesting data that the producers had already collected regularly. Data-driven production management in Danish pig production is done through software systems, allowing pig producers to record and monitor a wide range of production data, including weight at weaning (SEGES, 2020). Monitoring weaning weight and age is essential for evaluating piglet health and allows producers to optimise management practices and improve piglet survival and growth post-weaning.

It is also plausible that the sample of 52 pig herds was insufficient to detect statistically significant associations between the AMU level and the predictor variables. A larger sample might elicit different results. There was no significant difference in median herd size between the participating and non-participating herds. However, more non-participating herds were enrolled in the SPF system than those participating (70% vs. 58%). Therefore, the impact of biosecurity measures in both the univariable and multivariable models might have resulted in a different outcome if more herds with SPF status had been included in the study.

This study found that most herds assigned a Yellow Card between 2016 and 2020 for weaners treated newly weaned pigs primarily for gastrointestinal disorders. However, in Denmark, respiratory disorders are the second most common reason for antimicrobial use (AMU) in weaners (Moura et al., 2023). Therefore, if all herds that received a Yellow Card for weaners during this period had been included in the study, rather than just 24, the most frequently reported herd diagnoses might have differed from those presented in this paper.

Between 2016 and 2020, the national average for AMU in weaners and the permitted limit values under the Yellow Card scheme were gradually reduced (Ministry of Food, 2018b). These changes complicated direct comparisons of AMU patterns across herds that were assigned a Yellow Card at different times during the study period, as herds assigned a Yellow Card in 2020 might not have been assigned a Yellow Card earlier in the study period. Consequently, the classification of case and control groups was not strictly consistent throughout this study. However, a study period of five years was necessary to ensure a reasonable number of cases were included in the study

The change in permitted limit values could also have influenced management behaviour and biosecurity schemes, prompting some producers to pre-emptively reduce AMU or adopt additional biosecurity measures to avoid receiving a Yellow Card, potentially masking actual AMU trends. Furthermore, external factors such as market conditions, changes in feed, and industry-led initiatives to reduce AMU could have influenced these trends, complicating the direct attribution of AMU reductions solely to the Yellow Card scheme.

Variations in the inclusion criteria for case and control herds might also impact the study results. Case herds were selected from herds with an AMU above the permitted limit value, which is assessed through a 9-month period in the Yellow Card scheme. However, control herds were selected due to AMU levels below the national average for at least 12 months. The 12-month period for control herds was chosen to capture seasonal variations and ensure consistent low AMU levels throughout the year. This longer timeframe may dilute short-term fluctuations in AMU that the 9-month period for case herds could capture, potentially leading to biased comparisons. Seasonal variations, such as higher disease pressure in winter, might cause an increase in AMU that a 12-month selection period does capture. In contrast, a 9-month period may miss these fluctuations if the 9-month period does not elapse throughout the wintertime. This discrepancy could mean that the observed differences in AMU between case and control groups are more reflective of these temporal differences rather than actual variations in biosecurity practices. Additionally, over time, producers may adapt management strategies to align more closely with the permitted limit values in the Yellow Card scheme, possibly optimising antimicrobial treatment protocols and practices rather than fundamentally improving biosecurity.
Geographical location and herd size are also critical factors that influence the transmission of pathogens and, thus, AMU (Cleveland-Nielsen et al., 2002). However, the potential impact was not fully explored in this study. Variables related to geographical distribution, age groups, and herd size were included to ensure uniformity between case and control groups and thus not analysed as independent variables. Previous research has shown that proximity to neighbouring farms and high pig density significantly contribute to the spreading of respiratory pathogens (Cleveland-Nielsen et al., 2002). Moreover, factors such as herd size and building age are particularly relevant (Kruse et al., 2020), as respiratory diseases are the second most common indication for AMU in Danish weaners (Moura et al., 2023).

Overall, the study aimed to improve the knowledge of the association between specific herd factors and the AMU level in Danish pig herds with a high AMU in weaners. In the present study, herds with a Yellow Card in weaners frequently treated gastrointestinal disorders. They relied on group medication and had fewer internal biosecurity measures and feeder spaces (**Tables 3** and **4**). These findings are highly relevant in a Danish context but may be less applicable in an international setting. Denmark has a relatively low AMU in livestock production compared to other EU countries (European Medicines Agency, 2022). What is considered high AMU in a Danish setting, especially under the Yellow Card scheme, may not be viewed the same in other MS. Herds unable to reduce group medication in favour of individual treatments must instead incorporate disease-preventive measures.

If the permitted limit values continue to be lowered under the Yellow Card scheme, it will become increasingly important for herds unable to shift from group medication to single animal treatments to focus on implementing more effective disease-prevention strategies. If these preventive measures prove insufficient, the next step should involve reducing the overall disease burden through partial or total herd eradication. Stamping out diseases through eradication measures is already implemented by SPF herds alongside biosecurity protocols to target and eradicate specific SPF diseases (SPF Health, 2021). Since October 2023, all Danish pig herds have been required to obtain an official status on Porcine Reproductive and Respiratory Syndrome (PRRS). Herds with a positive PRRS status can work towards achieving a negative status through eradication strategies (Ministry of Food, 2023b). Obtaining and maintaining a PRRS-negative status or using eradication measures to reduce disease pressure, in general, might be necessary to continue reducing AMU and ensure prudent AMU in pig production. This will also help improve herd welfare and sustainability. The results of this study should, therefore, be regarded as indicative of which areas have the potential to increase the risk of a Yellow Card. This knowledge can benefit future antimicrobial reduction schemes. Instead of solely relying on legislative restrictions, a targeted counselling approach could be adopted to offer advice to producers who experience increased AMU.

Conclusion

Herds with a Yellow Card in weaners primarily treated newly weaned pigs for gastrointestinal disorders and were more likely to rely on group medication than those with a very low AMU, who mainly used single-animal treatments.

The multivariable model indicated that management practices, such as the number of feeding spaces for newly weaned pigs and adherence to structured work routines, may influence AMU levels. Herds with a Yellow Card had fewer feeding spaces, which may increase stress and disease incidence, leading to higher AMU. Additionally, inadequate work routines, such as not attending to sick pigs last, can facilitate disease transmission within the herd. Improving these practices could help reduce AMU and strengthen biosecurity and herd health.

The findings indicate that herds experiencing high disease pressure may face challenges in maintaining stringent biosecurity measures, highlighting the importance of disease prevention and management strategies. Several limitations complicate the interpretation of the results. The small sample size, differing inclusion criteria for case and control herds, and changes in AMU thresholds during the study period make direct comparisons challenging. While this study did not establish a direct causal relationship between biosecurity and AMU levels, it indicates the need for targeted interventions to support herds in reducing AMU through enhanced internal biosecurity and improved management practices. A combined approach of legislative measures and targeted support for producers could help address management challenges and improve biosecurity practices to reduce AMU.

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9.2. Manuscript II

Anne-Sofie Glavind, Amanda Brinch Kruse, Helle Stege and Lis Alban (2024).
Association between antimicrobial use levels and meat inspection lesions in Danish finishers
Under revision at Preventive Veterinary Medicine

Parts of this study were previously presented at the annual meeting of the Society for Veterinary Epidemiology and Preventive Medicine (SVEPM) held in Uppsala, Sweden, from 20–22 March 2024 and published in: Vergne, T. and Robinson, P.A. (eds). Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine annual meeting held in Uppsala, Sweden, SVEPM. ISBN 978-0-948073-75-5

Association between antimicrobial use levels and meat inspection lesions in Danish finishers

ABSTRACT

In Denmark, the Yellow Card scheme enforces restrictions on pig herds exceeding official permitted limits for antimicrobial use (AMU). To assess if a Yellow Card is related to poor animal health, we examined the association between AMU and the health status of Danish finishers using meat inspection data as a proxy for pig health. We included meat inspection findings in 10.5 million finishers delivered to 9 Danish abattoirs between 2016 and 2020 from herds classified as having a low, medium, or high AMU (Yellow Card herds). The prevalence of meat inspection findings was calculated and analysed in mixed-effects logistic regression models to determine the associations between each of the eight selected lesions and AMU level while also considering the effect of herd size, location (east or west) and herd type (indoor or outdoor). Despite differences in AMU, only minor differences in meat inspection lesions were present. The overall result of the multivariable modelling was that herds with a medium AMU level were associated with the lowest risk of meat inspection lesions – observed for five out of the eight lesions investigated. Moreover, large herds were associated with a lower risk of meat inspection lesions than small herds – observed for seven out of eight lesions. The risk of lung and tail lesions was higher for herds in the western region of Denmark compared to the eastern regions, and the risk of tail lesions, arthritis and osteomyelitis was higher in outdoor herds compared to indoor herds. Hence, both a high and a low AMU could be linked with health and welfare issues. The results indicate that the advisory role of the herd veterinarian is pivotal in preventing adverse effects of antimicrobial reduction measures on animal health and welfare, in particular, if the Yellow Card limits are reduced further.

Keywords: Antimicrobials Lesions, Meat inspection, Pigs, Yellow Card

1. Introduction

The debate on prudent antimicrobial use (AMU) addresses usage patterns in livestock production and humans. The main concern is the emergence of antimicrobial resistance (AMR) in human and veterinary medicine. To mitigate this risk, several AMU-reducing measures have been implemented gradually in Danish pig production (DANMAP, 2021).

Following a voluntary phase-out of growth promoters in finishers and weaners in 1998 and 1999 (Moura et al., 2023), a restriction on the use of fluoroquinolones and polymyxin was introduced by the competent authorities in 2002 and 2016. In 2010, a voluntary ban was put on 3rd and 4th generation cephalosporins in 2010 (DANMAP, 2022). As a result, critically important antimicrobials (AMs) are seldom used in Danish pig production (DANMAP, 2022).

As in the rest of the EU, the use of veterinary AMs in Denmark is limited to prescription only, and all sales of veterinary medicinal products have been recorded in the Danish Veterinary Statistics database (VetStat) since 2000 (Stege et al., 2003). The database has a high level of granularity, as each record specifies animal species, age group, treatment indication, and route of administration, as well as product information, like active ingredient, ATC code, product name, amount, and concentration (Stege et al., 2003).

The VetStat database has enabled risk-mitigating initiatives, such as the Yellow Card scheme, introduced by the Danish Veterinary and Food Administration in 2010. The Yellow Card scheme is a legal intervention promoting the prudent use of AMs in Danish pig production (Ministry of Food, 2018a). In the Yellow Card scheme, data from VetStat are used as a proxy for AMU, presented as a 9-month rolling average. The AMU is measured in Animal Daily Doses (ADD) per 100 animals per day (ADD/100 animals/day), denoting the percentage of animals treated per day in each of the three age groups (sows with their piglets, weaners, and finishers). The scheme allows the Danish Veterinary and Food Administration to identify herds, where AMU exceeds the permitted limits, which results in a Yellow Card. This entails a mandatory reduction of AMU within the specific age group, accompanied by advisory visits from the authorities at the cost of the producer. Moreover, the option to keep and refill prescriptions for previously prescribed AMs for group treatment is abolished, which is costly for the producer (Ministry of Food, 2018b). Initially, the permitted limits for each age group were set as twice the mean of the AMU of all Danish pig farms. The permitted limit has been lowered several times, most recently in 2019, and it now stands at 4.4 ADD/100 animals/day for finishers (Ministry of Food, 2018a).

Since the Yellow Card was implemented in 2010, the total annual AMU in pig production decreased from 100.5 tons of active ingredient (DANMAP, 2011) to 71.3 tons in 2022 (DANMAP, 2023). In the meantime, the number of pigs produced increased from 28.6 million heads to 32.6 million (Danish Agriculture & Food Council, 2022). In 2021, weaners for export accounted for almost 44% of the total production, with around 14 million animals exported in that year (Danish Agriculture & Food Council, 2022). In comparison, 5.8 million people live in Denmark, hence, Denmark may be considered a pig country. Denmark is ranked fourth among EU Member States (MS) regarding pig population size, only surpassed by Spain, France, and Germany (Danish Agriculture & Food Council, 2022). According to Moura et al. (2023) the Danish pig sector registered an AMU of 43.3 mg per population correction unit (PCU) for 2020. In comparison, the national median of the AMU in all livestock in the 31 European countries that reported to ESVAC in that year was 51.0 mg/PCU (European Medicines Agency, 2021). In Denmark, sales of AMs for use in pig production account for 75% of the total sales of veterinary AMs (DANMAP, 2022). For more details about the AMU in Danish pigs, see Moura et al. (2023).

The profit margin for veterinary medicine in Denmark is restricted to 5% above the market value (Ministry of Food, 2023). Instead, Danish veterinary practitioners rely on health advisory contracts with producers. These contracts are a prerequisite for allowing the producers to initiate AM treatment without consulting a veterinarian first (Ministry of Food, 2021). Veterinary practitioners commit to making regular visits to the farm. Typically, there is a minimum of nine annual visits, but this can be reduced to four for finisher herds. During the visits, veterinarians document recurring diseases, perform necropsies, and conduct diagnostic sampling to identify infectious agents and test for AMR. These activities help diagnose herd problems, which guide AM prescriptions. Additionally, during the advisory visits, action plans are created to prevent disease and reduce the future need for treatment.

The main indications for AM treatment in commercially raised finishers are 1) gastrointestinal diseases, 2) conditions related to the skin and central nervous system (CNS), and 3) respiratory disorders (Moura et al., 2023). Gastrointestinal and respiratory infections influence herd-level AMU, as they are often treated using peroral preparations (Moura et al., 2023). Pigs can recover fully from such infections when they occur early in the animal's life, whereas infections occurring closer to the time of slaughter can result in lesions that might be detected at post-mortem meat inspection. Lesions related to respiratory disorders are frequent findings at meat inspection (Alban et al., 2015; Kongsted et al., 2017).

