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Risk factors for antimicrobial use in Dutch pig farms: A cross-sectional study

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ABSTRACT

Background: Antimicrobial use (AMU) has decreased significantly in Dutch pig farms since 2009. However, this decrease has stagnated recently, with relatively high AMU levels persisting mainly among weaners. The aim of this study was to identify farm-level characteristics associated with: i) total AMU and ii) use of specific antimicrobial classes.
Methods: In 2020, cross-sectional data from 154 Dutch pig farms were collected, including information on AMU and farm characteristics. A mixed-effects conditional Random Forest analysis was applied to select the subset of features that was best associated with AMU.
Results: The main risk factors for total AMU in weaners were vaccination for PRRS in sucklings, being a conventional farm (vs. not), high within-farm density, and early weaning. The main protective factors for total AMU in sows/sucklings were *E. coli* vaccination in sows and having boars for estrus detection from own production. Regarding antimicrobial class-specific outcomes, several risk factors overlapped for weaners and sows/sucklings, such as farmer's non-tertiary education, not having free-sow systems during lactation, and conventional farming. An additional risk factor for weaners was having fully slatted floors. For fatteners, the main risk factor for total AMU was PRRS vaccination in sucklings.
Conclusions: Several factors found here to be associated with AMU. Some were known but others were novel, such as farmer's tertiary education, low pig aggression and free-sow systems which were all associated with lower AMU. These factors provide targets for developing tailor-made interventions, as well as an evidence-based selection of features for further causal assessment and mediation analysis.

1. Background

Antimicrobial resistance (AMR) has been listed by the World Health Organization (WHO) as one of the top ten global threats to human health (World Health Organization, 2019), with one of the main reservoirs being livestock (Manyi-Loh et al., 2018). In the Netherlands, policies have been applied since 2009 that reduced AMU by over 77% in all livestock and by 72% in pigs specifically, based on farm-level prescription data analysed by the Netherlands' Veterinary Medicines Institute (in Dutch: Stichting Diergeneesmiddelenautoriteit - SDa), which monitors national antimicrobial usage in Dutch livestock sectors. However, that decreasing trend has stagnated in recent years (SDa, 2023) and weaners remain the highest consumers of antimicrobials (SDa, 2023) within the Dutch pig sector similar to other European countries (Dewulf et al., 2022)(Nunan, 2022). Specifically in 2022, the mean consumption of antimicrobials in weaners was 14.6 $DDDA_F$ (Defined Daily Dosage for Animals per livestock Farm), while AMU in sows and suckling piglets was 2.8 $DDDA_F$ and in fatteners 2.2 $DDDA_F$. As routine usage in the Netherlands is prohibited by law since 2011 (Speksnijder et al., 2015), i.e. only therapeutic AMU is allowed, AMU is driven by the actual herd

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health status, which is in turn affected by farm conditions and husbandry practices. Thus, technical factors on farm have predictive power over AMU.

In livestock, AMU is known to be influenced by various groups of factors related to farm management (Collineau et al., 2018), while it is not uncommon for antimicrobials to be used as "remedies" for improper biosecurity (Magnusson et al., 2021). Two literature reviews on studies on risk factors for AMU in pig farms have showed the importance of both internal and external biosecurity (Bokma et al., 2018)(Dhaka et al., 2023). For example, concerning internal biosecurity, lower within-farm stocking densities, vaccination, all-in/all-out systems (AIAO) at all stages, and older weaning age, have been associated with lower AMU. Conversely, shorter farrowing rhythms and poor air quality were associated with higher AMU. Regarding external biosecurity, mixing of animals from different farms is a known risk factor, whereas proper quarantine of incoming animals, being an organic/extensive farm and low chance of other pig herds presence within 500 m, have shown to have protective effects. Apart from biosecurity, other groups of factors are at play too, including nutrition, micro-climate conditions, environmental richness and behavioural factors, such as farmers' knowledge and awareness on AMU and AMR (McKernan et al., 2021), and farmers' perceived motivations and barriers (Houben et al., 2020).

In this study, we assessed the main technical characteristics of pig farms (i.e., rearing conditions and husbandry practices during production) that could influence AMU as to identify targets for further AMU reduction. We approached the epidemiological study of these factors with machine-learning, such as Random Forest. Thanks to the flexibility and non-parametric nature of these models, results can be provided both at the population and individual level (Foster et al., 2011) (Lu et al., 2018). The analysis aimed at answering the following questions: which modifiable farm characteristics and management-related factors are associated with i) total AMU in sows/sucklings, weaners, and fatteners, and with ii) use of specific antimicrobial classes in sows/sucklings and weaners? While weaners were the main focus of this study because they account for the highest AMU in the Dutch pig sector (SDa, 2023), the first research question included also sows/sucklings and fatteners to understand better how some factors influence total AMU throughout the whole production line. The second research question focussed on the different antimicrobial classes because class-specific AMU may entail different biological underpinnings that determine such AMU, and therefore provide additional insights for controlling it. The second research question was only addressed for sows/sucklings and weaners, as specific information for fatteners was lacking to perform these analyses. Moreover, sows and sucklings could not be analysed separately, as their AMU data are jointly collected.

2. Materials and methods

2.1. Study design and participants

In 2020, data on 154 multiplier pig farms in the Netherlands were collected using a cross-sectional study design. Of these farms, 151 kept weaners, 144 had sows/sucklings and 86 also had fatteners. The underlying farm census population was 1833, 1659 and 4005 farms, respectively (SDa, 2020a). Moreover, 16 enrolled farms with both weaners and sows/sucklings were organic under the "Better life 3" label (The Dutch Society for the Protection of Animals, 2023), whereas the other farms were conventional. All 154 farms included in the study were enrolled through their respective veterinarians. The five largest veterinary practices specialized in porcine medicine in the Netherlands were invited to participate, and a total of 34 veterinarians working in these practices contributed to the study. These veterinarians were selected internally by each veterinary practice based on their availability and interest in the study; no criteria were imposed by the researchers. In the Netherlands, there is a mandatory one-to-one relationship between veterinary practices and farms, meaning that a given farm can only receive veterinary care services by one registered veterinary practice. The enrolled veterinary practices were asked to randomly select farms from the ones under their care, with the only criteria being to have weaners and that they have complete overview of the characteristics and applied practices in those farms. Each veterinary practice was asked to enrol 50 farms, but not all practices managed to reach that target; the internal farm participation rate per practice was unknown to the researchers.

2.2. Data collection

Data for this study were collected through an offline digital questionnaire filled out by the participating veterinarians themselves. This could be done in the office or directly on site during their routine farm visits. Veterinarians could decide for themselves when and how to complete the questionnaire. Some questions might have required direct assessment during a farm visit and even a farmer's input, depending on the veterinarian's specific knowledge of the farm in question. The questionnaire gathered information on AMU, overall and per antimicrobial class, along with a wide range of farm's structural and management characteristics. As the main focus was on weaners, most questions were specific for weaners, and also for sows/sucklings given their close link to the weaning phase, but less specific over fatteners. The data collection period lasted 12 months and the data represented the farms' situation during the whole year of 2019. Both veterinarians and farmers were informed about the study aim and consented in analysing the anonymised data for the purpose of scientific research. For the labour time spent in completing the questionnaires, the veterinarians were compensated financially according to their standard hourly rates.

