Factors associated with antimicrobial use in pig and veal calf farms in the Netherlands: A multi-method longitudinal data analysis

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ABSTRACT

Minimizing antimicrobial use (AMU) in livestock is needed to control antimicrobial resistance (AMR). In the Netherlands, the livestock sector reduced AMU by almost 70 % since 2009, but this reduction stagnated in recent years. With only therapeutic AMU allowed, it is clear that besides socio-economic and behavioral factors, also the farm technical characteristics influence the conditions under which farmers need AMU. These characteristics pertain to farm management, including biosecurity, vaccination schemes, nutrition, micro-climate and husbandry practices. Identifying farm-related risk factors for AMU is needed to control AMR in a sustainable and pragmatic way. This need, often concerns the overall contribution of seemingly related (rather than individualized) factors. Here, risk factors for AMU in pig and calf farms were determined using two approaches: a typical risk factor analysis based on generalized estimating equations (GEEs) or hierarchical mixed-effects models and a multiblock partial least-squares regression analysis. These methods were applied to longitudinal data from two previous studies, i.e. a panel study and an intervention study involving 36 multiplier pig farms and 51 veal calf farms in the Netherlands, respectively. The multiblock analysis allowed us to quantify the importance of each factor and their respective block (i.e. farm management domain). For pigs, factors related to internal biosecurity had the highest impact on AMU, while for calves, these were mainly related to micro-climate. Structural characteristics, such as farm size and production type, followed in importance for both sectors. While both methods provided similar outcomes, the multiblock approach provided further insights by grouping and comparing factors believed to be inter-related.

1. Introduction

In the last decades, antimicrobial resistance (AMR) has become a public health concern. Antimicrobial use (AMU) in both human and animals has set the ground for the emergence and spread of AMR in bacterial populations, resulting in increasing antimicrobial therapy failure. Conservative estimates show that, every year, AMR causes over 700,000 human deaths worldwide (Neill, 2014), with 33,110 deaths in Europe alone (Cassini et al., 2019). The link between AMU in livestock and AMR in humans is due to resistant bacteria emerging by selection pressure of AMU in animals being transferred to humans through exposure to animals (Van Cleef et al., 2015), foods (De Boer et al., 2011) and the environment (Pikkemaat et al., 2016). A recent meta-analysis observed that reducing AMU in animals would decrease the prevalence of antimicrobial-resistant bacteria in animals by about 15 % and multidrug-resistant bacteria by 24–32 %. Comparable effects were also observed in humans, in which the pooled prevalence of AMR reported was 24 % lower in the intervention groups compared to the control groups, with a stronger association seen for humans with direct contact with food-producing animals (Tang et al., 2017). Additionally, a modelling study on the effects of curtailing AMU in animals on AMR in humans has shown not only that the response to any intervention is strongly determined by the rate of transmission from humans to animals, but also that failure to address AMU in animals limits the potential of tackling the problem from the human side (van Bunnik and Woolhouse, 2017).

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Studies on the sources and transmission pathways of AMR have shown that livestock production is among the main reservoirs (Iwu et al., 2020; Van Boeckel et al., 2015; Dorado-García et al., 2016). Between 2009 and 2019, Dutch farms (broilers, pigs, veal calves and dairy cattle) as a whole were able to reduce AMU by approximately 69.6 % (based on sales data). The main tools for achieving that were important legislative measures, such as prohibition of preventive use, strict rules for 3rd choice antimicrobials, mandatory reduction goals set by the Ministry of Agriculture (MoA), along with adoption of herd health and treatment plans, guidelines, benchmarking systems and transparency in prescriptions (Speksnijder, 2017). Yet, differences between livestock sectors and farms are extensively present. The pig farming sector in particular has achieved a 62 % AMU reduction since 2009 (i.e. from 21 to 8 defined daily dose animal ($DDDA_{NAT}$) approximately; $DDDA_{NAT}$ represents the amount of antimicrobials used within a particular livestock sector in the Netherlands and it is expressed in DDDA/animal-year), becoming the second lowest sector for AMU in the country in 2019, after dairy cattle. The veal farming sector, at the same period of 2009–2019 managed to reduce its AMU by 51 % (from 34 to 16 $DDDA_{NAT}$ approximately), with that decline having started already prior to 2009 (SDa, 2020). In general, all livestock sectors in the Netherlands have almost or at least halved their AMU since 2009, but this reduction has begun to stagnate in recent years. This indicates that a 70 % or higher AMU reduction (i.e. the current Dutch government's goal ("Policy on the use of antibiotics in food-producing," 2015) would require more fundamental changes at the farm level above those implemented, to be achieved. In the future, the Dutch MoA, apart from going beyond the aforementioned 70 % AMU reduction, also intends to implement sector-specific goals.