Tail-biting is widespread in both indoor and outdoor production systems (Alban et al., 2015). The condition is multifactorial, but health and housing-related stressors are important precursors (Schrøder-Petersen and Simonsen, 2001). The bite site provides an entry point through the skin and tail vertebrae, and the spread of pyogenic bacteria can result in several sequelae, including osteomyelitis in the pelvic area, abscesses in the hindquarters, and pyaemia (Schrøder-Petersen and Simonsen, 2001). In addition to increased AMU and reduced animal welfare, infectious diseases are also responsible for financial losses due to slower growth, poorer feed conversion, partial or total condemnation at the abattoir as well as higher mortality on-farm (Harley et al., 2012; Schrøder-Petersen and Simonsen, 2001).

Meat inspection is important for food safety, animal welfare, and animal health. Foremost, meat inspection ensures that only meat fit for human consumption enters the food chain (Ministry of Food, 2022). Pigs delivered to the abattoir are subjected first to ante-mortem inspection, and if accepted, the resultant carcass and organs also undergo post-mortem inspection. In Denmark, the inspections are undertaken by official veterinarians and official auxiliaries, and various findings detected during inspection are logged using a comprehensive coding system (Ministry of Food, 2022). Meat inspection findings determine whether parts of or the whole carcass need to be condemned, and this is based on pre-defined condemnation criteria (Vieira-Pinto et al., 2022). Hence, meat inspection can be seen as a surveillance system, providing a source of information on animal health at the time of slaughter. Moreover, it has been suggested that meat inspection has the potential to be used as part of animal welfare assessment in livestock production (Stärk et al., 2014).

It might be hypothesised that some herds have a high AMU due to compromised animal health and, therefore, a high need for AM treatment. However, the AM administered could effectively reduce the impact of infections other than those for which the AM treatment was intended, thereby reducing the overall disease burden in the herd. Conversely, some producers could intentionally delay or avoid treating animals needing AMs to maintain a low herd-level AMU. Politically, there is an interest in further lowering the AMU in Danish pig production. To better understand the possible outcomes of future AMU restrictions, it is important to examine the current health status of Danish finishers across different AMU levels and assess whether a high AMU is associated with reduced pig health. This study builds on preliminary findings previously presented in the Society for Veterinary Epidemiology and Preventive Medicine (SVEPM) conference proceedings (Glavind et al., 2024). Specifically, the objectives of the study were to:

- Explore the variations in meat inspection findings among finishers from herds with either a high, medium, or low AMU.

- Examine the association between meat inspection findings and AMU level, considering herd size, regional location within Denmark (east versus west), and herd type (indoor versus outdoor).

2. Materials and methods

2.1. AMU data and herd selection

The methodology applied in this study expands on the approach initially described by Glavind et al. (2024). Information on herds assigned a Yellow Card between 2016 and 2020 was provided upon request to the Danish Food and Veterinary Administration. The data contained the herd identify number and the date for each assigned Yellow Card. From these data, herds which had received a Yellow Card for the AMU in the finishers were selected and categorised as having a high AMU. Data on AMs sold for use in Danish pig herds were obtained from VetStat and used to select herds with medium and low AMU in finishers. First, herds that had not previously received a Yellow Card were assigned a random date between 2016 and 2020. Next, data on AMU covering a total of 9 months before each randomly assigned date were extracted from the VetStat data. This was done to select herds using similar criteria that are applied in the Yellow Card scheme. Herds with an AMU equal to or lower than 2.5 ADD/100 animals/day in all 9 months were classified as having a low AMU. This group consisted of herds with an AMU of half or less than half of the permitted limit values in the Yellow Card scheme. Herds with an AMU higher than 2.5 ADD/100 animals/day in at least 1 month out of the 9 months were classified as medium AMU. This group included herds that use AMs consistently but had not been issued a Yellow Card.

Information on herd type and geographical location was obtained from the Central Husbandry Register (CHR). Herd type indicated whether the pigs were raised indoors or outdoors. Outdoor herds included organic and conventional free-range systems. The geographical location indicated whether a herd was located in the western region of Denmark covering Jutland and Funen or the eastern region covering Zealand and the other islands, including Bornholm.

2.2. Meat inspection data

The meat inspection data originated from nine large Danish abattoirs in Jutland, Zealand, and Bornholm. The data covered monthly recordings of selected meat inspection lesions and the number of pigs slaughtered for each herd. A total of 16 meat inspection lesions were included in the study as indicators of pig health (**Table 1**). The lesions are primarily of infectious origin, which, in the live animal would require AM treatment. The lesion code "totally condemned" was also included, as it is used in cases of generalised or extensive disorders affecting the carcass. Lesions only found locally in the pelvis and lesions found beyond the pelvis as an indication of septicaemic spread were grouped as "tail lesions". Chronic pneumonia and chronic pleurisy were grouped as "lung lesions". Abscesses detected in the front, middle, or hindquarters were combined into "abscesses, trunk", while abscesses in the head or legs were grouped as "abscesses, extremities". Hereby, the 16 initial lesion codes were combined into 11 lesions. The number of pigs delivered to the abattoir from each herd during the study period was used as a proxy for herd size. Herds delivering equal to, or more than the median number of pigs were categorised as large herds, while herds delivering fewer than the median were characterised as small herds.

The study analysed meat inspection data for 9 consecutive months, the same time period used in the Yellow Card Scheme. This period was adjusted to cover 6 months before and 3 months after the specified dates for each herd. This was done because Danish finishing pigs usually reach their final slaughter weight within 10-12 weeks after entering the fattening unit. So, a herd with a Yellow Card could potentially have a high AMU from up to 9 months before receiving the Yellow Card. But pigs introduced to the fattening unit at the beginning of this period will not reach slaughter weight until 10-12 weeks later. The same applies to the period following the Yellow Card, where pigs will reach slaughter weight during the following 3 months.

2.3. Statistical analysis

Data were managed using the statistical software program R (version 4.3.0). Statistical analyses were carried out using the glmer function in the lme4 package.

The prevalence of the meat inspection lesions listed in **Table 1** was calculated for each of the three AMU levels. The statistical association between the lesions and the AMU levels were assessed by chi-square tests. Only lesions with a prevalence > 0.2% and P-value < 0.1 were considered for further analysis.

The pairwise associations between the lesions were identified using chi-square tests, where the P-value indicated the statistical association between the lesions. Moreover, the odds ratio (OR) measured the size and direction of the biological association, being either positive or negative. Due to many univariable comparisons increasing the probability of detecting association by chance, an odds ratio (OR) > 3 or < 0.33 and a P-value < 0.001 were used as limits to indicate either a strong positive or a strong negative association. All significant biological associations between the lesions for the three AMU levels are visualised in a hive plot.

The potential associations between AMU level and meat inspection lesions were investigated in mixed-effects logistic regression models. A model was fitted for each lesion, with AMU level, region, herd type, and herd size as covariates. Because meat inspection data are reported as the number of animals with a given lesion out of the total number of animals delivered for slaughter, the outcome was set as the number of pigs with a given lesion (y) detected by post-mortem inspection divided by the overall number (n) of finishers delivered from the herd during the selected 9-month period. To account for any differences in the use of the meat inspection code system by the nine abattoirs, herd and abattoir were included as random effects in all models. Model reduction was performed using stepwise backward elimination, retaining only statistically significant covariates. Because of the large dataset size, P < 0.01 was used to determine statistical significance. Confounding effects on AMU and modifying effects were assessed by re-entering covariates into the final models and adding interaction terms between significant covariates, respectively. The degree of statistical differences between a family of estimates for a given variable with more than two levels was revealed using the Tukey method in R. For control of model fit, residual plots and QQ plots for each model were inspected. For the random effects, the ranef function in R was applied to extract the conditional modes of the random effects.

3. Results

3.1. Descriptive statistics and univariate analyses

In total, 84 herds (348,124 pigs) with a high AMU (herds assigned a Yellow Card), 1332 herds with a medium AMU (5,976,589 pigs), and 1305 herds with a low AMU (4,186,343 pigs) were included in the analyses. The prevalence of the selected lesions for each AMU level is presented in **Table 2**. Overall, lung lesions were the most common (16-17%) among the lesions included. The other lesions were recorded less frequently (each at < 3%). The difference between the three AMU levels with respect to lesions was low, with less than 0.5% variation.

Still, the univariate analysis revealed that lung lesions, abscesses in the extremities, tail lesions, and arthritis were more common in herds with a high AMU. Scar/contusion/bursitis were more prevalent in herds with a medium AMU. Conversely, abscesses in the trunk, pericarditis, peritonitis, and osteomyelitis were more prevalent in herds with a low AMU. There was no effect of pyaemia (P-value = 0.21), scar/contusion/bursitis (P-value = 0.28) or condemnation (P = 0.13) on the AMU level. The remaining eight lesions were statistically associated with AMU level, implying P<0.001. Therefore, pyaemia, scar/bursitis/contusion and condemnation were left out from the subsequent multivariable analyses. Hence, eight mixed-effected models with each meat inspection lesion as a response were investigated in the multivariable analyses.

3.1. Biological associations

Only positive biological associations (OR > 3, P < 0.001) between the lesions in all three AMU levels were identified (**Fig. 1**), and most lesions were associated with each other. Pericarditis was the only lesion associated with only two other lesions (peritonitis and lung lesions). Total condemnation, which reflects the final judgement regarding all findings in a slaughtered animal, was associated with lung lesions, abscesses in the trunk or the extremities, scar/contusion/bursitis, osteomyelitis, and pyaemia.

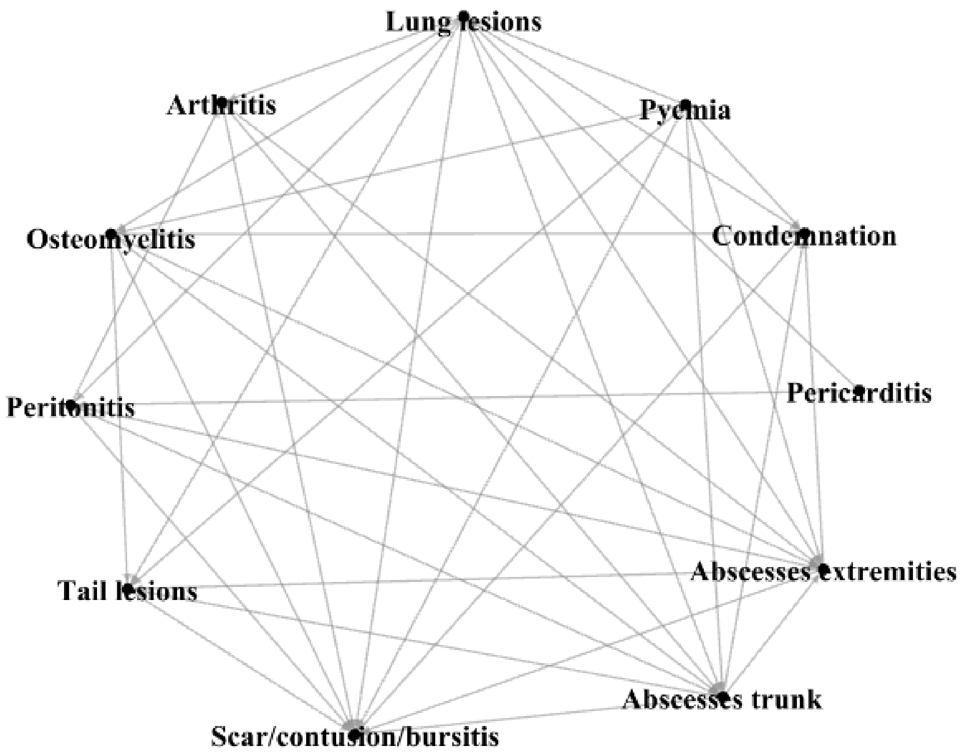

Fig. 1. Illustration of pairwise, statistically significant, positive biological associations between 11 meat inspection lesions (including total condemnation) for 2721 Danish pig herds, delivering finishers to nine Danish abattoirs between 2016 and 2020. Only statistically

significant associations were included (OR> 3, $P<0.001$). No negative associations were present.

3.2. *Mixed-effects logistic regression models*

The results of the eight mixed-effects logistic regression models are presented in **Table 3**. Consistent with the preliminary results in Glavind et al. (2024), the overall result of the multivariable modelling was that herds with a medium AMU level were generally associated with the lowest prevalence of meat inspection lesions. This was observed for five out of eight lesions, i.e. peritonitis, abscess in the trunk, abscesses in the extremities, tail lesions and arthritis. Moreover, large herds were associated with a lower prevalence of meat inspection lesions than small herds. This was observed for seven out of eight lesions, i.e. pericarditis, peritonitis, abscess in the trunk, abscesses in the extremities, tail lesions, osteomyelitis and arthritis. For osteomyelitis, an interaction was present between AMU level and herd type, which complicated the interpretation. Here, the lowest risk was associated with indoor herds with either a high or a medium AMU, whereas the highest risk was associated with outdoor herds with either a high or a medium AMU. Outdoor production, compared with indoor production, was associated with a higher prevalence of osteomyelitis, arthritis, and tail lesions (**Table 3**). The prevalence of lung lesions and tail lesions was higher for herds in the western region than in the eastern region of Denmark. The variance introduced to the models due to animals delivered from the same herds was greater than that due to the different abattoirs for all lesions except pericarditis and peritonitis (**Table 3**). No multicollinearity was present in any of the full models. A relatively good fit of all models was detected. In general, the fixed effects provided some explanatory power, but the random effects were very important in capturing the variability in the data. The effect of abattoir and herd was greater in the model for lung lesions, where the random effects had a stronger explanatory power than in the other models.