More specifically, the questionnaires collected AMU data for 11 antimicrobial classes: aminoglycosides, amphenicols, combinations, macrolides-lincosamides, other (here only spectinomycine was reported), penicillins, pleuromutilins, polymyxins, quinolones, tetracyclines and trimethoprim-sulphonamides. These classes were defined according to SDa classification (SDa, 2023) and guidelines published by the Dutch Royal Society for Veterinary Medicine (in Dutch: Koninklijke Nederlandse Maatschappij voor Diergeneeskunde - KNMvD) regarding AMU in pigs (KNMvD, 2019). The yearly AMU data, as total and per antimicrobial class, are recorded in a harmonized way for reporting to the SDa through the national farm quality assurance system of the pig sector. Veterinarians are obliged to register every medicine delivery in a database of that system, for check of compliance with veterinary medicines regulations, $DDDA_F$ calculation for reporting to the SDa and benchmarking of AMU with other farms. For the current study, the participating veterinarians could then simply extract the AMU data from the aforementioned database, to be then reported in the questionnaire. Total AMU and AMU per antimicrobial class were expressed as Defined Daily Dosage per Animal per Year (DDDA/Y). As for $DDDA_F$, AMU in DDDA/Y is equal to the amount of active substances divided by the total weight of the number of animals (the standardized weights for sucklings, weaners, fatteners and sows are 4.5, 17.51, 70 and 220 kg respectively (SDa, 2020b)) and mean authorized dosage (Lekagul et al., 2018), showing the average number of authorized dosages the average animal on the farm is exposed to. This can also be interpreted as the average number of days an animal is treated (with standardized daily dosages) in a farm within a year (Werner et al., 2018). The AMU outcomes of interest were: total DDDA/Y in sows/sucklings, weaners, and fatteners; use or not (i.e., class specific DDDA >0) of macrolides-lincosamides, penicillins, tetracyclines and trimethoprim-sulphonamides in sows/sucklings and weaners (separately); and total DDDA/Y of macrolides-lincosamides, tetracyclines and trimethoprim-sulphonamides in weaners. The aforementioned class-specific AMU outcomes were included because they were significantly associated with at least one disease aetiology for applying a group treatment in the respective group from previous analyses of the same data set (Stefanopoulou, 2022).

Previous research has shown that non-medical risk factors occur within several aspects of farming and can play a significant role in a farm's total AMU (Collineau et al., 2018)(Bokma et al., 2018)(Dhaka et al., 2023). Here, with the collected data about farm characteristics, it was possible to include an extensive list of factors providing an overview of the main technical aspects of pig production, as well as the possible pathogen transmission pathways, internal and external biosecurity standards, housing conditions, vaccination schemes, feed and water quality, husbandry practices, structural features (e.g., number of workers, production type, etc.) and a few characteristics of the farmer, such as educational background. The questions were in total 226 and comprised binary, categorical (mutually exclusive), custom input, continuous, count and Likert scale input variables. The latter had 5 predefined levels described within the survey. The survey was constructed based on input from the pig veterinarians and academic experts in the field, scientific literature and other similar surveys (e.g., Biocheck UGent (Gelaude et al., 2014)). Table S1 in supplementary material contains the descriptive statistics of all the variables included in the different models and Table S2 includes an English translation of the survey (Supplementary file 1), as well as the original Dutch questionnaire as used (Supplementary file 2).

2.3. Statistical analysis

The relationship between farm practices and AMU is mediated through diseases. However, as disease occurrence and AMU lay in the same causal pathway, including them in the same model-building process would interfere with the association between farm characteristics and AMU. Therefore, the analysis focussed on the direct associations between farm characteristics and AMU, without considering the mediation. Class-specific AMU outcomes were assessed both as binary and continuous variables. The binary AMU variables indicated only if a specific class was used or not, which was related to a specific group of diseases (e.g., respiratory diseases). However, the continuous AMU variables might provide more insights into the dosing needs. This is because for DDDA/Y to increase, it is required that either the kg of treated animals for the same amount of antimicrobials are reduced or that the antimicrobials used for the same amount of animals are increased. Either way, the need for increased dosage might be an indication of persistent health problems.

A variable selection procedure was applied to identify risk factors at farm level for different AMU outcomes. The analysis for sows/sucklings and weaners as mentioned in the introduction was performed both at the level of total DDDA/Y and DDDA/Y per antimicrobial class, while in fatteners only the former was assessed. For each age category, only biologically relevant questions were considered in each initial set, herd size (i.e. total reared sows and wearers within the year of 2019) was always forced as a-priori control covariate and the number of observations was adjusted accordingly. We used a mixed-effects conditional Random Forest analysis with “MixRF” (Wang and Chen, 2016) and “party” R packages (Hothorn et al., 2006). Two nested random intercepts for each veterinarian within each veterinary practice was applied to correct for potential clustering given the study design. A Random Forest framework was preferred to address the various interaction effects (Wright et al., 2016) and multicollinearity that are expected given the complexity of livestock farms. Variable selection was applied manually using the steps from the “binomialRF” R package (Zaim et al., 2020)(Zaim et al., 2019), but by using a conditional Random Forest with mixed-effects model instead (hereinafter referred to as CmRF model). This novel algorithm greatly reduces computational time as it uses significantly less models and iterations compared to others (Zaim et al., 2020). The steps of the selection algorithm were as follows:

i) Run a CmRF model with all X_j variables from the initial set.
ii) Tabulate the frequency counts of root node splitting variables.
iii) Calculate the probability of randomly selecting a X_j as a root node using Eq. (1).

$$p_{root} = \frac{1}{m}\left[1 - \prod_{g=1}^{m}\frac{P-g}{P-(g-1)}\right] \tag{1}$$

with P being the total number of X_j and m being the number of subsampled variables for each tree (i.e. P/3).

iv) Conduct a binomial exact test for significance of a variable X_j with Eq. (2).

$$P\left(X_j = F_j | V\right) = \binom{V}{F_j} p_{root}^{F_j}\left(1-p_{root}\right)^{V-F_j} \tag{2}$$

with F_j the frequency counts of X_j and V the total number of trees.

v) After correcting for the family-wise error, we selected X_j variables with a Bonferroni-adjusted *p*-value <0.05

The above procedure was iterated 10 times using the “binomialRF Model Averaging” method (Zaim et al., 2020)(Zaim et al., 2019), where in each run an additional 10% of total features was added randomly without replacement. Variables present in less than half of the iterations were considered noisy and thus not selected. A specific optimal subset for each AMU outcome was defined with the above steps. Furthermore, in all final selected models, the average effect size for each variable was estimated using the method of partial dependence as performed in the “rfUtilities” R package (Evans and Murphy, 2019)(Cafri and Bailey, 2016).

Due to randomness intrinsic to the random forest algorithm, the results varied slightly across runs; thus, the above two-step selection algorithm was applied iteratively 20 times and variables appearing in all iterations were kept. Subsequently, normal and Sidak-adjusted bootstrapped confidence intervals were calculated with 200 iterations for each of the selected variables in the different models. As conditional Random Forest (Hothorn et al., 2006) handles missingness using surrogate splits (Hothorn et al., 2015) the level of 60% in the predictors was allowed. However, due to missingness, it was not possible to calculate conditional importance of the predictors in each model, and because unconditional importance gives biased results when correlated variables are present (Strobl et al., 2009)(Levshina, 2020), this measure was avoided. Variables with low variation (<10%) were excluded. The open-source environment R version 4.0.3 (R Core Team, 2020) was used for all analyses.

3. Results

The average AMU per surveyed farm was 14.4 DDDA/Y (median = 9.8; SD = 15.9). In weaners, the mean AMU was 11.01 DDDA/Y (SD = 14.8), while in sows/sucklings and fatteners it was 2.5 (SD = 2.6) and 2.1 DDDA/Y (SD = 3.4), respectively. Overall, tetracyclines, penicillins, trimethoprim-sulphonamides and macrolides-lincosamides were the most used antimicrobial classes. Fig. 1 summarizes the total sum of DDDA/Y per antibiotic class and age group.

Associations between farm characteristics and different AMU outcomes are shown in Figs. 2, 3, 4 and 5. Hereinafter, when a result is expressed in DDDA/Y, it refers to the expected change in the quantity of AMU in total or of a specific antimicrobial class (i.e. similar to a b-coefficient in a regression), and when it is expressed in percentage, it refers to the change in the relative probability of using a specific class. In both cases, zero indicates no predicted change.