Prudent AMU can be promoted by applying a major two-step strategy (Speksnijder, 2017). First, to decrease the incidence of infections on farm (e.g. by strict biosecurity measures and vaccination protocols) under the paradigm that every infection avoided, is a treatment with antimicrobials prevented. Second, to apply antimicrobials only in those cases where preventive measures have failed and antimicrobial treatment is indicated. Previous research has shown that AMU is influenced by multiple factors (Dorado-García et al., 2016; Speksnijder, 2017; SDa, 2020; Sjölund et al., 2015). In traditional risk factor analyses, the individual variables in question tend to differ substantially (in meaning and structure) among studies; therefore, it is difficult to compare their information unless the same variable is used (i.e. derived from the same question). To work around this constraint, a multiblock analysis can be applied in which the different individual factors associated with AMU at the farm level can be grouped into fewer biologically meaningful groups (hereinafter referred to as "blocks") that are relevant to livestock production (Laanen et al., 2013; Collineau et al., 2018). The aim of each block is to capture the overall importance of seemingly related factors pertaining to the same farm management domain. This offers the opportunity to consider the importance of these factors as a whole, allowing for the observed differences in block importance among the different studies and livestock sectors to be appreciated because each block represents comparable management goals and priorities for the farmers. Some examples of these blocks are internal and external biosecurity, vaccination, husbandry practices, nutrition and herd performance, among others. To decrease AMU in a rational and sustainable way, it is important to identify the determinants of AMU in animals and to quantify their relative contributions in explaining the outcome.

In 2011–2013, two longitudinal studies were conducted in the Netherlands to determine the prevalence and risk factors for AMR in pigs and veal calves respectively. The study on pigs focused on methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL) carriage in pigs and pig farmers (Dohmen et al., 2017; Dorado-García et al., 2015a) and the veal calf study focused on control measures for MRSA (Dorado-García et al., 2015b). While both studies showed AMU to be among the significant predictors of AMR on farm, they did not study risk factors for AMU itself. Therefore, the aim of this study was two-fold i) to perform a comparative risk factor analysis for AMU in pig and veal calf farms, and ii) to assess the outcomes of a dimension reduction modelling approach as compared to a more traditional variable selection method. Both objectives were addressed by re-using the data from the previous two studies. However, given that the data were collected almost a decade ago, when some husbandry practices and the general attitude towards AMU in the Netherlands were different from the present day, the analysis performed here is not able to provide an updated picture of the current situation and, therefore, is not meant to provide actual targets for AMU-reducing interventions. While the age of the data may render them less suitable for certain management changes, they may still provide insights into the general relationship between AMU and farm management. Moreover, the data sets used here are well suited for the analysis from a methodological perspective (i.e. adaptation of a multiblock analysis to longitudinal data) and for providing a historical baseline for comparison with other studies on this topic, as well as for re-interpretation of existing data.

2. Materials and methods

2.1. Pig study

2.1.1. Study design

During 2011–2013, an 18-month panel longitudinal study aimed at investigating the prevalence and determinants of livestock-associated MRSA (LA-MRSA) and Extended-Spectrum Beta-Lactamase (ESBL), was conducted on 36 multiplier pig farms (sows and piglets present, with or without finishing pigs) in the Netherlands. A detailed description of the study design, including type of farms enrolled, data collection methods applied and information collected, is available in (Dohmen et al., 2017) and (Dorado-García et al., 2015a) along with information on data availability. Each farm was assessed four times during that period (6-month intervals) using a survey about on-farm practices and characteristics such as internal and external biosecurity, swine husbandry and vaccination and their AMU. Total AMU for each farm was expressed as defined daily dosages per animal per year (DDDA/Y) for the four periods preceding each sampling time. The different age categories present on farm were taken into account by including standardized weights for each category within the DDDA formula. The DDDA/Y indicates the number of days of antimicrobial drug use per year for an average animal on the farm.