4. Discussion

4.1. *Prevalence of meat inspection lesions*

The first objective of this study was to assess variations in meat inspection lesions among herds with a high, medium, or low AMU. This was done to investigate a hypothesis of potential associations between a high AMU and poor health in finishers. Preliminary findings are presented in Glavind et al. (2024). Despite the large differences in AMU, the prevalence of each lesion differed by less than 1% between the three AMU levels (**Table 2**). There are several possible reasons for this finding. As described in the introduction, the AMU in the Danish pig sector is below the median among the 31 European countries that reported to ESVAC in 2020 (European Medicines Agency, 2021). The relatively low AMU can be explained by the extensive collaborating efforts undertaken by Danish policymakers, the livestock industry, and other stakeholders in working toward prudent AMU, including establishing the VetStat database and implementing the Yellow Card scheme. Therefore, herds with high AMU in Denmark may not necessarily reflect herds with high AMU in other countries. Although several countries have introduced AMU monitoring systems, there is considerable variation in coverage, funding, and implementation. Apart from Denmark, only Austria, Belgium, Norway, Switzerland, and Sweden have government-funded systems with full sector coverage (Sanders et al., 2020).

4.2. Biological associations

As illustrated in **Fig. 2**, statistically strong, positive biological associations were observed among all meat inspection lesions included in the study, suggesting that none of the selected lesions occur independently at the herd level. Several lesions were also positively associated with carcass condemnation indicating complex disease patterns. In addition to influencing animal health, lesions associated with carcass condemnation can have financial repercussions for the producer due to reduced settlement at the abattoir.

4.3. Associations between antimicrobial use and meat inspection lesions

In Denmark, ongoing efforts are dedicated to reducing AMU in pig production, with a significant achievement of a 16% reduction in 2019 compared to 2014 (DANMAP, 2023). This highlights the second objective of this study, which aimed to investigate the potential associations between AMU levels and meat inspection lesions. The findings expand on preliminary results in Glavind et al. (2024) and showed that a medium AMU was associated with a lower risk for five out of the eight meat inspection lesions included in the multivariable analyses. It is plausible that herds with a medium AMU do not experience similar or as severe health challenges, as those with a high AMU. In contrast, herds with a low AMU probably encompass labelled production systems such as organic (which takes place outdoors) and “raised without antimicrobials”, which usually takes place indoors. Financial incentives are in place for these two production systems to maintain a low AMU.

Outdoor production was associated with higher odds of delivering pigs with arthritis compared to indoor production. Similar findings are described in previous Danish studies (Alban et al., 2015; Hansson et al., 2000; Kongsted and Sørensen, 2017). As suggested by Alban et al. (2015), there could be several reasons for arthritis in pigs from outdoor production, including infection pressure caused by humid conditions and poor hygiene. It is unknown if arthritis was the cause of the high AMU in this study. Arthritis may have gone undetected, or treatment could have been delayed to avoid a greater increase in AMU. In addition to compromising animal welfare, arthritis is costly and time-consuming for the producer.

This study found that outdoor herds were more likely to deliver pigs with tail lesions than indoor herds, which was also seen by Alban et al. (2015). Moreover, herds with a low AMU were more likely to deliver pigs with tail lesions than herds with a medium AMU (P=0.004). The same was somewhat also seen for osteomyelitis, a common sequela of tail lesions (Fertner et al., 2017; Schrøder-Petersen and Simonsen, 2001). For both tail lesions and osteomyelitis, the likelihood of pigs being delivered with these conditions was higher in outdoor production than in indoor production. Other studies have also reported greater odds for tail lesions in herds with outdoor production and in herds where pigs have undocked tails (Alban et al., 2015; Gomes et al., 2022; Kongsted & Sørensen, 2017). The close association between tail lesions, pyaemia, and abscessation, which were also observed by Huey (1996), provides another justification for minimising disease prevalence at the herd level and thus potentially reducing the number of condemned carcasses at the abattoir.

For all three AMU levels, lung lesions were the most prevalent (16-17%). However, this prevalence is slightly below previous findings from Denmark and Finland (Alban et al., 2015; Cleveland-Nielsen et al., 2002; Hälli et al., 2020). Since the introduction of the Yellow Card scheme, the use of vaccines against pulmonary infections has increased (Alban et al., 2013; Kruse et al., 2017), which is likely to have had an effect on reducing lung lesions. Additionally, non-infectious risk factors are considered significant in spreading lung diseases, including proximity to neighbouring farms and pig density (Cleveland-Nielsen et al., 2002), enhancing the airborne spread between herds. The density of pigs could have had a decisive impact on the results of our study. This was confirmed in the model for lung lesions showing that the herds in the western region of the country were associated with a higher risk compared to herds in the eastern region. This is consistent with the current pig density in Denmark, which is higher in the western part of Denmark (Danish Agriculture & Food Council, 2022). Finally, large herds were associated with a lower risk of all lesions studied except lung lesions. This likely is a result of the many actions taken in Danish pig farms, including widespread acceptance of SPF requirements to external biosecurity, sectioning, all-in-all-out production, and extensive use of vaccination. An association between herd size and the age of buildings (implying larger herd sizes are generally seen in newer buildings) was also found by Kruse et al. (2020), whereas Gardner et al. (2002) argued for the inclusion of herd size in all studies.

4.4. Limitations and perspectives

This study evaluated 9 months of data for each herd collected between 2016 and 2020, mirroring the method used by the Danish Food and Veterinary Administration when assigning a pig herd a Yellow Card. The availability of monthly AMU data for all Danish herds in the VetStat database allowed for a large study population. The group of herds with a high AMU was notably smaller than those with a medium or a low AMU. This discrepancy arose from our selection criterion that restricted the high AMU level to include only herds with a Yellow Card. However, in the Yellow Card scheme, these herds are defined as having an unacceptably high AMU, which is why it is particularly important to examine meat inspection lesions in this group.

The potential for meat inspection data to be included in national welfare indices has previously been explored by Nielsen et al. (2017). Based on our study, we conclude that the utility of using meat inspection data for animal health, animal welfare and AMU varies between the different lesions. As most pigs in Denmark are subjected to visual-only post-mortem inspection, only lesions that are present macroscopically on the surfaces of the carcass or the organs can be found. Moreover, lesions due to disease early in the pig’s life may not be visible at the time of slaughter. Treatment with AMs for one disease condition may also influence other diseases and, thereby, the lesions that are detected at meat inspection. This makes it necessary to consider which meat inspection data to use for what purpose. Since the Yellow Card was introduced, the permitted limits have been lowered several times as a part of national action plans to reduce AMU (DANMAP, 2023). If the permitted limits are to be lowered again, the role of the veterinarian with regular advisory visits to the herd will become even more important to ensure that less AMU does not inadvertently contribute to a decline in animal health reflected in the meat inspection records.

The study raises the concern that some herds may struggle to provide the necessary treatments due to regulatory restrictions. A low AMU could reflect inadequate health management, including lack of treatment where indicated, thus compromising animal welfare.

In 2017, the use of neomycin rose due to changes in the weighting of colistin under the Yellow Card scheme. The weighting of colistin by a factor of 10 meant that gastrointestinal diseases in pig production are no longer treated with colistin (DANMAP, 2023). However, antimicrobial-reducing initiatives implemented through the permitted limits in the Yellow Card scheme are not the only measures affecting Danish pig production. Medicinal zinc oxide was primarily used to prevent post-weaning diarrhoea in piglets. The phasing out of medicinal zinc oxide in 2022 has led to increased use of certain AMs, such as neomycin, which belongs to the aminoglycoside class (DANMAP, 2023). The European Medicines Agency (EMA) places aminoglycosides in the "Caution" category, for which there are only a few alternatives in human medicine (European Medicines Agency, 2017). It is currently under investigation to evaluate to which extent these measures have impacted the prevalence of meat inspection lesions. Such evaluations form part of a system's thinking approach which is needed to understand the potential impact of a given measure to be introduced, preferably before the introduction (Aenishaenslin et al., 2019; Anderson and Johnson, 1997).

Danish pig producers thus face challenges in balancing animal and public health. In 2022, a new EU regulation on veterinary medicine was implemented, directly affecting the options for the herd veterinarian to ensure a prudent AMU. The new EU regulation mandates veterinarians to prescribe AMs according to the treatment duration and dose set by the summary of the product characteristics (SPC) (European Union, 2019). As a result, veterinarians can no longer specify the treatment duration independently, which, in the Danish case, may lead to an unnecessary increase in AMU in some Danish herds. In VetStat, the accuracy of calculating AMU at the herd level is also limited due to the use of standardised measures. Here, the number of pen places registered in the CHR database is used as a proxy for herd size. However, this method may lead to inaccuracies since it does not consider the number of pigs produced per pen place (Dupont and Stege, 2014). These issues are currently being addressed in the next step of our research.

5. Conclusion

This study investigated the association between AMU and meat inspection lesions in Danish finisher pigs as a proxy for animal health. Despite notable differences in AMU among finisher pig herds, there were only minor differences in the overall prevalences of meat inspection lesions among herds with a high, medium, or low AMU. Meat inspection data may have a limited value as indicators for AM treatments, which occur early in life, such as to control diarrhea, which in most cases do not result in findings that can be detected macroscopically at meat inspection.

The overall result of the multivariable modelling was that herds allocated to the medium group regarding AMU were associated with the lowest risk of meat inspection lesions – observed for five out of the eight lesions. Moreover, large herds were associated with a lower risk of meat inspection lesions than small herds – observed for seven of the eight lesions. If the permitted limits for AMU are further reduced, the veterinary advisory role should become even more vital in preventing adverse effects on animal health and welfare.

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Table 1

List of selected meat inspection lesions from the Danish pig slaughterhouse database used to study the association of AMU and animal health, 2016 - 2020.

Group lesion name	Original lesion code name	Description
Pyaemia	Pyaemia	Pyaemia, blood poisoning, embolic pneumonia, pyaemia related to splenitis, and kidney inflammation
Pericarditis	Pericarditis	Chronic pericardial infection
Lung lesions	Pneumonia Pleuritis	Chronic pneumonia, aerogenous lung abscesses, chronic pleurisy, serositis
Peritonitis	Peritonitis	Chronic peritonitis, abscess in the peritoneum
Osteomyelitis	Osteomyelitis	Acute, chronic, localised, and healed bone marrow inflammation, immersion abscesses, infected bone fracture
Arthritis	Arthritis	Chronic arthritis, osteoarthritis
Abscess, trunk	Abscess, front part Abscess, mid-part Abscess, hind part	Abscess in the front part Abscess in the middle part Abscess in the hindquarters
Abscesses, extremities	Abscess, leg/toe Abscess, head region	Abscess in leg/toe, elephantiasis leg, abscess in the head, blood ear, crooked ear, elephantiasis ear
Tail lesions	Tail bite Tail infection	Tail bite with local infection Tail infection proximal to the os sacrum
Scar/contusion/bursitis	Scar/contusion/bursitis	Scar, contusion, bursitis
Condemnation	Condemnation	The entire carcass is discarded

Table 2

Prevalence[a] and univariable association between lesions recorded during meat inspection in finishing pigs from herds with a high, medium and low AMU during 9-month periods from 2016 to 2020 from 9 Danish abattoirs.

Lesion	Antimicrobial use level			P-value[c] for association
	High	Medium	Low	
	No. of lesions (%)[b]	No. of lesions (%)[b]	No. of lesions (%)[b]	
Lung lesions	57589 (16.54)	958302 (16.00)	687801 (16.40)	< 0.001
Scar/contusion/bursitis	9758 (2.80)	168697 (2.83)	117690 (2.81)	0.28
Abscess, extremities	8261 (2.37)	124357 (2.08)	97370 (2.32)	< 0.001
Abscess, truncus	5664 (1.63)	83734 (1.40)	70201 (1.67)	< 0.001
Pericarditis	4332 (1.24)	72244 (1.21)	56580 (1.35)	< 0.001
Peritonitis	2402 (0.70)	36145 (0.61)	30159 (0.72)	< 0.001
Tail lesions	2305 (0.66)	28831 (0.48)	24923 (0.60)	< 0.001
Arthritis	1397 (0.40)	14494 (0.24)	12222 (0.29)	< 0.001
Osteomyelitis	656 (0.19)	8623 (0.14)	8282 (0.20)	< 0.001
Pyemia	73 (0.02)	1166 (0.02)	884 (0.02)	0.21
Total no. of lesions[d]	92437 (26.55)	1496593 (25.01)	1106112 (26.38)	< 0.001
Condemnation	623 (0.18)	10516 (0.18)	7605 (0.18)	0.13

[a] The prevalence for each antimicrobial level was calculated from the number of registered lesions divided by the total number of finishers delivered to the abattoir during the 9 months. [b]Total number of finishers slaughtered in the study period: high AMU herds = 348,124 heads, medium AMU herds = 5,976,589 heads, low AMU herds = 4,186,343 heads. [c]P-value based on chi-square test. [d]More than one lesion per animal could have been recorded.

Table 3 - Results of the multivariable analyses of the associations between eight selected meat inspection lesions and AMU level, herd size, region, and herd type in 10.5 million finishing pigs delivered to 9 Danish abattoirs between 2016 and 2020.