3.1. Results for overall antimicrobial use

Overall, for weaners' total AMU, the largest risks (i.e. increased

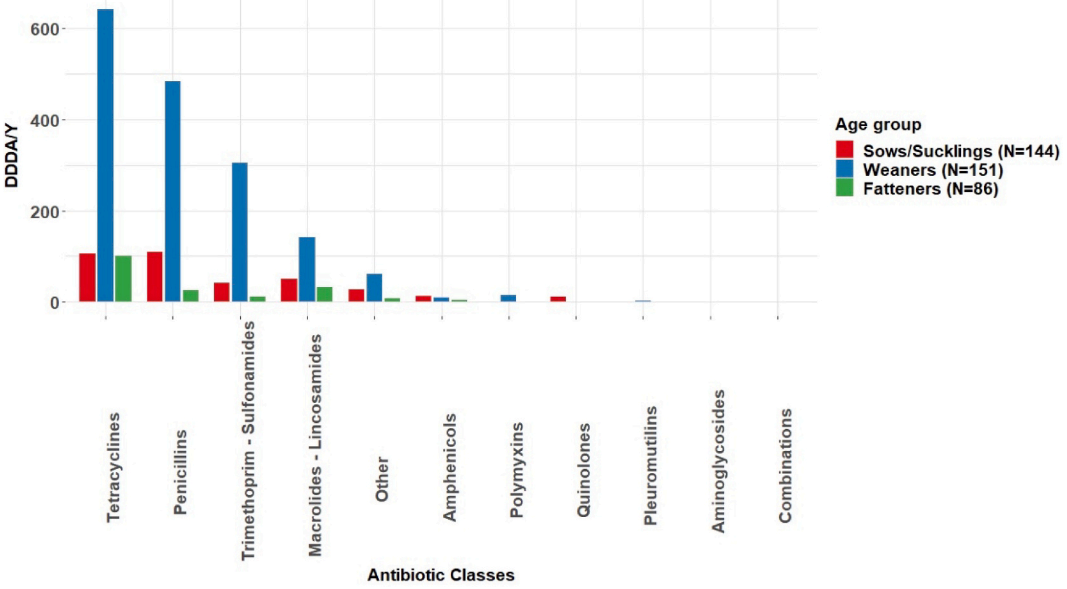

Fig. 1. Total sum of antimicrobial use of all farms per class and age category (N is the number of farms with those age groups); the three groups appear in the same order as in the legend. DDDA/Y is a harmonized metric for quantifying AMU and it is defined as the ratio of total mg of active substance divided by the standard dosing level (mg/kg) and total kg of animals on farm. The mean AMU per antimicrobial class per farm can be inferred by dividing each bar level with the number of farms in each age group.

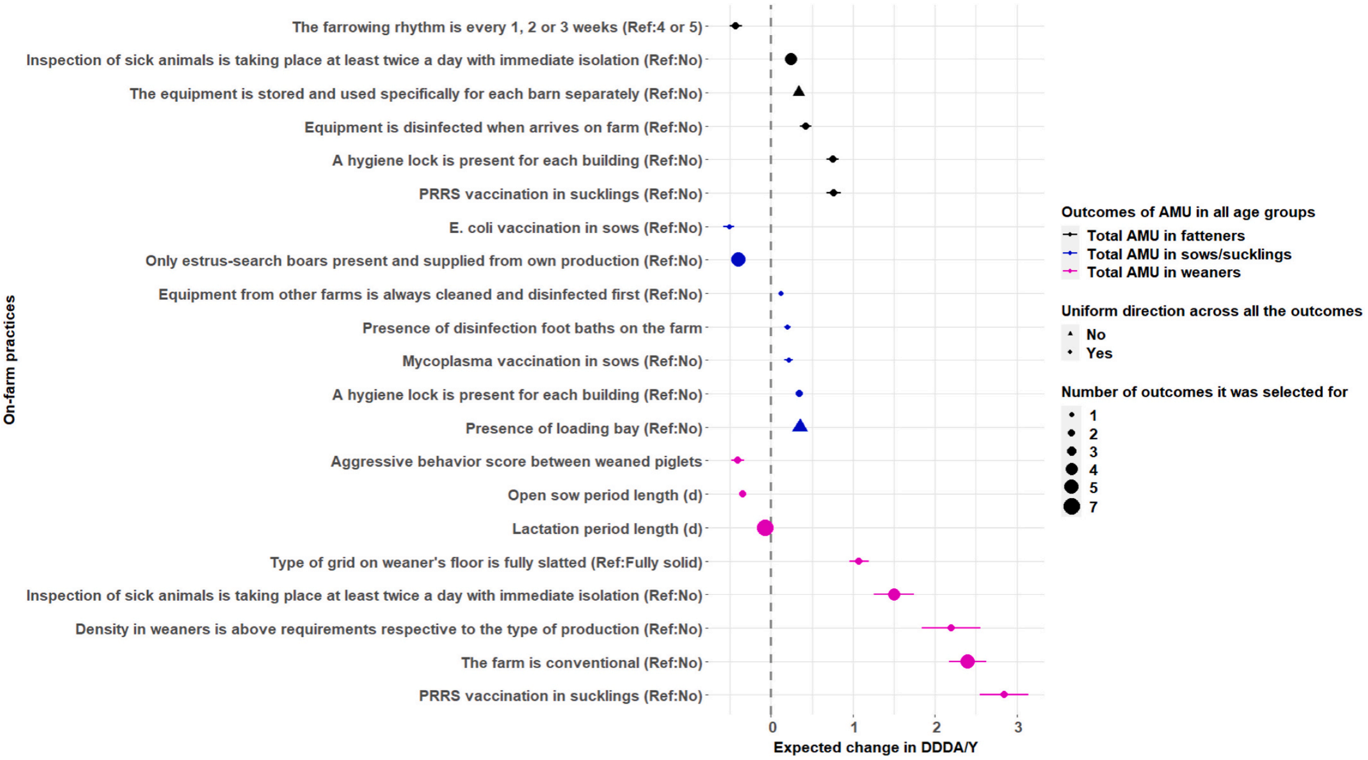

Fig. 2. Farm characteristics associated with the total AMU in weaners, sows/sucklings and fatteners. Colours represent the outcomes, the size of the symbols represents the number of AMU outcomes this characteristic was associated with across all models (including also the rest AMU outcomes in Figs. 3, 4 and 5), and the shape shows whether in any of these cases (affecting more than two outcomes) the association was of the same direction (triangle signifies different directions and circle same direction). Effect sizes are shown in increasing order for each outcome. The effect size shows the expected change in DDDA/Y; if it is positive it is a risk, if it is negative it is protective and if it is 0 no effect is expected; the three outcomes appear in the same order as in the legend.

AMU) were observed for application of PRRS vaccination in sucklings, being a conventional farm (vs. not; i.e. organic but also “Better Life label 1” (The Dutch Society for the Protection of Animals, 2023)), and having higher stocking densities than the farm production scheme allows (2.8, 2.4 and 2.2 higher DDDA/Y, respectively). Inspection of clinically diseased animals at least twice a day with immediate isolation and presence of fully slatted floors in weaner buildings followed (1.5 and 1.1 DDDA/Y, respectively). The last three variables, in terms of effect size,