2.1.2. Risk factor analysis

Given the longitudinal nature of the data set, generalized estimating equation (GEE) was used to determine factors associated with AMU. GEE is a marginal modelling approach (Ziegler, 2011; Lee and Nelder, 2004) that can handle correlated, non-normally distributed and heteroscedastic data. Analyzing the data under the independence correlation structure showed that the model residuals had a decreasing correlation over time; thus, the correlation structure was set to be first-order autoregressive (AR-1). In all analyses, the outcome variable was the transformation of DDDA/Y values by Eq. 1 (Busse and Hefeker, 2007), as this transformation provided normally distributed residuals like the traditional approach of taking the natural logarithm, but with maintaining a 0 (instead of minus infinity) when DDDA/Y values were 0.

$$y = \ln(x + \sqrt{x^2 + 1}) \tag{1}$$

From the full survey, explanatory variables with more than 10 % missing values or less than 10 % variation were excluded from the analysis (Dorado-García et al., 2015a). Then the variables were assessed univariably, with those having a p-value <0.1 for their association with the outcome, being selected for inclusion in multivariable analysis. Variables selected after the univariable analysis were grouped into biologically meaningful blocks. Specifically, four blocks of variables were created, namely: a block for internal biosecurity (block X_1),

external biosecurity (block X_2), husbandry practices (block X_3) and nutritional practices (block X_4). Within these blocks separately, variables were checked for collinearity using the Variance Inflation Factor (VIF) (O'Brien, 2007); for variables with a VIF > 10, the Corrected Quasi-likelihood under Independence Model Criterion (QICC) was used to retain only one of the collinear variables (Boruff et al., 2012). Then manual backward elimination procedure using multivariable GEE analysis was applied for further selection of significant variables (p-value <0.05) for building the best fitted model within each block independently. Subsequently, those significant variables retained in each block (i.e. block specific models) were combined and tested for significance altogether again with manual backward elimination (p-value <0.05) to conclude in an overall and final multivariable GEE model. Three factors that defined the profile of the farm at large, being associated with both the outcome and the other covariates of interest based on literature, were included in all model as a-priori control covariates (Table 1): i) total number of animals in the farm per year (i.e. sows, suckling piglets, weaned piglets, gilts and fatteners) as continuous variable; ii) type of production, a binary variable defining an "open" or "closed" farm; and iii) type of farm, a binary variable defining a "farrowing" or "farrow-to-finish" farm. A farm was defined as "open" when it received external supply of gilts more than once a year from at least one supplier and as "closed" when gilts were not supplied externally. A "farrowing" farm did not produce fatteners and delivered growers (25 kg live weight) to finishing farms (with the exception of one farm that delivered gilts for farrowing), while "farrow-to-finish" farms integrated farrowing and finishing production and delivered fattening pigs to the abattoir. The model building approach is schematized in Fig. 1.

2.1.3. Multiblock variable importance analysis

The relative contribution of each block of variables to the DDDA/Ys was quantified by combining partial least-squares (PLS) and GEE analyses (Fig. 2). Our approach resembled the previously applied multiblock PLS (mbPLS) regression method on cross-sectional data (Collineau et al., 2018; Wold, 1984; Bougeard et al., 2011a; Bougeard et al., 2011b), as in our case it needed to be adapted to longitudinal data. Briefly, multiblock regression analysis is suited to data organized in (K + 1) blocks of variables, consisting of a block of variable(s) to be explained (i.e. block Y), and an extended number of explanatory variables (i.e. potential risk factors, organized in K meaningful blocks $X_1,...,X_k$) (Collineau et al., 2018). MbPLS then performs the following three steps:

i) An overall factor analysis, between each explanatory block and the response variable, to summarize the information contained in each block X_k into K partial components t_k (i.e. here the first dimension extracted from the PLS in each block was used)

$$t_k = \sum_i X_{ki} * w_{ki} \qquad (2)$$

where k refers to the block number, i refers to the variable number within the block X_k and w_{ki} to the weight (i.e. loading) of X_{ki} within t_k. The squared loading of an original variable in a component reflects the proportion of the explained variance of that component by that variable. The score t_k is the value that each observation obtains for that PLS component which can be used to predict y.