Model with lesion as outcome	Fixed effects					Random effects and relevant contrasts		
	Covariates[a]	Categories	Estimate	Odds ratio (95% CI)	P-value for covariate	Levels	Variance	Standard deviation
Pericarditis	Herd size	Intercept	-6.73		<0.001	Herd	0.59	0.77
		Small	0.00	1	0.005	Abattoir	7.71	2.78
		Large	-0.11	0.89 (0.83 – 0.96)				
Lung lesions[b]	Region	Intercept	-2.74		<0.001	Herd	1.27	1.13
		East	0.00	1	<0.001	Abattoir	0.06	0.25
		West	0.80	2.22 (1.99 – 2.47)				
Peritonitis	AMU level	Intercept	-5.07		<0.001	Herd	0.25	0.50
		High	0.00	1	<0.001	Abattoir	0.38	0.62
		Medium	-0.11	1.00 (0.79 – 1.02)		<u>Contrasts</u>	<u>Estimate</u>	<u>P-level</u>
		Low	-0.005	1.00 (0.87 – 1.13)		H vs M	0.11	0.23
	Herd size	Small	0.00	1	<0.001	H vs L	0.006	1.00
		Large	-0.22	0.80 (0.77 – 0.84)		M vs L	-0.10	<0.001
Osteomyelitis		Intercept	-6.67		<0.001	Herd	0.57	0.75
	AMU *herd type	High*Indoor (HI)	0.00	1	<0.001	Abattoir	0.12	0.34
		High*Outdoor (HO)	2.26	9.56 (3.26-28.20)		<u>Contrast</u>	<u>Estimate</u>	<u>P-level</u>
		Medium*Indoor (MI)	0.06	1.06 (0.86-1.31)		HI vs HO	-2.26	<0.001
		Medium*Outdoor (MO)	1.03	2.81 81.53-5.18)		HI vs MI	-0.06	0.99
		Low*Indoor (LI)	0.36	1.43 (1.16-1.77)		HI vs MO	-1.03	0.01
		Low*Outdoor (LO)	0.74	2.09 (1.47-2.98)		HI vs LI	-0.36	0.01
	Herd size	Small	0.00	1	<0.001	HI vs LO	-0.74	<0.001
		Large	-0.33	0.72 (0.67-0.77)		HO vs MI	2.20	<0.001
						HO vs MO	1.23	0.34
						HO vs LI	1.90	0.006
						HO vs LO	1.52	0.07
						MI vs MO	0.98	0.01
						MI vs LI	-0.30	<0.001
						MI vs LO	-0.68	<0.001
						MO vs LI	0.67	0.20
						MO vs LI	0.30	0.94
						MI vs LO	-0.38	0.11

Arthritis		Intercept	-5.91		<0.001	Herd	0.38	0.62
	AMU level	High	0.00	1	<0.001	Abattoir	0.16	0.41
		Medium	-0.16	0.85 (0.73 – 1.01)				
		Low	-0.04	0.96 (0.82 – 1.14)		Contrasts	Estimate	P-level
	Herd size	Small	0.00	1	<0.001	H vs M	0.16	0.15
		Large	-0.29	0.75 (0.71 – 0.79)		H vs L	0.04	0.90
	Herd type	Indoor	0.00	1	<0.001	M vs L	-0.12	<0.001
		Outdoor	0.92	2.51 (2.10 – 3.01)				
Abscesses,		Intercept	-4.30		<0.001	Herd	0.26	0.51
trunk[c]	AMU level	High	0.00	1	<0.001	Abattoir	0.07	0.27
		Medium	-0.04	0.96 (0.85 – 1.09)		Contrasts	Estimate	P-level
		Low	0.16	1.17 (1.04 – 1.33)		H vs M	0.04	0.80
	Herd size	Small	0.00	1	<0.001	H vs L	-0.16	0.02
		Large	-0.19	0.82 (0.79 – 0.86)		M vs L	-0.20	<0.001
Abscesses,		Intercept	-3.70		<0.001	Herd	0.30	0.55
extremities[d]	AMU level	High	0.00	1	<0.001	Abattoir	0.10	0.32
		Medium	-0.14	0.87 (0.77 – 0.99)		Contrasts	Estimate	P-level
		Low	-0.04	0.96 (0.85 – 1.09)		H vs M	0.14	0.08
	Herd size	Small	0.00	1	<0.001	H vs L	0.04	0.83
		Large	-0.23	0.79 (0.76 – 0.83)		M vs L	-0.10	<0.001
Tail lesions[e]		Intercept	-5.69		<0.001	Herd	1.10	1.05
	AMU level	High	0.00	1	0.006	Abattoir	0.12	0.35
		Medium	-0.09	0.94 (0.73 – 1.21)				
		Low	0.08	1.09 (0.85 – 1.40)		Contrasts	Estimate	P-level
	Herd size	Small	0.00	1	<0.001	H vs M	0.09	0.75
		Large	-0.39	0.68 (0.62 – 0.74)		H vs L	-0.05	0.91
	Herd type	Indoor	0.00	1	<0.001	M vs L	-0.14	0.004
		Outdoor	0.56	1.75 (1.38 – 2.23)				
	Region	East	0.00	1	<0.001			
		West	0.21	1.23 (1.10 – 1.38)				

[a] Covariates with a statistically significant ($P < 0.01$) association with the outcome, [b] Combined code for chronic pneumonia and chronic pleurisy, [c] Combined code for abscesses in the front, middle, or hindquarter, [d] Combined code for abscesses in the head, leg, or toe, [e] Combined code for tail bite and tail infection

Appendix A

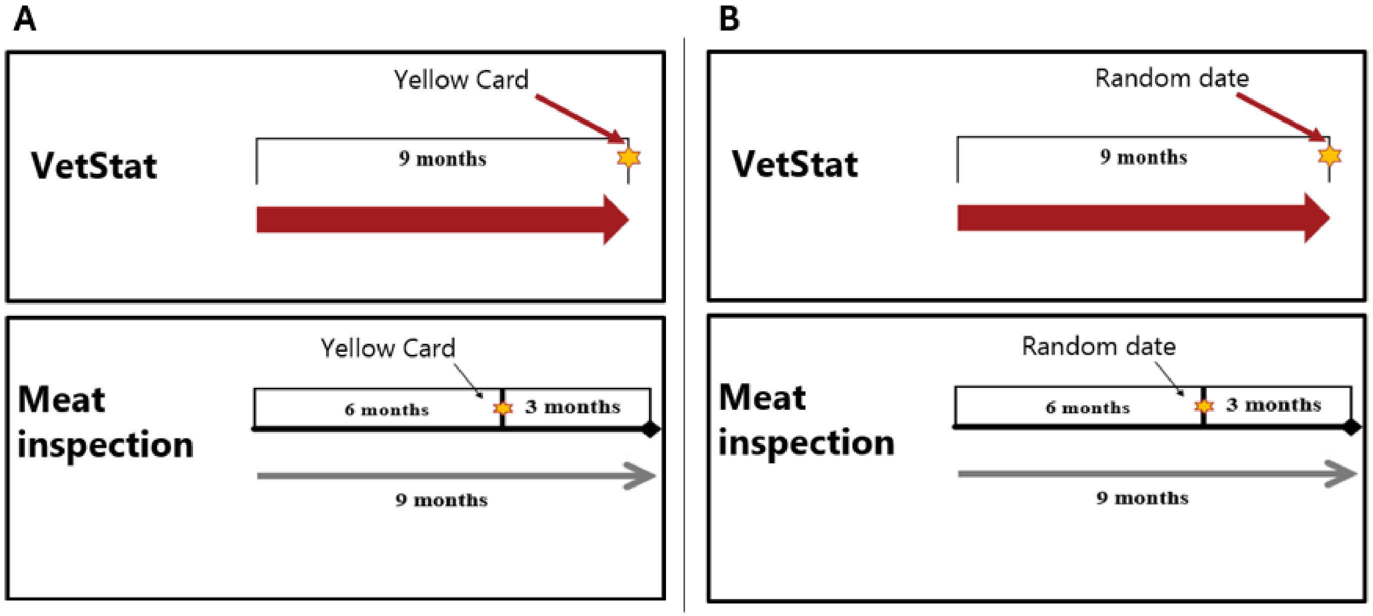

Figure A1. Overview of the study design according to the combination of VetStat and meat inspection data. For each herd, meat inspection data for 9 months was included to align with the time period in the Yellow Card scheme. The time period relating to the meat inspection data was adjusted to cover 6 months before and 3 months after the specified dates (or the assigned Yellow Card) in the VetStat data. This was done because Danish finishing pigs usually reach their final slaughter weight within 10-12 weeks after entering the fattening unit

9.3. Manuscript III

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RESEARCH

Open Access

Monitoring antimicrobial usage in companion animals: exploring the use of the Danish VetStat database

Anne-Sofie Glavind*, Amanda Brinch Kruse, Liza Rosenbaum Nielsen and Helle Stege

Abstract

Background: In the Danish Veterinary Statistics Program, VetStat, sales data on medicinal products prescribed for veterinary consumption is collected. The Danish Food and Veterinary Administration (DVFA) manages the database and each purchase contains detailed product-specific information linked with a species-specific ID. National surveillance systems are also implemented or being developed in the other European Union Member States. By 2029, all Member States are required to report data on antimicrobial usage for companion animals to the European Medicines Agency. This study aimed to assess the challenges encountered when using the VetStat database to quantify antimicrobial use in Danish companion animals. Raw VetStat data were propagated by the DVFA and originated from veterinary practitioners and Danish pharmacies.

Results: Comprehensive estimates of antimicrobial use in Danish companion animals were not readily available due to database construct. Antimicrobials sold for use in companion animals (linked to a companion animal ID) comprised a large number of products licensed solely for horses or livestock, while data assigned a replacement code encompassed both topical and peroral antimicrobials licensed for companion animals. Additionally, antimicrobials sold from pharmacies to veterinary practitioners presented the biggest challenge in data retrieval and validation. Treatment data are only transferred to VetStat through the billing systems when Danish veterinarians are treating livestock, but not companion animals. Information on products sold for in-house use in companion animals is only available from pharmacy records without a species-specific ID. As a result, parenteral antimicrobials with multi-species authorization utilized by small animal veterinary practitioners are not accounted for in the overall estimate for companion animals.

Conclusions: Owing to the database structure and requirements for data entry, antimicrobial use in companion animals is an approximation. The actual consumption may be significantly higher than what is currently calculated from the database, as the majority of parenteral products are not included. Consumption data can be measured more accurately provided treatment data from veterinary practitioners in small or mixed practices are transferred to the database through the billing system. This would equal the legal requirements for Danish veterinary practitioners treating livestock.

Keywords: Antimicrobial, Antimicrobial stewardship, Prescriptions, Register data, Surveillance systems, Validation, Veterinary sales data

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Background

Antimicrobial resistance (AMR) is a global public health threat that requires monitoring of usage patterns in both human and veterinary medicine [1–3]. The selective spread of AMR-resistant pathogens from livestock to humans is a primary concern [2–4], but companion animals are increasingly recognized as potential reservoirs [5–10]. Advanced specialization in small animal medi- cine has generated a demand for larger referral hospi- tals allowing for longer-term admissions, and treatment of geriatric or critically ill patients, giving rise to more high-risk veterinary patients in the veterinary field [11]. Nosocomial infections and clinical outbreaks of AMR-resistant pathogens can reduce the treatment efficacy [9, 10] and compromise animal welfare. The zoonotic properties in many of the pathogens are a potential workplace hazard for veterinarians, whilst the close cohabitation between owners and their pets facilitates the spread of AMR genes and pathogens outside hospital settings [5, 10–12].

The use of critically important antimicrobials (CIA) for human medicine in the veterinary field is another con- cern, drawing attention to the stewardship of these in small animal medicine [5]. Current classification by the World Health Organization (WHO) has ranked 3rd to 5th generation cephalosporins, polymyxins, quinolones, glycopeptides, and macrolides as those of highest prior- ity [13]. In Europe, many of these are commonly applied to treat companion animals [10], and in Denmark, almost all prescriptions of fluoroquinolone for veterinary pur- poses are made by small animal practices [14].

In the European Union (EU), The European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) provides an overview of AMU in the veterinary field. Data on sales of veterinary antimicrobials from each member state (MS) are published in yearly reports as milligrams per Population Correction Unit (mg/PCU) [15, 16]. However, consumption data for companion animals is not yet included since data is not available in all MS [15].

Reporting of total consumption in the veterinary field is often limited to national surveillance schemes, and at the time of writing, 16 countries had systems in place to monitor AMU in livestock [17]. The systems apply different indicators and metrics [18], and only a fraction includes some variations of usage data on companion animals with data originating from pharmacy recordings, voluntary admissions, selected clinics, or surveys [19].

In Denmark, a national database (the Veterinary Statistics program, VetStat) collects sales data on drugs prescribed for veterinary consumption from three main sources; pharmacies, feed mills, and veterinar- ians [20–22]. Veterinary antimicrobials are available by prescription-only [21], and a purchase is linked with information on prescribing veterinarian, the report- ing pharmacy, Anatomical Therapeutic Chemical (ATC) codes or ATCvet-codes, and a species-specific ID. Sales recorded without a species-specific ID are listed with a replacement code (animal species code 0). Antimicrobials are sold from Danish pharmacies to veterinary practitioners or directly to farmers or pet owners on prescription. Products purchased by veterinary practitioners are often recorded in the VetStat database with only a veterinary practice ID. Danish veterinarians treating livestock are obliged to record each treatment in VetStat with information on species, age, and disease group. The veterinarian must also register a Central Husbandry Register code (CHR-number), which refers to the location and species of the farm property [20, 21]. Data is often transferred automatically to VetStat from the electronic billing system of the veterinary practice. Veterinarians treating companion animals are not subject to the same requirements, and there is no legal obligation for veterinarians to transfer data from Danish companion animals to VetStat [21, 22], which means that only sales from pharmacies to pet owners are linked with an animal species code.