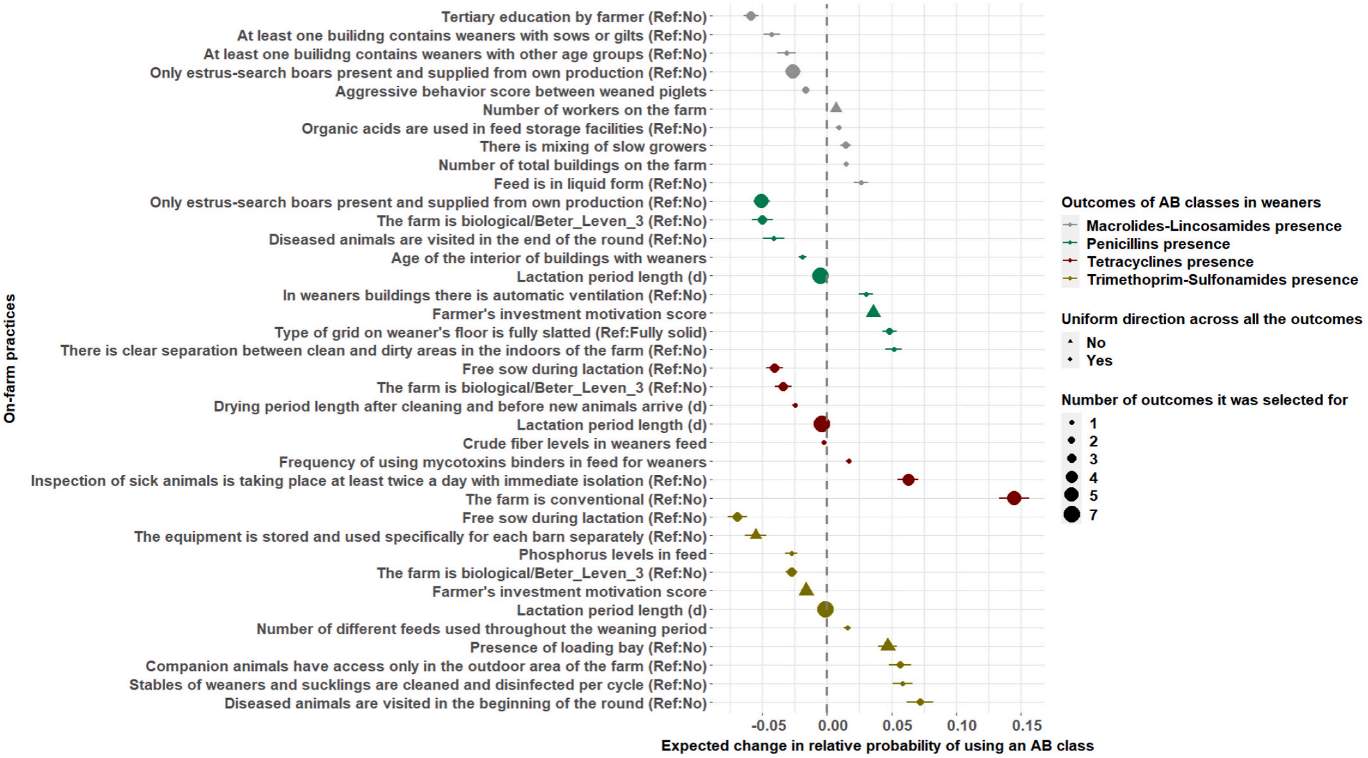

Fig. 3. Farm characteristics associated with the use of specific antimicrobial (AB) classes in weaners. Colours represent the classes, the size of the symbols represents the number of AMU outcomes this characteristic was associated with across all models (including also the rest AMU outcomes in Figs. 2, 4 and 5), and the shape shows whether in any of these cases (affecting more than two outcomes) the association was of the same direction (triangle signifies different directions and circle same direction). Effect sizes are shown in increasing order for each outcome. Each point shows the expected change (%) in the relative probability of using an AB class, if it is positive it is a risk, if it is negative it is protective and if it is 0 no effect is expected; the four outcomes appear in the same order as in the legend.

were lower post-weaning aggressive behaviour (based on Likert scale score given by the veterinarian), longer lactation and open sow periods (i.e. period from weaning to next insemination) (−0.4, −0.3, −0.1 DDDA/Y respectively). For AMU in sows/sucklings, the largest effects were observed for *Escherichia Coli* (*E.coli*) vaccination in sows, having estrus-search boars from own production, presence of a loading bay and having a hygiene lock per stall (vs. having one lock for the whole farm) (−0.5, −0.4, 0.4 and 0.3 DDDA/Y, respectively). For fatteners, PRRS vaccination in sucklings, having a hygiene lock per stall and following a farrowing cycle of one, two or three (vs. four or five) rounds, showed the three largest effect sizes (0.76, 0.75 and −0.43 DDDA/Y, respectively). Fig. 2 shows all results for these three outcomes.

3.2. Results per antimicrobial class

Figs. 3, 4 and 5 summarize all results from the class-specific AMU models. Looking at the top effect size, increased tetracycline use (which also had the highest DDDA/Y values) in weaners was associated with being a conventional farm (14.5%). The highest risk for quantity used was observed for farms with higher pig densities than the farm production scheme allows (1.96 DDDA/Y). For tetracycline use in sows and sucklings, farmer's tertiary education was the most protective factor (−10.9%). For penicillin use in weaners, having clear separation of clean and dirty zones in the indoor area of the farm was the main risk factor (5.2%). For sows/sucklings, penicillin use was associated with being a conventional farm (9.6%). Both use and quantity of trimethoprim-sulphonamides in weaners was positively associated with starting the farm round by visiting the diseased animals first (7.2% and 0.7 DDDA/Y respectively). For the same class in sows/sucklings, contact of companion animals with production animals had the largest effect (−10.6%). Lastly, for macrolides-lincosamides, their probability of being used in sows/sucklings was associated with having a loading bay (−7.6%). For both amount and probability of being used in weaners, farmer's tertiary education had the top effect size (−0.30 DDDA/Y and −5.8%, respectively).

3.3. Results for both total AMU and antimicrobial classes

Given the relatively large number of outcomes and predictors in this study, the epidemiological importance of a variable was not necessarily based on its effect size alone, but also on whether it appeared as significant both for total AMU and the probability of using an antimicrobial class in a specific age group. Factors consistently associated with at least two AMU outcomes (i.e. total AMU and a specific class) were adopting a longer average lactation length, which was associated with decreased use of trimethoprim-sulphonamides, tetracyclines and penicillins (−0.1%, −0.4% and −0.05%, respectively), total use of the former two classes (−0.02 and −0.01 DDDA/Y, respectively) and total AMU in weaners (−0.074 DDDA/Y). Having an estrus-search boar from own production was associated with less total AMU in sows/sucklings (−0.4 DDDA/Y) and lower relative probability of using macrolides/lincosamides in the same age group (−3.6%). Being a conventional farm was associated with increased total AMU (2.4 DDDA/Y) and tetracyclines in weaners (14.5%, 1.24 DDDA/Y). Inspecting diseased animals at least twice a day with immediate isolation showed a positive association with total AMU in weaners (1.5 DDDA/Y) and increased the probability of using tetracyclines and the quantity used (6.3% and 1.2 DDDA/Y). Lower post-weaning aggression was associated negatively with total AMU in weaners (−0.41 DDDA/Y) and the probability of using macrolides/lincosamides (−1.6%), while the presence of a fully slatted floor in the same age group appeared as a risk factor for both total AMU and the probability of using penicillins (1.07 DDDA/Y and 4.9%, respectively). Higher pig density than the farm production scheme allows was a risk factor for total AMU and quantity of tetracyclines used in weaners (2.2,