ii) Afterwards, a global component t is defined as a linear combination of the t_k, oriented towards explaining Y and summing up the partial components t_k for (K = 1,...,K)

$$t = \sum_k a_k * t_k = \sum_k a_k * X_k * w_k = X * w \qquad (3)$$

iii) Finally, a multiple linear regression of Y against the global component t is performed:

$$Y = t * c + \varepsilon \qquad (4)$$

where c is the regression coefficient of Y upon t, and ε represents the residuals of the regression models. The mbPLS method provides also two interpretation tools for block and variable importance called 'block importance index (BlockImp)' and 'variable importance index (VarImp)' respectively. If K is the number of explanatory blocks, $BlockImp_k$ is defined as:

$$BlockImp_k = a_k^2 \qquad (5)$$

With a_k^2 being the squared weight for the explanatory block $k = (1,..., K)$. As a_k^2 satisfies $\sum_k a_k^2 = 1$ $BlockImp_k$ can also be expressed as a percentage. If P is the total number of explanatory variables included in the model, $VarImp_p$ is defined as:

$$VarImp_p = a_k^2 * w_p^2 \qquad (6)$$

With w_p^2 being the squared weight for the explanatory variable $p = (1, ...,P)$. $VarImp_p$ can also be expressed as a percentage since w_p^2 satisfies $\sum_p w_p^2 = 1$ too.

In our analysis, the second and third step were replaced by directly combining the partial components t_k in a GEE model, to account for the longitudinal intercorrelation of the observations. Subsequently, block importance was calculated by dominance analysis (Azen and Budescu, 2003) based on the percentage of adjusted R^2 of the full GEE model (i.e. $adj.R^2_{full-model}$) explained by each partial component (i.e. $adj.R_k^2$ for (K = 1,...,K)), instead of using a global component, and the threshold value of $1/K$ was used to identify significant associations between the blocks and Y (Bougeard et al., 2011b; Bougeard and Dray, 2018) (i.e. a block X_k is considered to be significantly associated with the Y block, if Block_Importance_k is larger than $1/K$ and its 95 % tolerance interval does not include this threshold value).

$$Block\_Importance_k(\%) = adj.R_k^2 \Big/ adj.R^2_{full-model} * 100\% \qquad (7)$$

Variable importance was calculated in a similar manner to $VarImp_p$ and expressed as a percentage too. Here again the threshold value of $1/P$ can also be used to identify significant associations between original explanatory variables and Y (Bougeard et al., 2011b; Bougeard and Dray, 2018) (i.e. a variable X_{kp} is considered to be significantly associated with the Y block, if Variable_Importance_p is larger than $1/P$ and its 95 % tolerance interval does not include this threshold value).

$$Variable\_Importance_p(\%) = Block\_Importance_k * w_p^2 * 100\% \qquad (8)$$

In this case, apart from the results of univariable screening, authors' expertise was also used to define the biologically meaningful blocks (Table S1 in supplementary material, Fig. 2). Thus, to the four blocks present, a fifth was added for including variables related to vaccination (block X_5) even though they were not significant in the univariable screening. In the null model, the three control variables (Table 1) were included to account for the different types and sizes of farms. Moreover, collinear variables within each block were not excluded from the PLS analysis. Finally, a bootstrap procedure was used to compute 95 % confidence intervals (95 % CI) with 1000 iterations.
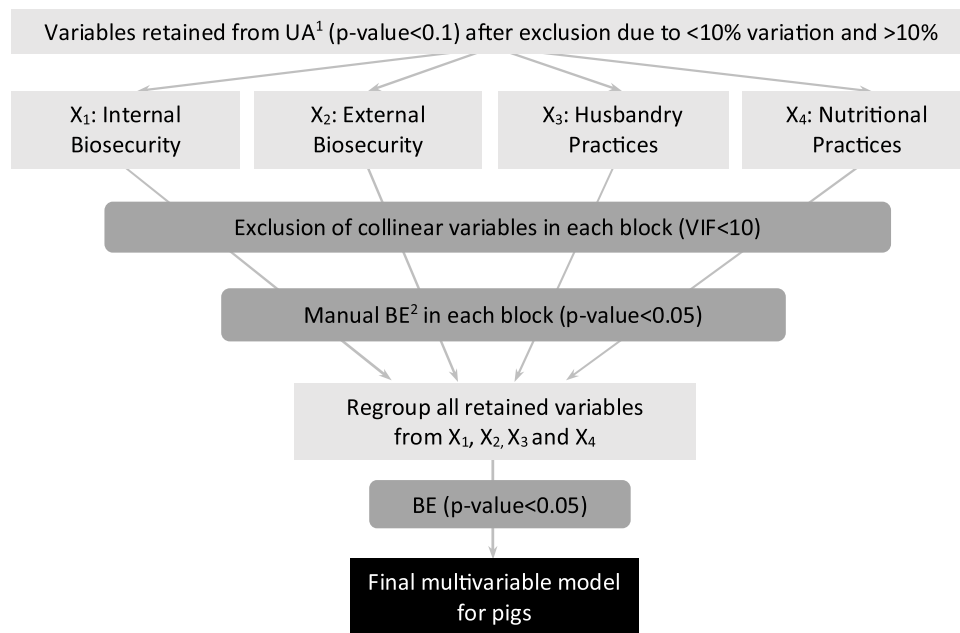
Table 1
Control variables of the null model for 36 pig farms and their descriptive statistics.