The governmentally supported Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) publish yearly reports on AMU in Danish animals based on VetStat data [23]. Given the challenges connected to animal species codes omitted in part of the VetStat data, DANMAP has adopted a method of retrieving sales data on companion animals from several different VetStat tables [23–25]. Antimicrobials without an animal species code are allocated to companion animals based on product license (dogs and cats only) or preparations (tablets, capsules, ear- and eye drops). Additionally, oral preparations recorded as prescribed under the companion animal species code are omitted provided the products are licensed solely for livestock or horses. Parenteral preparations with multi-species authoriza- tion recorded without an animal species code are not assigned to companion animals [24]. This suggests that several obstacles emerge in retrieving accurate sales data for companion animals.

By 2029, all MS are required to report national AMU in companion animals [6, 26]. In addition, several countries are currently in the process of establishing national surveillance systems, with varying inclusion of animal species [17]. To comply with the forthcoming EU requirements, it is necessary to strengthen and validate existing national systems and to test the usability of each database in retrieving valid information on usage data on companion animals.

Therefore, the present study aimed to assess the usability of the VetStat database for estimating national sales of antimicrobials in Danish companion animals and to present data in mg/PCU as suggested by ESVAC [15]. More specifically the objectives were to: (1) quantify the total sales of AMs in Danish companion animals (dogs and cats) in 2018 from data available in the VetStat database, (2) stratify sales data based on antimicrobial classes, preparations, and licenses, (3) use the national sales data to calculate mg/PCU, and (4) describe and discuss the main challenges in using VetStat data to quantify total AMU in companion animals.

Methods

Descriptive analysis

Data were extracted from VetStat in august 2019 and descriptive analysis was performed in R (version 4.0.3 of 2020—The R Foundation for Statistical Computing) and in Microsoft Excel.

Extracting and processing VetStat data

To estimate the total amount of AMs sold for use in Danish companion animals in 2018, raw data from VetStat were extracted in august 2019 by the DVFA. Informa- tion on products purchased at the pharmacy (by animal owners or veterinary clinics) and products used by vet- erinary practitioners treating livestock are automatically transferred to the VetStat database. Data used in the present study thus originated from Danish pharmacies and veterinarians.

The methodology presented in DANMAP [24] was applied in the present study as a basic principle for the examination of VetStat data. In DANMAP [23], consumption data related to companion animals were compiled from AMs with no specified animal species (animal group code 0) provided that license, preparation, or concentration were applicable to companion animals and from products prescribed for companion animals with the exclusion of oral AMs licensed solely for horses and livestock. DANMAP does not allocate parenteral antimicrobials with multi-species approvals recorded without a specified animal species [24].

In the present study, two datasets were created from the tables in the raw VetStat data; one covering AMs sold directly from pharmacies for use in companion animals (recorded on animal group code 90) and another cover- ing AMs sold from pharmacies with no specified animal species (recorded on animal group code 0, i.e. a replacement code). Data entries are accepted in the database regardless of animal species. Consequently, AMs licensed for one animal species may erroneously be recorded under a conflicting animal group code. Each dataset thus contained the same variables (product ID, product name, prescribing veterinarian, receiving practice, CHR num- ber, and amount of active compound) to enable examina- tion of possible errors (Fig. 1). Consumption data were calculated as a weight-based unit (kg active compound). Information on product license was included by official

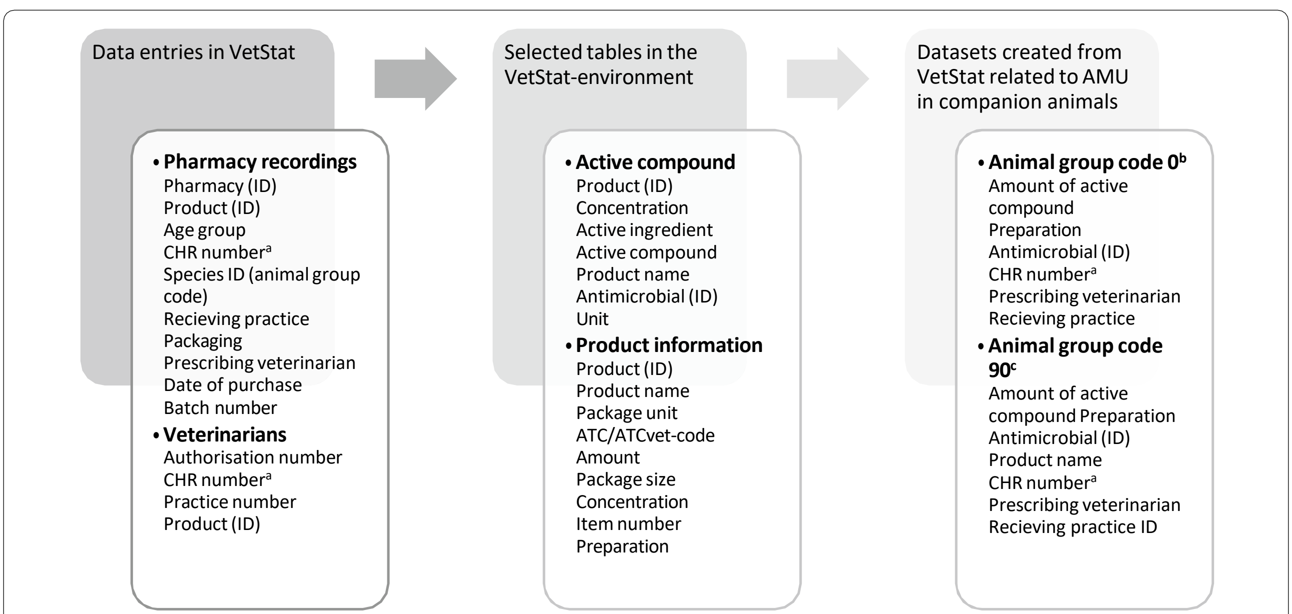

Fig. 1 Data entries and product details from selected tables in the VetStat environment. A simplified version of raw data in the VetStat environment. Data entries from pharmacies and veterinary practitioners are combined with variables from various tables in the VetStat environment to create datasets with information on antimicrobial use in companion animals. The datasets are created by extracting sales data recorded with either a companion animal ID or a replacement code. [a]Central Husbandry Register. The CHR number refers to the location, size, and type of a specific farm property [b]Corresponds to a species-specific ID [c]Anatomical Therapeutic Chemical classification system/ Anatomical Therapeutic Chemical classification system for veterinary medicinal products [d]Corresponds to records without a species-specific ID (replacement code). [e]Corresponds to records with a species-specific ID (animal group code) assigned to companion animals

product descriptions and approvals [27] validating February 2020. Both datasets were subdivided by license and preparation.

Because the database allows products to be recorded under conflicting animal group codes, AMs from animal group code 90 were excluded provided license or preparation indicated that the products in question were recorded under an erroneous animal group code. In the present study, intramammary, intrauterine, and topical preparations for livestock were excluded in addition to oral preparations licensed for horses or livestock. This differs slightly from DANMAP [23].

A similar procedure was applied to the data covering animal group code 0. Antimicrobials were transferred to the remaining data from animal group code 90, provided that license or preparation were pertinent to compan- ion animals. The quantity of AMs sold for use in Dan- ish companion animals was thus a calculated estimate, referred to hereafter as AMU_{calc}.

Furthermore, each dataset was examined for the presence of invalid practice numbers and omitted veterinary authorization numbers to evaluate product traceability.

AMU_{calc} covering 2018 was then stratified according to antimicrobial classes (aminoglycosides, amphenicols, cephalosporins, fluoroquinolones, lincosamides, macrolides, penicillins, pleuromutilins, sulphonamides, trimethoprim, and tetracyclines), preparation (parenteral, oral powder, tablets, oral paste, ointments, eye, and ear drops), and specific product name.

Parenteral antimicrobials

Parenteral antimicrobials sold from pharmacies for use in veterinary clinics were examined to assess if data alone were sufficient to indicate the quantity used for companion animals. New datasets were created from the raw VetStat data. The datasets covered parenteral AMs sold from pharmacies to veterinary practitioners (recorded with a veterinary practice number, but no CHR number) and parenteral AMs used by veterinary practitioners to treat livestock (recorded with a CHR number and livestock species ID and transferred to VetStat through the billing system). Sales and consumption data were calculated as a weight-based unit (kg active compound), and products with multi-species authorization were identified. The difference between the amount of parenteral AMs sold from pharmacies to veterinary practitioners and the parenteral AMs recorded by veterinary practitioners treating livestock was calculated. The difference between sold and consumed amount thus covered parenteral AMs sold to small animal veterinary practitioners, wastage, or products still on the shelf. It may indicate the magnitude of parenteral AMs administered for in-house treatment of companion animals.

Calculating the population correction unit

National sales data from each of the ESVAC participating countries are harmonized by including the population at risk in the denominator. The metric applied is the "population correction unit (PCU)", which serves as a standardized measure for the population potentially treated in each MS [15]. In the present study, PCU for Danish companion animals was calculated using a simplified version of the PCU for livestock [15]. Only dogs and cats are considered, as the significance of rodents and birds is negligi- ble in small animal practices in Denmark.

Calculation of the Population Correction Unit (PCU) for Danish companion animals:

$$PCU_{Companion\,animals} = (n_d * AW_d) + (n_c * AW_c)$$

where n is the estimated population size and AW is the average standard weight (in kg) at the time of treatment for dogs (d) and cats (c) respectively.

In the present study, $PCU_{Companion\ animals}$ were calculated using standard live weights of dogs (20 kg) and cats (4 kg) [25] and the individual population size of Danish dogs (810,000 heads) and cats (730,000 heads) [28].

The overall national consumption can thus be further approximated by adding sales data from companion animals (peroral AMs) and biomass ($PCU_{companion\ animals}$) separately to that of livestock already presented in the ESVAC reports.

Calculation of mg sold per PCU for Danish livestock and companion animals:

$$mg/PCU_{national} = \frac{national\ sales\ of\ antimicrobials\ (mg)}{PCU_{livestock}\ (kg) + PCU_{companion\ animals}\ (kg)}$$

Results

National antimicrobial sales data

Sales data assigned a companion animal ID (animal group code 90) covers sales of AMs from pharmacies for intended use in companion animals (mainly prod- ucts purchased by the pet owner). The consumption amounted to 515 kg active compound in 2018. However, 53% (275 kg active compound) were products licensed solely for use in livestock or horses. Because the database accepts sales data even though the license or preparation does not match the assigned animal group code, the products in question were regarded as registration errors, and thus not prescribed for companion animals. The products included oral paste licensed for horses (241 kg active compound), intramammary and intrauterine AMs licensed for cattle only (< 1 kg active compound), AMs solely for aquaculture (1 kg active compound), parenteral preparations licensed for horses or livestock (3 kg active compound), topical sprays for livestock (< 1 kg active compound), and feed additives for livestock (30 kg active compound). After deducting sales data inconsistent with AMU in companion animals, the remaining amount of AMs recorded on animal group code 90 was more than halved (240 kg active compound remaining).

Sales data assigned a replacement code (animal group code 0), hence recorded in the database without a species-specific ID, covers sales of AMs from pharmacies to veterinary practitioners and occurrences where a species-specific ID has been erroneously omitted. This amount was 5956 kg active compound in 2018.

Parenteral preparations represented more than half (3451 kg active compound), and the majority were products with multi-species authorization (2609 kg active compound). The remaining products recorded without a species-specific ID comprised peroral and topical antimicrobials. From license and preparations (tablets, ointment, ear- and eye drops, or licenses solely for dogs and cats), a total of 706 kg active compounds were identified and assigned to companion animals.

Hence, aggregated sales data from the different VetStat tables provided the source for a calculated estimate of AMU in Danish companion animals, and AMU_{calc} amounted to 946 kg active compound in 2018. AMU_{calc} is clearly an approximation and does not include parenteral AMs with multi-species approval assigned a replacement code.

Veterinary practice and authorization IDs provided information on presumed product distribution. The majority of AMs assigned a specific companion animal ID were sold from Danish pharmacies to pet owners from veterinary prescriptions (no assigned CHR number in the data). Less than three kg active compound was sold directly to veterinary practitioners or farmers (only a veterinary practice ID or CHR number is available in the data).

More than 90% of AMs assigned a replacement code (no species-specific animal ID) were products sold from pharmacies directly to veterinary practitioners (practice ID recorded). A small fraction was sold to farmers from veterinary prescriptions (CHR number recorded) or to individual veterinary practitioners (veterinary ID).

Parenteral antimicrobials

Examination of parental antimicrobials assigned a replacement code revealed that products with multi-species approval purchased for use in veterinary clinics greatly exceeded the amount retrieved in the veterinary recordings, i.e. treatment of livestock recorded by the veterinary practitioners.

Deducing consumption data recorded by veterinary practitioners from the pharmacy sales of antimicrobi- als directly to veterinary clinics yielded a difference of 552 kg active compound. This amount covers parenteral AMs that have not been used to treat livestock and there- fore contains the number of products with multi-species approvals used for the in-house treatment of companion animals. It covered purchases from 697 veterinary clinics (697 different veterinary practice IDs), but to which extent the amount also covered wastage or wrongly omit- ted recordings of CHR numbers could not be explained solely from the VetStat data.