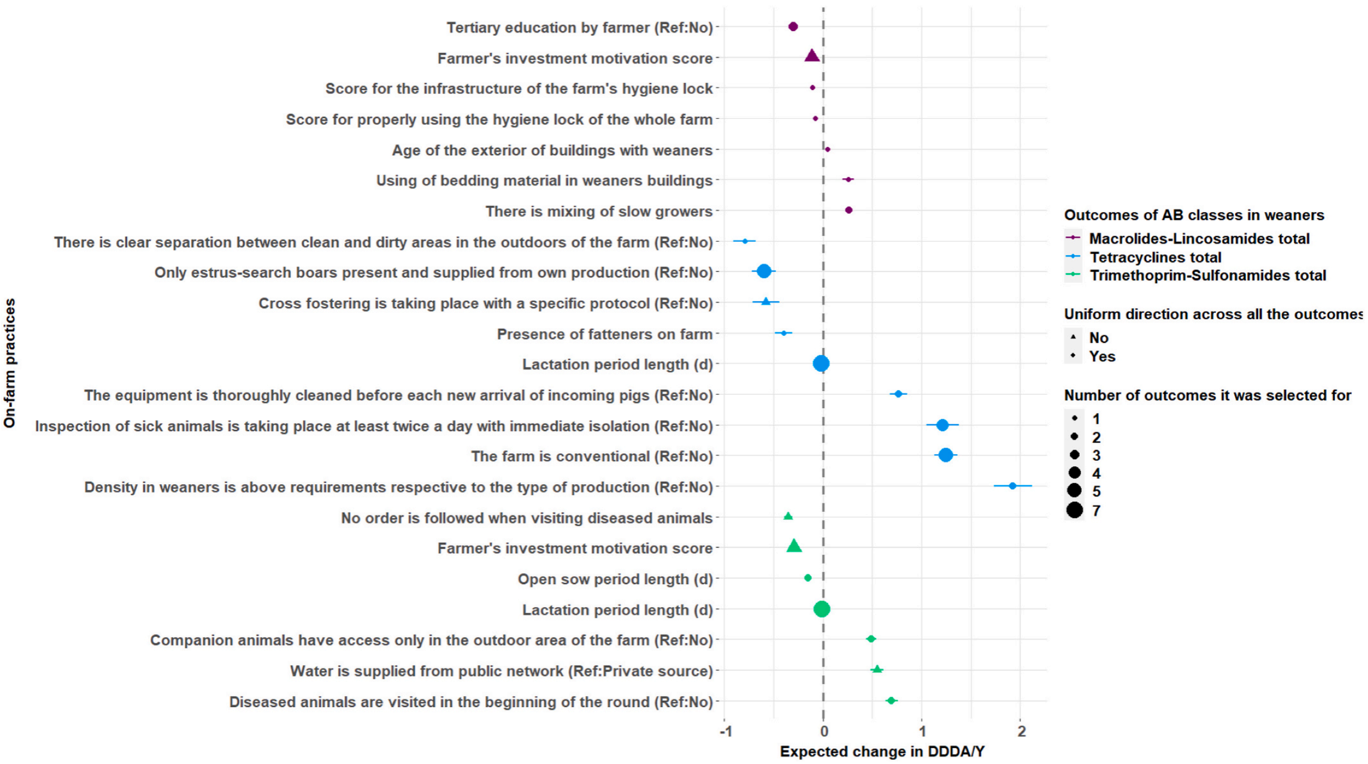

Fig. 4. Farm characteristics associated with the total quantities of specific antimicrobial (AB) classes in weaners. Colours represent the classes, the size of the symbols represents the number of AMU outcomes this characteristic was associated with across all models (including also the rest AMU outcomes in Figs. 2, 3 and 5), and the shape shows whether in any of these cases (affecting more than two outcomes) the association was of the same direction (triangle signifies different directions and circle same direction). Effect sizes are shown in increasing order for each outcome. The effect size shows the expected change in DDDA/Y; if it is positive it is a risk, if it is negative it is protective and if it is 0 no effect is expected; the three outcomes appear in the same order as in the legend.

1.93 DDDA/Y). Lastly, a longer open-sow period appeared as a protective factor against total AMU and quantity of trimethoprim-sulphonamides in weaners (−0.35 and −0.15 DDDA/Y, respectively).

4. Discussion

In this study, associations between farm characteristics and AMU were assessed using cross-sectional data from 154 Dutch pig farms in 2019. The focus of the study was on weaners, as they appear to be the highest antimicrobial consumers in the Dutch pig sector (SDa, 2023). Apart from total AMU, the DDDA/Y levels per antimicrobial class were also of interest. As the suckling period is critical for the weaning stage afterwards, the class-specific analysis also included sows/sucklings, while for fatteners only one model was built for total AMU.

Overall, the main protective factors for AMU in sows and suckling piglets were *E. coli* vaccination in sows, having estrus-search boars from own production, farmer's tertiary education and not being a conventional farm (either organic or “Better life 1” label). *E. coli* vaccination in sows is known to protect from neonatal diarrhoea (Riising et al., 2005). Another issue related to vaccination is needle management. Here, changing the needle for every pen was protective against penicillin use. Repeated use of needles is of concern for pathogen transmission, as well as inflicting larger punctures (Owen et al., 2022). To further reduce stress and increase pig welfare, needle-free vaccines can be considered (Dalmau et al., 2021)(Temple et al., 2020), although their use was not associated here with decreased AMU either. Having a closed herd is among the best farm practices in terms of external biosecurity (Alarcón et al., 2021)(Maes et al., 2008). Also farmer's education is a key (indirect) determinant of prudent AMU, as it promotes suitable practices and awareness about AMR (McKernan et al., 2021). Interestingly, although lower education tends to be a problem in farmers from developing countries, in developed ones, despite the higher education level, misconceptions still persist among them (e.g., doubts on impact of extensive livestock AMU on AMR and public health) (McKernan et al., 2021). The higher AMU risk in conventional vs. organic farms may derive from higher animal densities (Leeb et al., 2014), which act as stressor and favour pathogen transmission, and/or from policy on organic production that requires the farm to be extremely prudent with AMU in general (Regulation (EU) 2018/848 (European Commission, 2018)). Given that organic farms indeed are quite different in terms of practices compared to conventional ones, stratified analysis was also performed. The results were similar and thus it was chosen to include organic and conventional farms in one analysis. Previous studies have shown that organic farms have also lower resistance levels in their *E. coli* isolates compared to conventional ones (Mencía-Ares et al., 2021) (Österberg et al., 2016). Next, frequent checks of drinking water appeared as a protective factor, but obtaining water from a public supplier (instead of a private water well or similar) showed mixed results. In general, biosecure drinking water management is vital for pig health, as it can be a common transmission pathway of pig pathogens and resistance (Schmithausen et al., 2018).

In weaners, several factors associated with their AMU outcomes were shared with the other age groups, such as being a conventional farm, having estrus-search boars from own production and farmer's tertiary education. Among the main risk factors associated with total AMU in weaners, we found PRRS vaccination in sucklings, higher within-farm densities than the adopted production scheme requires, inspecting diseased animals frequently and presence of fully slatted floors. Although the finding for PRRS vaccination could be a result of reverse causality (as farms experiencing recurrent outbreaks also tend to vaccinate more), there are vaccine related drawbacks too, including failure to prevent infection/transmission, as well as modified-live PRRS vaccines that may revert to virulence (Charerntantanakul, 2012)(Chae, 2021)(Zhou et al., 2021). The finding regarding the health inspection is