A comparison of 2018 with previous years revealed that the quantity varied from 128 kg active compound in 2016 to 583 kg active compound in 2017.

Preparations and antimicrobial classes

By reference to AMU_{calc}, antimicrobials on peroral preparations (mainly tablets and capsules) were by far the most applied route of administration (915 kg active com- pound) for companion animals in 2018, followed by par- enteral (20 kg active compound) and topical preparations (11 kg active compound).

In Table 1, AMU_{calc} is further subdivided into antimicrobial classes. Extended-spectrum penicillins

Table 1 Calculated estimate (AMU_{calc}) of national sales of antimicrobials for use in Danish companion animals (dogs and cats) in 2018

Antimicrobial class	Preparations[1]			Total
	Peroral	Topical	Parenteral	
Penicillin (ext.)[a]	675	–	2	677
Penicillin (sim.)[b]	4	–	16	20
Cephalosporin (1st gen)	96	–	–	96
Cephalosporin (3rd & 4th gen)	–	–	1	1
Lincosamides	64	–	–	64
Tetracycline	23	< 1	–	24
Amphenicols	–	< 1	–	< 1
Aminoglycosides	–	1	< 1	1
Quinolones[c]	11	< 1	< 1	11
Macrolides	< 1	–	–	< 1
Sulfonamide[d]	1	–	< 1	2
Other[e]	40	8	–	48
Polymyxin	–	< 1	–	< 1
Total	915	11	20	946

[a] Penicilins with extended-spectrum (amoxicillin-clavulanate acid)

[b] Simple penicillins

[c] Specified values comprise fluoroquinolones

[d] Including sulfonamide/trimethoprim combinations

[e] Fucidic acid, sulfasalazine, and metronidazole

[1] Active compound (kg)

represented more than two-thirds in 2018 (677 out of 946 kg active compound). Cephalosporins accounted for 97 kg active compound, lincosamide, and tetracycline for 64 and 24 kg active compound, respectively. The remain- ing 84 kg active compound was distributed on amphen- ichols, aminoglycosides, fluoroquinolones, macrolides, simple penicillins, sulfonamide, trimethoprim combina- tions, tiamulin, and *others* (fusidic acid, sulfasalazine, and metronidazole). From AMU_{calc}, 12 kg active compound (1.3%) were antimicrobials categorized by WHO [13] as the highest prioritized CIAs. Administration of cephalosporins for companion animals was mainly 1st generation cephalosporin for peroral administration (96 kg active compound), whereas 3rd and 4th generation cephalosporin accounted for 1 kg active compound. Fluoroquinolones and macrolides were 11 and < 1 kg active compound, respectively.

Calculating the population correction unit

The population correction unit for Danish companion animals ($PCU_{companion\ animals}$) calculated in the present study was 19,120 tonnes. As a result, the approximated contribution from Danish companion animals to the overall national consumption amounts to 915 kg active compound (peroral AMs) and 19,120 tons of live weight.

In 2018, the magnitude of veterinary AMs for Danish livestock amounted to 38.2 mg sold per PCU [15]. The metric covers sales of 93.6 tonnes of active compound and a PCU of 2,446,700 tonnes. Adding the approximated contribution from companion animals brings the Danish consumption ($mg/PCU_{national}$) to 38.3 mg sold per PCU.

Data issues

Applying VetStat data as a means to estimate total sales of AMs in companion animals presented a number of challenges. The challenges, which are summarized in Table 2, refer to the extraction, analysis, and validation of data.

The main challenge in extracting total sales of veterinary AMs for companion animals was encountered in the data covering sales of antimicrobials to veterinary practitioners. Products sold from pharmacies to veterinary practitioners and used for the treatment of livestock were readily available by the veterinary recordings, but veterinary practitioners treating companion animals are not obliged to transfer data to VetStat, so the database does not contain any treatment data for Danish companion animals.

Consequently, products sold from pharmacies to veterinary practitioners are often recorded without a species ID (hence assigned the replacement code 0). Each sale is recorded with a veterinary practice ID, but the database does not cross-reference with the practice type (small, large, or mixed practice). Consumption data for companion animals, therefore, relies on a calculated estimate (in the present study referred to as $AMU_{calc)}$ with sales data retrieved and calculated from both animal group codes 0 and 90. If sales data were extracted solely from animal group code 90, companion animals would mainly account for pharmacy recordings of products sold to pet owners (i.e. veterinary prescriptions). Peroral and topical preparations distributed by veterinary practitioners (706 kg active compound in 2018) would be unaccounted for. It complicates data extraction and increases the risk of erroneous data analysis. This is in particular demonstrated by the lack of species-specific ID in parenteral AMs used in-house by small animal veterinary practitioners (recorded with only a practice number), which complicates any register data-based estimate of multispecies approved AMs for companion animals.

General data validation also proved challenging. From animal group code 0, 10.5% of the veterinary practice numbers were invalid (less than four digits) and a small segment of entries (approximately 1 kg active compound) was recorded with only product-specific information, but no CHR number, veterinary, or veterinary practice ID.

Table 2 Challenges encountered in using VetStat data to estimate antimicrobial sales data in Danish companion animals

Data entry	Data extraction	Analysis and interpretation of raw data
Only treatment data from livestock are transferred to VetStat	Data from several animal group codes may be necessary when assessing AMU[a] in one species	Knowledge of the nature of data and construction of the database is necessary
Missing or faulty recordings of Danish veterinary practice or authorization numbers	Raw data from a specific animal group code may contain sales data for other species than those relevant for the group code in question	Calculation of sales data from several animal group codes may be necessary
The database does not receive treatment data for companion animals		Interpretation of raw data from only one group code may lead to faulty conclusions
Information on animal species (animal group code) are not recorded for antimicrobials sold to veterinary practitioners		

[a] Antimicrobial use

Discussion

VetStat data related to companion animals is currently used as the data source for the yearly DANMAP reports. However, due to the EU regulation, it is expected that the focus will be directed more towards AMU in companion animals. This may lead to an increased interest in VetStat data on companion animals from both the veterinary authorities, researchers, and other stakeholders.

In this study, the method of estimating AMU in Dan- ish companion animals presented in DANMAP [24] was tested. Sales data from 2018 were reviewed with a thorough evaluation of specific products recorded both with and without an animal group code related to companion animals, and the study depicts the large disparity of recorded AMs for companion animals between animal group codes 0 and 90. AMU_{calc} is presented in kg active compound. Due to sales from pharmacies to veterinary practitioners often being recorded without an animal species code, parenteral antimicrobials from the pharmacy and veterinary recordings were examined to pre- sent an estimate of antimicrobials that could have been used in-house for companion animals. Furthermore, the study identified several challenges encountered when using register data such as the Danish VetStat database.

Quantification of national antimicrobial sales data

An objective of this study was to apply national sales data from the VetStat database as a means to estimate total sales of AMs in Danish companion animals. The VetStat database provided detailed information on overall AMs in both animal categories (i.e. group codes 90 and 0) selected for the present study.

The various tables in the VetStat environment enable national sales data to be extracted as prescription data recorded under a specific animal group code [22], which provides an easy overview of records for selected animal species. One approach to estimate AMU in companion animals would therefore be to extract sales data recorded solely on animal group codes related to companion animals, but the present study made it evident that it would generate insufficient estimates. These findings support the method applied in DANMAP and emphasize the necessity of retrieving usage data from several tables and animal group codes, although this may seem redundant in a database that separates data by animal species.

An important finding in this study was how oral preparations licensed solely for companion animals were recorded in the database. A skewed distribution between animal group codes 0 and 90 was evident as the amount of peroral preparations, mainly tablets, recorded on animal group code 0 exceeded the total amount recorded at animal group code 90 with more than 300 percent. The products were recorded with a veterinary practice ID, hence sold to veterinary practitioners, which emphasizes the issue of traceability occurring because small animal practitioners are not obliged to transfer data on AMU to VetStat. Similar challenges may be encountered in other national surveillance systems built with the primary aim of recording consumption data for livestock.

The difference in total recorded amount between ani- mal group codes greatly increases the risk of underesti- mating the actual AMU for companion animals provided data from animal group code 90 are used solely to quan- tify total AMU. Thus, the validity of results based solely on records from the VetStat database will rely greatly on the researcher's knowledge of the nature of the data and the structure of the database itself.

However, a database such as the Danish VetStat database with records of all products sold for use in companion animals is a vital resource in AM surveillance since it also includes preparations licensed for humans only. Other national monitoring systems do not include preparations licensed solely for humans in the surveillance of sales data for companion animals or total AMU is measured based on peroral preparations only [29, 30]. Overall, this entails an imminent risk of underestimating the overall veterinary AMU. In the VetStat database, 58 kg active compound of parenteral AMs licensed for humans were recorded without an animal species, however, it is still included in the overall national estimate from DANMAP [23].

Parenteral preparations

The study aimed to quantify the total sales of AMs for Danish companion animals. Therefore, parenteral AMs recorded without an animal species ID (replacement code 0) were examined to assess, whether VetStat data alone could elucidate the scope of products used in-house for companion animals by veterinary practitioners. Deduction of veterinary consumption data from pharmacy sales records provided an estimate of 552 kg active compounds of parenteral products with a multi-species license sold to 697 different veterinary practices. A valid estimate of the actual amount used to treat companion animals could not be inferred from data alone, since the amount most likely also covered unused products, wastage, or omitted usage recordings from large animal veterinarians. However, these findings strongly emphasize that allocation of the parenteral AMs in question to the correct animal species is crucial in order to produce valid estimates of companion animal sales data in the future.

The Netherlands Veterinary Medicines Authority (SDa) has addressed similar issues in estimating veteri- nary AMU based on sales and consumption data. Due to multi-species authorization, approximately 17 kg active compound could not be assigned a correct animal species. The SDa conducted a questionnaire survey among 100 veterinarians to elucidate the correct animal species to which the AMs were used [31]. Plausible solutions under Danish settings could be data validation through questionnaires distributed to each of the veterinary practitioners with purchases of the products in question. However, a solution like that is labor-intensive and dependent on a high response rate. A more sustainable solution would instead be found by including small animal veterinarians in the statutory data reporting to VetStat.

Antimicrobial classes and preparations

Products from the beta-lactam class were the most commonly prescribed AM for companion animals. The largest quantity was extended-spectrum penicillins, making up 72% of the total amount. Simple penicillins accounted for 2% and cephalosporins for 10%. This preponderance compares with data from Europe and the UK [29, 30, 32–35]. Lincosamides accounted for 8% of the total prescribed amount, and tetracyclines and fluoroquinolones for 3 and 1 percent respectively. In regards to lincosamides and fluoroquinolones, the results from the present study are in agreement with reports from Norway and Finland [29, 30], but Denmark differs in the use of tetracyclines when compared with neighboring countries. The larger amount of tetracyclines recorded in Denmark may be linked to those preparations often being licensed for human consumption, which contrary to VetStat, is not included in the estimates from Norway and Finland [29, 30]. Results from Spain and Italy show a different use of fluoroquinolones, as those preparations are reported as the most commonly used following penicillins and cephalosporins [34, 35].

AMs sold for use in Danish companion animals were most frequently for oral administration. The same is evident in other European countries [29, 30, 33, 34, 36].

Applied metrics

In VetStat, sales data for companion animals are presented as a weight-based indicator (kg active compound). There is currently no international consensus on appropriate metrics to report AMU or accommodate comparison of exposure data. Numerous metrics are proposed in the literature, but a lack of standardization may compromise the comparability of data [18, 37, 38]. Weight-based indicators are currently applied by DANMAP [23] and EMA [15] to describe trends in sales data stratified by nation or species. Presenting the weight of active compounds (mg or kg) alone or adjusted by a standardized correction unit (PCU) makes for an intuitive measure of overall sales data [39]. Although it does not provide information on drug potency [39] it does elucidate possible interspecies differences in AMU. Several dose-based metrics are applied in research or surveillance of AMU in livestock, with the "average defined daily dose" (DDDvet) presented in the ESVAC reports and "animal daily doses" (ADD) and the percentage of animals treated per day (ADDs per 100 animals per day) in Denmark [15, 23]. Such metrics require accurate estimates of population size, which is currently not available for companion animals in many MS.

Several studies have described AMU in small animal practice by quantifying usage data in selected clinics [34, 36, 40–43]. This enables detailed information on the population at risk, including weight, but does not yield information on the overall population size. The current method in [15] for quantifying AMU in livestock production is the use of a Population Corrected Unit (PCU) as the denominator. Companion animals are not yet included due to difficulties with consistent and valid information on population size and average weight from each MS [44]. The denominator presented in this study is a rough estimate, which emphasizes the challenges in achieving a valid estimate of the population at risk. The challenges of obtaining valid estimates from the currently available data are further reinforced by the highly approximated contribution of Danish companion animals to the overall national sales of AMs. For now, it is currently not possible to define the consumption of parenteral AMs in Danish companion animals, which means that valid comparison across member states remains limited. Transfer of usage data from in-house treatments of companion animals to VetStat and other national databases through the billing system could ensure that Denmark and other MS can provide valid data on AMU for companion animals to the ESVAC as is expected from 2029 [6].