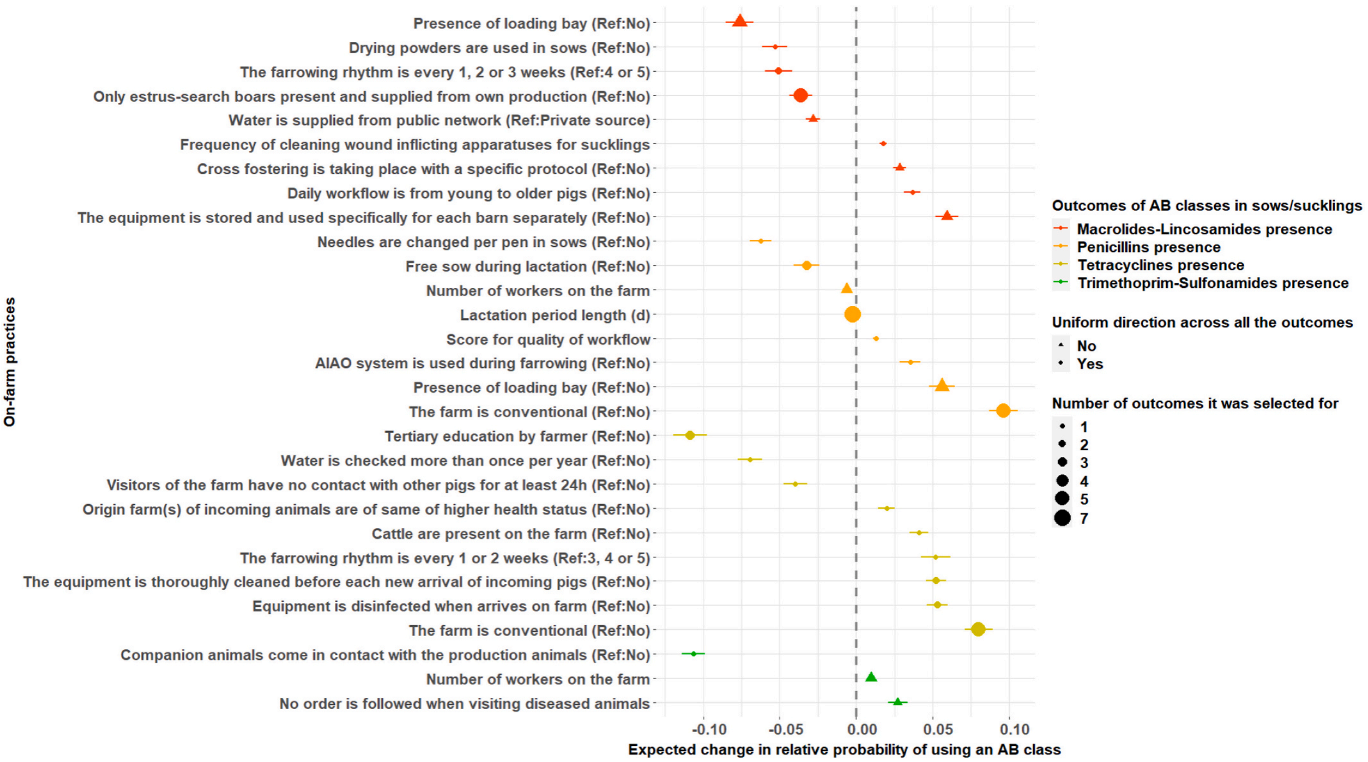

Fig. 5. Farm characteristics associated with the use of specific antimicrobial (AB) classes in sows/sucklings. Colours represent the classes, the size of the symbols represents the number of AMU outcomes this characteristic was associated with across all models (including also the rest AMU outcomes in Figs. 2, 3 and 4), and the shape shows whether in any of these cases (affecting more than two outcomes) the association was of the same direction (triangle signifies different directions and circle same direction). Effect sizes are shown in increasing order for each outcome. Each point shows the expected change (%) in the relative probability of using an AB class, if it is positive it is a risk, if it is negative it is protective and if it is 0 no effect is expected; the four outcomes appear in the same order as in the legend.

also most likely due to reverse causality and it mirrors the importance of regular checks and immediate isolation of animals that need additional care while, unsuitable flooring causing wounds and limiting normal pig behaviour, is a well-known risk factor (Kilbride et al., 2009). In particular fully slatted floors have been associated with higher lameness, but also with lower *Salmonella* prevalence (Vermeij et al., 2009). In our data, fully slatted floor was associated with group treatments for musculoskeletal/neurological diseases, which mostly comprised arthritis/meningoencephalitis by *Streptococcus suis* (*S. suis*) infections, while no group treatments for salmonellosis took place in weaners. Here the exact width of the grid was not assessed, only if it was partially solid-partially slatted or fully solid/slatted floor. Overall, it was observed that from fully solid to partially slatted floors, and from partially slatted to fully slatted floors, there was a gradual increase in risk of total AMU in weaners (5% and 18% mean bootstrapped increase in DDDA/Y respectively). Better animal welfare due to less stress has been linked to lower AMU (Albernaz-Gonçalves et al., 2022) and overall here we found comparable evidence. Specifically, lower aggression in weaners was associated with decreased AMU, and it is known that aggression is associated with various health issues (Boyle et al., 2022). Longer lactation lengths (mainly the contrast between early and late weaning; i.e. 21–28 vs. 37–50) showed a protective effect and it is known to provide more immunocompetent piglets, which show better performance and viability later on (López-Vergé et al., 2019)(Cabrera et al., 2010). Interestingly, based on our models, AMU in weaners was higher for farms that bought suckling piglets of 9 kg (thus, lactation did not take place there) even from farms that applied short lactation periods of 21 to 28 days (see Fig. S1 in Supplementary file 1). Having a free-sow system during lactation appeared to be protective against use of tetracyclines and trimethoprim-sulphanamides in weaners and penicillins in sows/sucklings. Farms with weaners in our data set used tetracyclines mostly to treat respiratory infections, while for trimethoprim-sulphanamides the aetiologies varied. Penicillins in sows/sucklings were used mainly for musculoskeletal/neurological diseases, which were mostly comprised by *S. suis* infections in sucklings and coronary band infections in sows. Free-sow systems seem to be an alternative option to the farrowing crates, allowing for more natural behaviour (Zhang et al., 2020). Although then the risk of crushed piglets is higher, overall survival is comparable between the two types of systems. This is because, at least in part, the crushed piglets usually are the weakest ones, which are also those more likely to succumb from other causes anyway (Spörri-Vontobel et al., 2023). In our analysis, disentangling this effect from being an organic farm was difficult and underpowered because all 16 organic farms applied free-sow systems, while within conventional farms only five did. Nevertheless, by exploring the structure of the relevant RF models it cannot be excluded that these five farms improve the predictive ability of each model. Whether free-sow systems reduce AMU even in non-organic farms would require further inquiry. However, this would concur with current knowledge on the subject. Microbiological air quality between the different farrowing systems does not appear to differ (Lühken et al., 2019), however, farrowing crates in particular have been associated with lower cardiovascular, bone and muscle health, and in heavier sows also with lameness and as such increase the need of inessential antimicrobials for “unnecessary infections” (Alliance to Save Our Antibiotics, 2024). Mixing of slow growers (i.e. ‘leftover pigs’) after delivery of normal pigs was also identified as a risk factor for total use of macrolides-lincosamides. In general, this type of risk can occur not only on the last production stage, but throughout the whole production flow, even in farms with AIAO systems, and it is associated with impaired health and growth performance (Díaz et al., 2017). Lastly, visiting diseased animals at the beginning of the farm-round was also an important risk factor for use of trimethoprim-sulphonamides, while it is known to be a practice of low internal biosecurity standards (Laurent, 2018).

For total AMU in fatteners, having a hygiene lock per building and PRRS vaccination in sucklings appeared as risk factors, and having a farrowing system of one, two or three weeks (vs. four or five), as protective factor. The latter appeared to be protective also for macrolides-lincosamides in sows/sucklings, but was in contrast with the finding of one and two weeks being a risk factor for use of tetracyclines in the same age group and previous research (Postma et al., 2016). This inconsistency is most likely due to residual confounding or sampling bias, while literature suggests that four to five weeks farrowing rhythm are preferable because shorter cycles favour within-farm transmission (Nathues et al., 2018), although this implication is interrelated with the length of the lactation period.

The main limitations of the current study are to be found in its cross-sectional design that is prone to reverse causality, as well as residual confounding and selection bias, considering that this is an observational study. Next, the combined AMU measurements of sows and sucklings is less biologically representative, as those groups face different types of diseases and thus their risk factors do not necessarily overlap. Moreover, the crude effects of the risk factors were quantified but we could not discriminate between indirect and direct effects (i.e. whether the causal pathway contains or not a disease as mediator, respectively). Of added value was the use of Random Forest, which allowed for the quantification of both population and individual level effects, which are more suitable for providing tailor-made AMU reduction plans. Such a model can be used within an algorithm to generate intervention scenarios where AMU is reduced for a specific farm but it is out of the scope of the current paper. Lastly, the focus here was on practices that are associated with AMU and thus related to spread of bacterial pathogens, but many pig diseases are also due to viral infections and some are polymicrobial in nature. However, important farm-level transmission routes of both viral, bacterial and parasitic pathogens overlap, forming common intervention targets. Combining these results with a risk assessment of specific diseases, such as those found in earlier research (Stefanopoulou, 2022), can provide an epidemiologically informed selection of most relevant variables for future mediation and causal analysis.

5. Conclusions

Several risk factors for total and class-specific AMU in pigs were identified, providing a comprehensive overview of factors being associated with AMU, and therefore potential targets for farm-level interventions to reduce it. While some of the factors identified here were confirmatory in nature, as they have been described before, there were some exceptions, such as weaners' aggression, free-sow systems during lactation, and farmer's tertiary education, which were associated with decreased AMU. Practical application of these findings would require additional research for the development of intervention scenarios to reduce AMU in a given farm. This is because not all interventions would influence AMU in situations where specific factors are at play. Based on our results, however, we can conclude that AMU among weaners in conventional farms is likely to benefit from limiting stocking density, implementing late weaning, improving flooring and implementing free-sow lactation systems, and the same can be said for providing training opportunities for farmers.

Ethics approval and consent to participate

This study was based on de-identified farm registry data. No sampling of animals or human subjects was performed, so no ethical approval was required.

Consent for publication

Not applicable.