Data issues

The primary purpose of the present study was to test the usability of the VetStat database to quantify AMs sold for use in Danish companion animals. The VetStat database proved useful, as data is easily accessible, detailed, and does not require extensive time to collect. However, data processing presented several challenges. The challenges arose in extracting a complete dataset covering the actual sales data for companion animals. In the present study, data from animal group code 90 proved incomplete, as it also contained preparations licensed for livestock only. To achieve an approximated estimate of total sales data for companion animals, a large amount of sales data had to be retrieved from another group code in the database. Although in this case, AMU_{calc} is incomplete, as it does not contain parenteral AMs used for in-house treatments by veterinary practitioners. In the present study, antimicrobials were grouped according to product preparation and license. Each product license was recorded and coded manually, as it is not part of the VetStat environment. To reproduce the study on a yearly basis, or if similar methods are used for retrospective studies, updating product licenses manually will be very labor-intensive.

The present study illustrates that the main challenge is how data on antimicrobials for companion animals are recorded and stored in VetStat. AMs prescribed by the veterinarian for intended use in companion animals are most often purchased at the pharmacy by the owner of the animal. Here, the animal species and veterinarian's authorization number are recorded in VetStat [20, 21] under animal group code 90. However, AMs sold to veterinary practitioners for use in-house are rarely recorded with a species-specific ID since no distinction is made by practice type. Most often, these products are only recorded with a veterinary practice number and the consumption data are subsequently transferred through the billing system when veterinarians treat livestock. This implies that parenteral antimicrobials used to treat companion animals, either in small animal or mixed practices, and peroral antimicrobials (often tablets) sold to the owner directly from the veterinary practice will only appear in VetStat at the pharmacy records (under the replacement code 0). For now, only peroral antimicrobials from the replacement code are transferred manually to companion animals.

A permanent solution to this issue would be to make it mandatory for Danish veterinarians in small animal and mixed practices to record each in-house treatment of companion animals in VetStat with species-specific information similar to what applies to veterinarians treating livestock. A change of this magnitude would require that the individual billing programs used in small animal practices are set up to automatically transfer data to the VetStat database. This would ensure that parenteral AMs used for in-house treatments of companion animals would be accounted for as well as peroral antimicrobials sold by veterinary practitioners.

A change of this magnitude will also allow researchers to extract valid estimates on AMU for companion animals from the veterinary recordings in the VetStat database. Transferring consumption data from small animal veterinary practitioners to the VetStat database will provide essential knowledge on the actual use of AMs in companion animals. It may also alleviate the challenges related to continuous monitoring of the population size and weight of the domesticated population of dogs and cats.

The present study also identified multiple data entries of peroral AMs with invalid veterinary practice IDs making it impossible to ensure valid product traceability. These findings demonstrate a profound issue related to the entry of sales data into the VetStat database as well as the indication of erroneous entries made at the pharmacy at the time of purchase. Entries of invalid veterinary identification ID or animal species ID have also been addressed and discussed by [45] in connection with AMU in Danish livestock. AMs prescribed by veterinary practitioners for use in livestock production are also purchased at the pharmacy. Pharmacies, therefore, partake an important role in entering valid and correct information on the sales of all veterinary antimicrobials to the VetStat database, but because the pharmacist conducts recordings manually [45], errors or omissions in the information entered in the VetStat database can occur.

Outlines of national medicinal databases and lessons learned from their use may serve as a prerequisite for how other databases can be designed and implemented. The importance is emphasized by the requirement for EU Member States to report valid estimates of AMU in companion animals. A database like the Danish VetStat is advantageous because all sales of antimicrobials for veterinary consumption are recorded and accounted for. Although the discrepancy between how detailed usage data in livestock is recorded and the fact that usage data in companion animals is not accounted for demonstrates that improvements are still required. If Denmark extends the mandatory transmission of consumption data to small animal veterinarians, it will also facilitate that outcomes of national measures and guidelines on prudent use of AMs in small animal medicine can be monitored from secondary data.

Conclusions

Owing to the structure of the VetStat database, quantification of AMU in Danish companion animals is an approximation. The actual consumption may be significantly higher than what is currently calculated from the database, as the majority of parenteral products used in-house by small animal veterinarians are not included. National AMU in companion animals can be measured more accurately provided treatment data from veterinary practitioners in small or mixed practices are transferred to the database through the billing system. This will equal the legal requirements for Danish veterinary practitioners treating livestock.

Transfer of usage data from in-house treatments of companion animals to VetStat will also ensure that Denmark can provide valid data on AMU for companion animals to the ESVAC as is expected from 2029 since population size and standard weight remain uncertain.

Abbreviations
AM: Antimicrobial; AMU: Antimicrobial use; AMU_{calc}: A calculated estimate of antimicrobials sold for use in Danish companion animals; AMR: Antimicrobial resistance; ATC: Anatomical therapeutic chemical classification system; ATCvet: Anatomical therapeutic chemical classification system for veterinary medicinal products; CIA: Critically important antimicrobials for human medicine; CHR: Central husbandry register code; DANMAP: Danish integrated antimicrobial resistance monitoring and research program; DVFA: The Danish food and veterinary administration; ESVAC: The European surveillance of veterinary antimicrobial consumption; MS: European member states; PCU: Population correction unit.

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Author contributions
AG contributed by conceptualization, data interpretation, and drafting of the manuscript. ABK contributed by conceptualization, data extraction, resources, and revision of the manuscript. LRN contributed to the methodology and revision of the manuscript. HS contributed by methodology and revision of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and analyzed during the current study are available in anonymized form from the corresponding author on reasonable request. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Declarations

Ethics approval and consent to participate
This study did not require official or institutional ethical approval.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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Publisher's Note

Appendix A

Table A1. Mg of antimicrobials per kg pig produced in 2014

2014	Number of heads[a]	Weight of age group (DVFA Standard weights)	Antimicrobials sold[b]	Mg/kg of produced pig
Number of pigs delivered to Danish abattoirs or exported for slaughter	19.2 mio	50 kg 19.2 mio x 50 kg = 960,000,000 kg	25,749 kg	26.82 mg/kg finisher pig for slaughter
Weaners exported for rearing or breeding purposes	10.9 mio	15 kg 10.9 mio x 15 kg = 163,500,000 kg	36,131 kg	220.98 mg/kg weaners for export
Total production of finishers for slaughter and weaners exported for rearing and breeding	30.1 mio	960,000,000 kg + 163,500,000 kg = 1,123,500,000 kg	86,020 kg[c]	76.56 mg/kg pig produced

[a]Danish Agriculture & Food Council, 2019
[b]DANMAP, 2015
[c]Antimicrobials sold for use in sows and piglets included

Table A2. Mg of antimicrobials per kg pig produced in 2012

2022	Number of heads[a]	Weight of age group (DVFA Standard weights)	Antimicrobials sold[b]	Mg/kg of produced pig
Number of pigs delivered to Danish abbatoirs or exported for slaughter	18 mio	50 kg 18 mio x 50 g = 900,000,000 kg	15,249.5 kg	16.94 mg/kg finisher pig for slaughter
Weaners exported for rearing or breeding purposes	13.8 mio	15 kg 13.8 mio x 15 kg = 207,000,000 kg	39,023.4 kg	188.52 mg/kg weaners for export
Total production of finishers for slaughter and weaners exported for rearing and breeding	31.8 mio	900,000,000 kg + 207,000,000 kg = 1,107,000,000 kg	71,355.9 kg[c]	64.46 mg/kg pig produced

[a]Danish Agriculture & Food Council, 2023
[b]DANMAP, 2023
[c]Antimicrobials sold for use in sows and piglets included

Table A3. Mg of antimicrobials per kg pig produced according to DVFA and EMA standard weights

2022	**Number of heads**[a]	**Antimicrobials sold**[b]	**Weight of age group (DVFA Standard weights)**	**Mg/kg of produced pig (DVFA)**	**Weight of age groups (EMA standard)**[c]	**Mg/kg of produced pig (EMA)**
Number of pigs delivered to Danish abbatoirs or exported for slaughter	18 mio	15249.5 kg	50 kg 18 mio x 50 g = 900,000,000 kg	16.94 mg/kg finisher pig for slaughter	65 kg 18 mio x 65 kg = 1,170,000,000 kg	13,03 mg/kg finisher pig for slaughter
Weaners exported for rearing or breeding purposes	13.8 mio	39023.4 kg	15 kg 13.8 mio x 15 kg = 207,000,000 kg	188.52 mg/kg weaners for export	25 kg 13.8 mio x 25 kg = 345,000,000 kg	113,11 mg/kg weaners for export
Number of sows	945,000	17083.0 kg	200 kg 945,000 x 200 kg = 189,000,000 kg	90.34	240 kg 945,000 x 240 kg = 226,800,000 kg	75.32 mg/kg for live sows
Mg/PCU						
			Standard weights (DVFA)	**Mg/PCU (Vetstat)**	**Standard weights (EMA)**	**Mg/PCU (EMA)**
Mg/PCU		71355.9 kg	900,000,000 + 207,000,000 + 189,000,000 = 1,296,000,000 kg	55,06 mg/PCU	1,170,000,000 + 345,000,000 + 226,800,000 = 1,741,800,000 kg	40.97 mg/PCU

[a]Danish Agriculture & Food Council, 2023
[b]DANMAP, 2023
[c]European Medicines Agency, 2011

Appendix B

An English version of the questionnaire used for the telephone interviews to collect data on herd characteristics in Danish herds with weaners for objective 1.

Appendix B: Questionnaire

English version of the questionnaire used for telephone interviews of 52 Danish pig producers with a Yellow Card in weaners enrolled in the case-control study. The results of the questionnaire are presented in detail in Manuscript I. All interviews were conducted by one interviewer (the author of this thesis). For all herds, the person interviewed was either the herd owner or the person responsible for management and antimicrobial treatment.

Section 1

1. **What age groups are on the property (more than one possible answer)**
    - Sows (including piglets and gilts)
    - Weaners
    - Finishers (including non-gestating gilts)

2. **The number of pen places for weaned pigs**

    ____

3. **How many staff members work primarily within the weaner section?**

    ____

4. **How many years of experience in working with pigs does the person responsible for working with the weaners have? (if the owner is responsible, state their experience)**

    ____

5. **Are weaners being introduced to the premises from stables outside the property (i.e. from another CHR number)?**
    - Yes (including sometimes)
    - No

6. **Are herd health diagnoses commonly treated in newly weaned pigs < 4 weeks post-weaning** (explanation: a herd health diagnosis is determined by the veterinary practitioner based on recurring clinical symptoms)
    - Yes
    - No

7. **What are the most treated herd health diagnoses in newly weaned pigs (< 4 weeks post-weaning)**

    - Gastrointestinal diseases
    - Respiratory diseases
    - Musculoskeletal disorders
    - Neurological disorders

8. **Are herd health diagnoses commonly treated in older weaned pigs 4-8 weeks post-weaning?**
    - Yes
    - No

9. **What are the most treated herd health diagnoses in older weaned pigs (4-8 weeks post-weaning)**

    - Gastrointestinal diseases
    - Respiratory diseases
    - Musculoskeletal disorders
    - Neurological disorders
    -

10. **Which antimicrobial preparation type is typically used to treat the most common herd diagnosis?**
    - Peroral preparations (group medication)
    - Parenteral preparations (single animal treatment)
    - Both (if both – then it is recorded under peroral preparation)
    -

11. **Has there been an outbreak of disease requiring antimicrobial treatment in weaners for which there is no prior herd health diagnosis?**
    - Yes
    - No

12. **When treating the most common herd health diagnosis in weaners < 4 weeks post-weaning - what is the average age (in weeks)?**

    ____

13. **When treating the most common herd health diagnosis in weaners < 4 weeks post-weaning - what is the average weight (in kilograms)**

    ____

14. **When treating the most common herd health diagnosis in weaners > 4 weeks post-weaning - what is the average age (in weeks)?**

    ____

15. When treating the most common herd health diagnosis in weaners > 4 weeks post-weaning - what is the average weight (in kilograms)

____

16. How is the weight of the weaners assessed before antimicrobial treatment is initiated?
- Weighing (weighing is accepted if batches are weighed regularly)
- Visual

17. Are there written instructions for flock medication (dose, number of treatments, clinical signs) available at the site where the antimicrobials are measured and mixed?
- Yes
- No (written instructions but on computer elsewhere equals no)

18. What is the average weaning weight (weight at entry into the weaner unit)

____

19. What is the average weaning age (in days)

____

20. Where does the feed for weaned piglets mainly come from?
- Bought
- Homegrown (also accepted if only vitamin mixed are purchased)

21. What type of feed is used for newly weaned piglets (1-3 weeks post-weaning)?
- Dry/pelleted
- Wet/gruel
- Other

22. What feeding strategy is used in the first 3 weeks post-weaning
- Ad libitum (including multi-phase feeding systems)
- Restrictive

23. Is there room for all weaned pigs to always eat at the same time the first 4 weeks post-weaning?
- Yes
- No

24. How is the changeover between feed mixes?
- Abrupt
- Gradually (including multi-phase feeding systems)

25. Are organic acids added to water or feed?

- Yes
- No

26. Are drinking spouts checked for functionality before newly weaned pigs are introduced to the weaner unit?

- Yes
- No
- Sometimes (sometimes equals no)

27. Is there an all-in/all-out structure in the weaner unit (= sectional all-in/all-out)?

- Yes (all in is also accepted as a yes provided no "not all out" is followed by cleaning and empty time)
- No
- Sometimes (sometimes equals no)

28. How often are weaners introduced to the weaner sections (when there are already pigs)? (this question is used to control for the use of all-in/all-out management)

- *Never*
- *Sometimes*
- *Regularly*

29. How many days are the sections in the weaner unit left empty between each batch? (from the last pig leaving until the next pig is brought in)

____

30. Is heating used to dry each section?

- Yes
- No
- Sometimes (sometimes equals no)

31. Are sections in the weaner unit washed between the batches?

- Yes
- No
- Sometimes (sometimes equals no)

32. Are the sections in the weaner unit disinfected between the batches?

- Yes
- No
- Sometimes (sometimes equals no)

33. Are the sections in the weaner unit dried between the batches?

- Yes (only accepted if completely dry)
- No
- Sometimes (sometimes equals no)

34. Are activity/rooting materials washed/replaced between batches?
(if hay/straw is used then “yes”)

- Yes
- No
- Sometimes (sometimes equals no)

35. Are the aisles washed before moving weaners into the weaner unit?

- Yes (including if always washed after moving pigs of all agegroups)
- No
- Sometimes (sometimes equals no)

36. Are there routines for cleaning the water pipes?

- Yes
- No
- Sometimes (sometimes equals no)

37. Is footwear washed daily (at the end of the workday)?

- Yes
- No
- Sometimes (sometimes equals no)

38. Is there separate equipment used exclusively in the weaner unit?

- Yes
- No
- Sometimes (sometimes equals no)

39. Are work routines such that you move from youngest to oldest, handling sick pigs last?

- Yes
- No
- Sometimes (sometimes equals no)

40. Are there any mixing of age groups? (mixing of age groups also includes moving of older pigs through sections of younger pigs and moving younger pigs through sections of older pigs)

- Yes
- No
- Sometimes (sometimes equals no)

41. Are designated entry rooms used when entering and leaving the stables?

- Yes
- No
- Sometimes (sometimes equals no)
-

Section 2:

For case herds:

- A1: What do you consider to be the main reasons why the herd was assigned a Yellow Card in weaners?
- A2: What changes do you think have had the biggest impact on reducing AMU in weaners following the Yellow Card?