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CRediT authorship contribution statement

Panagiotis Mallioris: Methodology, Data curation, Formal analysis, Writing – review & editing. **Roosmarijn E.C. Luiken:** Methodology, Data curation, Writing – review & editing. **Tijs Tobias:** Writing – review & editing. **John Vonk:** Writing – review & editing. **Jaap A. Wagenaar:** Conceptualization, Methodology, Data curation, Writing – review & editing. **Arjan Stegeman:** Conceptualization, Methodology, Data curation, Writing – review & editing. **Lapo Mughini-Gras:** Conceptualization, Methodology, Data curation, Formal analysis, Writing – review & editing.

Declaration of competing interest

All authors confirm that there are no potential conflicts of interest.

Data availability

Anonymized data related to the current study are available from authors at reasonable request and R code for the models and graphs developed here is available at https://github.com/forestiy/CIAOCIAO_pigs.

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Appendix A. Supplementary data

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References

Alarcón, L.V., Alberto, A.A., Mateu, E., 2021. Biosecurity in pig farms: a review. Porc. Health Manag. 7, 1–15. https://doi.org/10.1186/s40813-020-00181-z.

Albernaz-Gonçalves, R., Antillón, G.O., Hötzel, M.J., 2022. Linking animal welfare and antibiotic use in pig farming—a review. Animals 12, 1–21. https://doi.org/10.3390/ani12020216.

Alliance to Save Our Antibiotics, 2024. How to End the Misuse of Antibiotics in Farming.

Bokma, J., Dewulf, J., Deprez, P., Pardon, B., 2018. Risk factors for antimicrobial use in food-producing animals: disease prevention and socio-economic factors as the main drivers? Vlaams Diergeneeskd. Tijdschr. 87, 188–200. https://doi.org/10.21825/vdt.v87i4.16066.

Boyle, L.A., Edwards, S.A., Bolhuis, J.E., Pol, F., Šemrov, M.Z., Schütze, S., Nordgreen, J., Bozakova, N., Sossidou, E.N., Valros, A., 2022. The evidence for a causal link between disease and damaging behavior in pigs. Front. Vet. Sci. 8 https://doi.org/10.3389/fvets.2021.771682.

Cabrera, R.A., Boyd, R.D., Jungst, S.B., Wilson, E.R., Johnston, M.E., Vignes, J.L., Odle, J., 2010. Impact of lactation length and piglet weaning weight on long-term growth and viability of progeny. J. Anim. Sci. 88, 2265–2276. https://doi.org/10.2527/jas.2009-2121.

Cafri, G., Bailey, B.A., 2016. Understanding variable effects from black box prediction: quantifying effects in tree ensembles using partial dependence. J. Data Sci. 14, 67–96.

Chae, C., 2021. Commercial prrs modified-live virus vaccines. Vaccines 9, 1–16. https://doi.org/10.3390/vaccines9020185.

Charerntantanakul, W., 2012. Porcine reproductive and respiratory syndrome virus vaccines: immunogenicity, efficacy and safety aspects. World J. Virol. 1, 23. https://doi.org/10.5501/wjv.v1.i1.23.

Collineau, L., Bougeard, S., Backhans, A., Dewulf, J., Emanuelson, U., Grosse Beilage, E., Lehébel, A., Lösken, S., Postma, M., Sjölund, M., Stärk, K.D.C., Visschers, V.H.M., Belloc, C., 2018. Application of multiblock modelling to identify key drivers for antimicrobial use in pig production in four European countries. Epidemiol. Infect. 146, 1003–1014. https://doi.org/10.1017/S0950268818000742.

Dalmau, A., Sánchez-Matamoros, A., Molina, J.M., Xercavins, A., Varvaró-Porter, A., Muñoz, I., Moles, X., Baulida, B., Fàbrega, E., Velarde, A., Pallisera, J., Puigredon, A., Contreras-Jodar, A., 2021. Intramuscular vs. intradermic needle-free vaccination in piglets: relevance for animal welfare based on an aversion learning test and vocalizations. Front. Vet. Sci. 8, 1–13. https://doi.org/10.3389/fvets.2021.715260.

Dewulf, J., Joosten, P., Chantziaras, I., Bernaerdt, E., Vanderhaeghen, W., Postma, M., Maes, D., 2022. Antibiotic use in European pig production: less is more. Antibiotics 11. https://doi.org/10.3390/antibiotics11111493.

Dhaka, P., Chantziaras, I., Vijay, D., Bedi, J.S., Makovska, I., 2023. Can Improved Farm Biosecurity Reduce the Need for Antimicrobials in Food Animals? A Scoping Review, pp. 1–22.

Díaz, J.A.C., Diana, A., Boyle, L.A., Leonard, F.C., McElroy, M., McGettrick, S., Moriarty, J., Manzanilla, E.G., 2017. Delaying pigs from the normal production flow is associated with health problems and poorer performance. Porc. Health Manag. 3, 1–6. https://doi.org/10.1186/s40813-017-0061-6.

European Commission, 2018. Regulation (EU) 2018/848 on organic production and labelling of organic product. Off. J. Eur. Union 2018, 1–92.

Evans, J.S., Murphy, M.A., 2019. rfUtilities: Random Forests Model Selection and Performance Evaluation.

Foster, C.J., Taylor, M.G.J., Ruberg, J.S., 2011. Subgroup identification from randomized clinical trial data. Stat. Med. 30 https://doi.org/10.1002/sim.4322.

Gelaude, P., Schlepers, M., Verlinden, M., Laanen, M., Dewulf, J., 2014. Biocheck.UGent: a quantitative tool to measure biosecurity at broiler farms and the relationship with technical performances and antimicrobial use. Poult. Sci. 93, 2740–2751. https://doi.org/10.3382/ps.2014-04002.

Hothorn, T., Bühlmann, P., Dudoit, S., Molinaro, A., Van Der Laan, M.J., 2006. Survival ensembles. Biostatistics 7, 355–373. https://doi.org/10.1093/biostatistics/kxj011.

Hothorn, T., Hornik, K., Wien, W., Zeileis, A., 2015. Ctree: conditional inference trees. Compr. R Arch. Netw. 8, 1–34.

Houben, M.A.M., Caekebeke, N., Van Den Hoogen, A., Ringenier, M., Tobias, T.J., Jonquiere, F.J., Sleeckx, N., Velkers, F.C., Stegeman, J.A., Dewulf, J., Postma, M., 2020. The ADKAR® change management model for farmer profiling with regard to antimicrobial stewardship in livestock production. Vlaams Diergeneeskd. Tijdschr. 89, 309–314. https://doi.org/10.21825/VDT.V89I6.17413.

Kilbride, A., Gillman, C., Ossent, P., Green, L., 2009. Impact of flooring on the health and welfare of pigs. In Pract. 31, 390–395. https://doi.org/10.1136/inpract.31.8.390.

KNMvD, 2019. Formularium varken. https://www.knmvd.nl/app/uploads/sites/4/2019/09/formularium-varken_230919.pdf.

Laurent, J.W., 2018. Alternatives to Common Preventive Uses of Antibiotics for Cattle, Swine, and Chickens. NRDC Rep.

Leeb, C., Hegelund, L., Edwards, S., Mejer, H., Roepstorff, A., Rousing, T., Sundrum, A., Bonde, M., 2014. Animal health, welfare and production problems in organic weaner pigs. Org. Agric. 4, 123–133. https://doi.org/10.1007/s13165-013-0054-y.

Lekagul, A., Tangcharoensathien, V., Yeung, S., 2018. The use of antimicrobials in global pig production: a systematic review of methods for quantification. Prev. Vet. Med. 160, 85–98. https://doi.org/10.1016/j.prevetmed.2018.09.016.

Levshina, N., 2020. Conditional inference trees and random forests. In: A Practical Handbook of Corpus Linguistics. https://doi.org/10.1007/978-3-030-46216-1_25.

López-Vergé, S., Gasa, J., Coma, J., Bonet, J., Solà-Oriol, D., 2019. Effect of lactation length caused by the management production system on piglet performance until slaughter. Livest. Sci. 224, 26–30. https://doi.org/10.1016/j.livsci.2019.04.003.

Lu, M., Sadiq, S., Feaster, J.D., Ishwaran, H., 2018. Estimating individual treatment effect in observational data using random forest methods. J. Comput. Graph. Stat. 27 https://doi.org/10.1080/10618600.2017.1356325.

Lühken, E., Nicolaisen, T., Stracke, J., Schulz, J., Kemper, N., 2019. Microbiological air quality in free-farrowing housing systems for sows. Vet. Anim. Sci. 8, 100065 https://doi.org/10.1016/j.vas.2019.100065.

Maes, D., Segales, J., Meyns, T., Sibila, M., Pieters, M., Haesebrouck, F., 2008. Control of *Mycoplasma hyopneumoniae* infections in pigs. Vet. Microbiol. 126, 297–309. https://doi.org/10.1016/j.vetmic.2007.09.008.

Magnusson, U., Moodley, A., Osbjer, K., 2021. Antimicrobial resistance at the livestock–human interface: implications for veterinary services. OIE Rev. Sci. Tech. 40, 511–521. https://doi.org/10.20506/rst.40.2.3241.

Manyi-Loh, C., Mamphweli, S., Meyer, E., Okoh, A., 2018. Antibiotic Use in Agriculture and its Consequential Resistance in Environmental Sources: Potential Public Health Implications. https://doi.org/10.3390/molecules23040795.

McKernan, C., Benson, T., Farrell, S., Dean, M., 2021. Antimicrobial use in agriculture: critical review of the factors influencing behaviour. JAC Antimicrob. Resist. 3 https://doi.org/10.1093/jacamr/dlab178.

Mencía-Ares, O., Argüello, H., Puente, H., Gómez-García, M., Manzanilla, E.G., Álvarez-Ordóñez, A., Carvajal, A., Rubio, P., 2021. Antimicrobial resistance in commensal *Escherichia coli* and Enterococcus spp. is influenced by production system, antimicrobial use, and biosecurity measures on Spanish pig farms. Porc. Health Manag. 7, 1–12. https://doi.org/10.1186/s40813-021-00206-1.

Nathues, C., Janssen, E., Duengelhoef, A., Nathues, H., Grosse Beilage, E., 2018. Cross-sectional study on risk factors for porcine reproductive and respiratory syndrome virus sow herd instability in German breeding herds. Acta Vet. Scand. 60, 1–8. https://doi.org/10.1186/s13028-018-0411-7.

Nunan, C., 2022. Ending routine farm antibiotic use in Europe. Achieving responsible farm antibiotic use through improving animal health and welfare in pig and poultry production. Eur. Public Health Alliance 1–77.

Österberg, J., Wingstrand, A., Jensen, A.N., Kerouanton, A., Cibin, V., Barco, L., Denis, M., Aabo, S., Bengtsson, B., 2016. Antibiotic resistance in *Escherichia coli* from pigs in organic and conventional farming in four european countries. PLoS One 11, 1–12. https://doi.org/10.1371/journal.pone.0157049.

Owen, K., Blackie, N., Gibson, T.J., 2022. The effect of needle reuse on piglet skin puncture force. Vet. Sci. 9 https://doi.org/10.3390/vetsci9020090.

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

R Core Team, 2020. R: A Language and Environment for Statistical Computing.

Riising, H.J., Murmans, M., Witvliet, M., 2005. Protection against neonatal *Escherichia coli* diarrhoea in pigs by vaccination of sows with a new vaccine that contains purified enterotoxic *E. coli* virulence factors F4ac, F4ab, F5 and F6 fimbrial antigens and heat-labile *E. coli* enterotoxin (LT) toxoid. J. Vet. Med. B Infect. Dis Vet. Public Health 52, 296–300. https://doi.org/10.1111/j.1439-0450.2005.00857.x.

Schmithausen, R.M., Schulze-geisthoevel, S.V., Heinemann, C., Bierbaum, G., Exner, M., Petersen, B., Steinhoff-wagner, J., 2018. Reservoirs and Transmission Pathways of Resistant Indicator Bacteria in the Biotope Pig Stable and along the Food Chain: A Review from a One Health Perspective. https://doi.org/10.3390/su10113967.

SDa, 2020a. Usage of Antibiotics in Agricultural Livestock in the Netherlands in 2019 Trends and Benchmarking of Livestock Farms and Veterinarians, pp. 1–40.

SDa, 2020b. Standard Operating Procedure; Calculation of the DDDA for Antimicrobials by the SDa for the Cattle, Veal, Pig, Broiler, Turkey and Rabbit Farming Sectors. https://cdn.i-pulse.nl/autoriteitdiergeneesmiddelen/userfiles/overige%20rapporten/sop-rekensystematiek-website-03032020.pdf.

SDa, 2023. Usage of Antibiotics in Agricultural Livestock in the Netherlands in 2022.

Speksnijder, D.C., Mevius, D.J., Bruschke, C.J.M., Wagenaar, J.A., 2015. Reduction of veterinary antimicrobial use in the Netherlands. The dutch success model. Zoonoses Public Health 62, 79–87. https://doi.org/10.1111/zph.12167.

Spörri-Vontobel, C., Simmler, M., Wechsler, B., Scriba, M.F., 2023. Risk factors differ for viable and low viable crushed piglets in free farrowing pens. Front. Vet. Sci. 10, 1–9. https://doi.org/10.3389/fvets.2023.1172446.

Stefanopoulou, M., 2022. Swine Diseases Associated with Antimicrobial Usage in Dutch Pig Farms (MSc Thesis). Utrecht University.

Strobl, C., Hothorn, T., Zeileis, A., 2009. Party on! A new, conditional variable-importance measure for random forests available in the party package. R J. 1, 14–17.

Temple, D., Jiménez, M., Escribano, D., Martín-Valls, G., Díaz, I., Manteca, X., 2020. Welfare benefits of intradermal vaccination of piglets. Animals 10, 1–12. https://doi.org/10.3390/ani10101898.

The Dutch Society for the Protection of Animals, 2023. Beter Leven (Better Life label) [WWW Document]. URL. https://beterleven.dierenbescherming.nl/zakelijk/en/.

Vermeij, I., Enting, J., Spoolder, H.A.M., 2009. Effect of Slatted and Solid Floors and Permeability of Floors in Pig Houses on Environment, Animal Welfare and Health and Food Safety; A Review of Literature, Rapport 186. Wageningen, The Netherlands.

Wang, Jiebiao, Chen, L.S., 2016. MixRF: A Random-Forest-Based Approach for Imputing Clustered Incomplete Data.

Werner, N., McEwen, S., Kreienbrock, L., 2018. Monitoring antimicrobial drug usage in animals: methods and applications. Microbiol. Spectr. 6 https://doi.org/10.1128/microbiolspec.arba-0015-2017.

World Health Organization, 2019. Ten Threats to Global Health in 2019 [WWW Document]. World Health Organ.. URL. https://www.who.int/news-room/spotlight/ten-threats-to-global-health-in-2019

Wright, M.N., Ziegler, A., König, I.R., 2016. Do little interactions get lost in dark random forests? BMC Bioinformatics 17, 145. https://doi.org/10.1186/s12859-016-0995-8.

Zaim, S.R., Kenost, C., Lussier, Y.A., Zhang, H.H., 2019. BinomialRF: Scalable Feature Selection and Screening for Random Forests to Identify Biomarkers and their Interactions. bioRxiv, p. 681973.

Zaim, S.R., Kenost, C., Berghout, J., Chiu, W., Wilson, L., Zhang, H.H., Lussier, Y.A., 2020. BinomialRF: interpretable combinatoric efficiency of random forests to identify biomarker interactions. BMC Bioinformatics 21, 1–22. https://doi.org/10.1186/s12859-020-03718-9.

Zhang, X., Li, C., Hao, Y., Gu, X., 2020. Effects of different farrowing environments on the behavior of sows and piglets. Animals 10. https://doi.org/10.3390/ani10020320.

Zhou, L., Ge, X., Yang, H., 2021. Porcine reproductive and respiratory syndrome modified live virus vaccine: a "leaky" vaccine with debatable efficacy and safety. Vaccines 9. https://doi.org/10.3390/vaccines9040362.