For control herds

- B1: What do you consider having been the main reasons why you maintain a low AMU in weaners?
- B2: What have been the biggest challenges in ensuring a low AMU in weaners?

Appendix C

The biological associations between meat inspection lesions in herds with either a high, medium and low AMU in finishers. Data used for Figure 2 in Manuscript II.

***Table B.1**. The biological associations between meat inspection lesions in herds with a high AMU in* finishers

High AMU class	Pyemia	Pericarditis	Lung lesions	Peritonitis	Hernia	Osteomyelitis	Arthritis	Abscesses, trunkt	Abscesses, extremities	Tail lesions	Scar, contusion, bursitis,
Pyemia											
Pericarditis	P = 0.26 OR: 1.44										
Lungs lesions	P = 0.17 OR: inf	P <0.001 OR: 8.21									
Peritonitis	P = 0.0018 OR: 4.28	P <0.001 OR: 4.45	P <0.001 OR: 8.53								
Hernia	P = 0.0019 OR: 4.60	P = 0.4 OR: 1.18	P <0.001 OR: 6.38	P <0.001 OR: 4.95							
Osteomyelitis	P <0.001 OR: 6.12	P = 0.0003 OR: 1.76	P <0.001 OR: 16.29	P <0.001 OR: 3.06	P <0.001 OR: 2.49						
Arthritis	P <0.001 OR: 4.01	P <0.001 OR: 1.9	P <0.001 OR: 6.26	P <0.001 OR: 3.24	P <0.001 OR: 4.16	P <0.001 OR: 3.02					
Abscesses, trunk	P = 0.003 OR: 5.57	P = 0.02 OR: 1.62	P <0.001 OR: 13.03	P <0.001 OR: 3.81	P <0.001 OR: 5.33	P <0.001 OR: 9.08	P <0.001 OR: 3.62				
Abscesses, extremities	P = 0.002 OR: Inf	P <0.001 OR: 3.22	P <0.001 OR: 24.66	P <0.001 OR: 4.69	P <0.001 OR: 5.61	P <0.001 OR: 9.60	P <0.001 OR: 5.74	P <0.001 OR: 8.33			
Tail lesions	P <0.001 OR: 6.75	P <0.001 OR: 1.80	P <0.001 OR: 10.27	P <0.001 OR: 2.80	P <0.001 OR: 2.53	P <0.001 OR: 6.76	P <0.001 OR: 2.63	P <0.001 OR: 6.29	P <0.001 OR: 6.91		
Scar, contusion, butsitis	P = 0.003 OR: Inf	P <0.001 OR: 4.51	P <0.001 OR: 27.55	P <0.001 OR: 7.04	P <0.001 OR: 7.40	P <0.001 OR: 5.37	P <0.001 OR: 5.12	P<0.001 OR: 9.47	P <0.001 OR: 11.09	P<0.001 OR: 3.33	
Condemnation	P <0.001 OR: 9.93	P = 0.42 OR: 1.14	P=0.0003 OR: 5.32	P <0.001 OR: 2.65	P <0.001 OR: 3.55	P <0.001 OR: 3.88	P <0.001 OR: 3.08	P <0.001 OR: 4.05	P <0.001 OR: 6.81 (	P<0.001 OR: 2.93	P<0.001 OR: 5.39

Table B.2. *The biological associations between meat inspection lesions in herds with a low AMU in* finishers

Low AMU class	Pyemia	Pericarditis	Lung lesions	Peritonitis	Hernia	Osteomyelitis	Arthritis	Abscesses, trunk	Abscesses, extremitis	Tail lesions	Scar, contusion, bursitis
Pyemia											
Pericarditis	P = 0.03 OR: 0.82										
Lung lesions	P <0.001 OR: 5.44	P <0.001 OR: 5.83									
Peritonitis	P <0.001 OR: 1.79	P <0.001 OR: 4.66	P <0.001 OR: 13.93								
Hernia	P <0.001 OR: 2.69	P <0.001 OR: 1.82	P <0.001 OR: 8.77	P <0.001 OR: 4.41							
Osteomyelitis	P <0.001 OR: 3.81	P <0.001 OR: 1.62	P <0.001 OR: 5.84	P <0.001 OR: 2.36	P <0.001 OR: 2.43						
Arthritis	P <0.001 OR: 2.03	P <0.001 OR: 1.75	P <0.001 OR: 9.33	P <0.001 OR: 3.25	P <0.001 OR: 3.87	P <0.001 OR: 2.49					
Abscesses, trunk	P <0.001 OR: 4.79	P <0.001 OR: 1.63	P <0.001 OR: 12.59	P <0.001 OR: 3.45	P <0.001 OR: 4.01	P <0.001 OR: 7.93	P <0.001 OR: 3.79				
Abscesses, extremities	P <0.001 OR: 3.97	P <0.001 OR: 2.65	P <0.001 OR: 18.14	P <0.001 OR: 4.97	P <0.001 OR: 5.49	P <0.001 OR: 4.99	P <0.001 OR: 6.17	P <0.001 OR: 8.46			
Tail lesions	P <0.001 OR: 3.26	P <0.001 OR: 1.51	P <0.001 OR: 7.47	P <0.001 OR: 2.36	P <0.001 OR: 2.76	P <0.001 OR: 4.57	P <0.001 OR: 2.40	P <0.001 OR: 5.69	P <0.001 OR: 4.90		
Scar, contusion, bursitis	P <0.001 OR: 3.41	P <0.001 OR: 2.66	P <0.001 OR: 17.95	P <0.001 OR: 5.08	P <0.001 OR: 5.17	P <0.001 OR: 3.41	P <0.001 OR: 4.52	P <0.001 OR: 7.65	P <0.001 OR: 10.86	P <0.001 OR: 4.51	
Condemnation	P <0.001 OR: 6.39	P <0.001 OR: 1.41	P <0.001 OR: 2.79	P <0.001 OR: 2.82	P <0.001 OR: 2.79	P <0.001 OR: 3.53	P <0.001 OR: 2.81	P <0.001 OR: 3.78	P <0.001 OR: 3.48	P <0.001 OR: 2.36	P <0.001 OR: 3.10

Table B.3. *The biological associations between meat inspection lesions in herds with a medium AMU in* finishers

Medium AMU class	Pyemia	Pericarditis	Lung lesions	Peritonitis	Hernia	Osteomyelitis	Arthritis	Abscesses, trunk	Abscesses, extremities	Tail lesions	Scar, contusion, bursitis
Pyemia											
Pericarditis	P = 0.12 OR: 0.88										
Lung lesions	P <0.001 OR: 7.88	P <0.001 OR: 5.79									
Peritonitis	P <0.001 OR: 2.08	P <0.001 OR: 4.21	P <0.001 OR: 14.23								
Hernia	P <0.001 OR: 2.70	P <0.001 OR: 1.71	P <0.001 OR: 9.75	P <0.001 OR: 4.92							
Osteomyelitis	P <0.001 OR: 3.70	P <0.001 OR: 1.57	P <0.001 OR: 7.98	P <0.001 OR: 2.50	P <0.001 OR: 2.61						
Arthritis	P <0.001 OR: 2.77	P <0.001 OR: 1.54	P <0.001 OR: 10.02	P <0.001 OR: 3.32	P <0.001 OR: 3.81	P <0.001 OR: 2.70					
Abscesses, trunk	P <0.001 OR: 5.24	P <0.001 OR: 1.45	P <0.001 OR: 16.30	P <0.001 OR: 3.66	P <0.001 OR: 4.77	P <0.001 OR: 8.08	P <0.001 OR: 4.32				
Abscesses, extremities	P <0.001 OR: 4.49	P <0.001 OR: 2.27	P <0.001 OR: 18.38	P <0.001 OR: 5.20	P <0.001 OR: 7.10	P <0.001 OR: 5.41	P <0.001 OR: 6.28	P <0.001 OR: 10.11			
Tail lesions	P <0.001 OR: 3.33	P <0.001 OR: 1.42	P <0.001 OR: 7.91	P <0.001 OR: 2.36	P <0.001 OR: 2.96	P <0.001 OR: 4.39	P <0.001 OR: 2.52	P <0.001 OR: 5.74	P <0.001 OR: 5.96		
Scar, contusion, bursitis	P <0.001 OR: 7.24	P <0.001 OR: 2.25	P <0.001 OR: 18.66	P <0.001 OR: 4.93	P <0.001 OR: 6.30	P <0.001 OR: 3.95	P <0.001 OR: 5.60	P <0.001 OR: 9.05	P <0.001 OR: 12.78	P<0.001 OR: 4.86	
Condemnation	P <0.001 OR: 5.84	P <0.001 OR: 1.40	P <0.001 OR: 3.35	P <0.001 OR: 2.80	P <0.001 OR: 3.11	P <0.001 OR: 3.44	P <0.001 OR: 2.82	P <0.001 OR: 3.96	P <0.001 OR: 4.05	P<0.001 OR: 2.43	P<0.001 OR: 3.94

Table B.4. *The biological associations between meat inspection lesions in herds with a high, medium and low AMU in* finishers

All AMU classes	Pyemia	Pericarditis	Lung lesions	Peritonitis	Hernia	Osteomyelitis	Arthritis	Abscesses, trunk	Abscesses, extremities	Tail lesions	Scar, contusion, bursitis
Pyemia											
Pericarditis	P = 0.01 OR: 0.87										
Lung lesions	P <0.001 OR = 6.82	P <0.001 OR: 5.89									
Peritonitis	P <0.001 OR = 1.99	P <0.001 OR: 4.43	P <0.001 OR: 13.90								
Hernia	P <0.001 OR: 2.76	P <0.001 OR: 1.74	P <0.001 OR: 9.13	P <0.001 OR: 4.68							
Osteomyelitis	P <0.001 OR: 3.80	P <0.001 OR: 1.60	P <0.001 OR: 6.71	P <0.001 OR: 2.45	P <0.001 OR: 2.51						
Arthritis	P <0.001 OR: 2.45	P <0.001 OR: 1.65	P <0.001 OR: 9.58	P <0.001 OR: 3.30	P <0.001 OR: 3.87	P <0.001 OR: 2.60					
Abscesses, trunk	P <0.001 OR: 5.15	P <0.001 OR: 1.55	P <0.001 OR: 14.23	P <0.001 OR: 3.58	P <0.001 OR: 4.40	P <0.001 OR: 8.01	P <0.001 OR: 4.05				
Abscesses, extremities	P <0.001 OR: 4.43	P <0.001 OR: 2.50	P <0.001 OR: 18.83	P <0.001 OR: 5.09	P <0.001 OR: 6.16	P <0.001 OR: 5.20	P <0.001 OR: 6.26	P <0.001 OR: 9.23			
Tail lesions	P <0.001 OR: 3.38	P <0.001 OR: 1.47	P <0.001 OR: 7.82	P <0.001 OR: 2.38	P <0.001 OR: 2.86	P <0.001 OR: 4.53	P <0.001 OR: 2.47	P <0.001 OR: 5.77	P <0.001 OR: 5.40		
Scar, contusion, bursitis	P <0.001 OR: 4.69	P <0.001 OR: 2.51	P <0.001 OR: 18.83	P <0.001 OR: 5.10	P <0.001 OR: 5.67	P <0.001 OR: 3.61	P <0.001 OR: 4.98	P <0.001 OR: 8.33	P <0.001 OR: 11.90	P <0.001 OR: 4.63	
Condemnation	P <0.001 OR: 6.22	P <0.001 OR: 1.40	P <0.001 OR: 3.16	P <0.001 OR: 2.82	P <0.001 OR: 2.99	P <0.001 OR: 3.48	P <0.001 OR: 2.84	P <0.001 OR: 3.92	P <0.001 OR: 3.87	P <0.001 OR; 2.43	P <0.001 OR: 3.56