Control variables of the null model for pigs	Number of farms (Frequency)
Open/Close farm	
Open	22 (61.1 %)
Close	14 (38.9 %)
Production type	
Farrow to finish	24 (66.7 %)
Multiplier	12 (33.3 %)
Number of animals	mean: 4410; range: 1,390−22,020

Fig. 1. Conceptual scheme of the risk factor analysis for antimicrobial use in the pig data set. Information about the variables present in the blocks can be found in Table S1 in the supplementary material. UA[1]: Univariable analysis; BE[2]: Backward elimination.
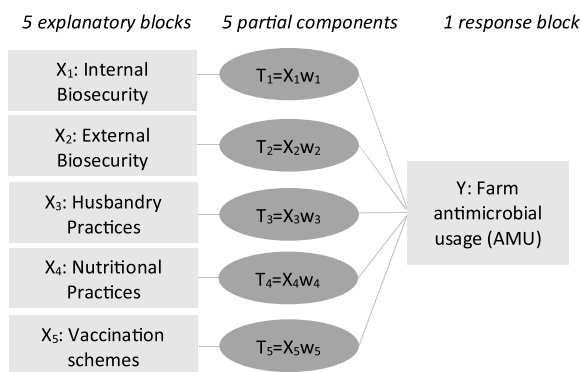

Fig. 2. Conceptual scheme of the relationships between explanatory blocks (X_1, …,X_5) and the response block Y in the pig data set.

2.2. Veal calf study

2.2.1. Study design

In 2012, 51 veal calf farms were followed up in an intervention study, over a period of 12 weeks starting at the beginning of two consecutive production cycles and they were assigned to three different study arms (i.e. 3×17). These three arms corresponded to two intervention groups and a control group. The farms were recruited in triplets to ascertain comparability. Each triplet was selected at a same time point within the same cooperative having the same breed of calves and comparable production parameters (mainly mortality and AMU in previous cycles). Each farm within a triplet was randomly assigned to one of the three following study arms: intervention farms reducing AMU by protocol (named RAB arm); intervention farms reducing AMU by protocol and applying cleaning and disinfection program of stables (RAB-CD arm); and control group of farms where no interventions were implemented (control arm). The protocol for AMU reduction in RAB and RAB-CD arms promoted individual treatments and focused on limiting group treatments by favoring a transition from treating whole herds to treating herds partially. A detailed description of the study is provided elsewhere (Dorado-García et al., 2015b) along with information on availability of the data.

AMU information was available for the four consecutive pre-study production cycles and the two study cycles. In each farm, AMU was calculated as defined daily dosages per animal per cycle (DDDA/C) for each of the four baseline and two study cycles. For interpretation of the results, a DDDA/C of 1 represents that the average animal in the population was exposed to antimicrobials for one day during the cycle (approximately six months). The values were transformed again with Eq. 1.

2.2.2. Risk factor analysis

For the analysis of the calf data set, a similar approach to the pig data set was used, but instead of a GEE model, a hierarchical mixed-effects model was applied to account for the two nested levels of clustering, as in the latter their coding was more straightforward. In the mixed-effects model, a random intercept was introduced to account for the clustering in the three arms and another random intercept was used to account for the farm clustering of the two repeated measurements. The total number of animals in the farm in a year (i.e. bulls, heifers, dairy cattle and calves combined) was always forced in all models as a control covariate. The model building approach is schematized in Fig. 3.

2.2.3. Multiblock variable importance analysis

The two procedures (i.e. the multivariable model reduced by backward elimination and the block and variable importance) were applied in a similar manner as in pigs with the main difference that in the second one, the marginal R^2 (R^2 due to fixed effects only) was used to calculate the importance (Table S2 in supplementary material, Fig. 4). During the analysis of both datasets linearity was explored visually where relevant. All analyses were performed in the open-source environment R version 3.6.2 (R Core Team, 2020) using the packages "geepack" (Yan, 2002), "lme4″ (Walker et al., 2015) and "pls" (Bjorn-Helge et al., 2019).

3. Results

3.1. Risk factors for AMU in pigs

The factors significantly associated with AMU in the final multivariable model for pig farms are reported in Table 2. Overall, three variables from the internal biosecurity block, one from the husbandry
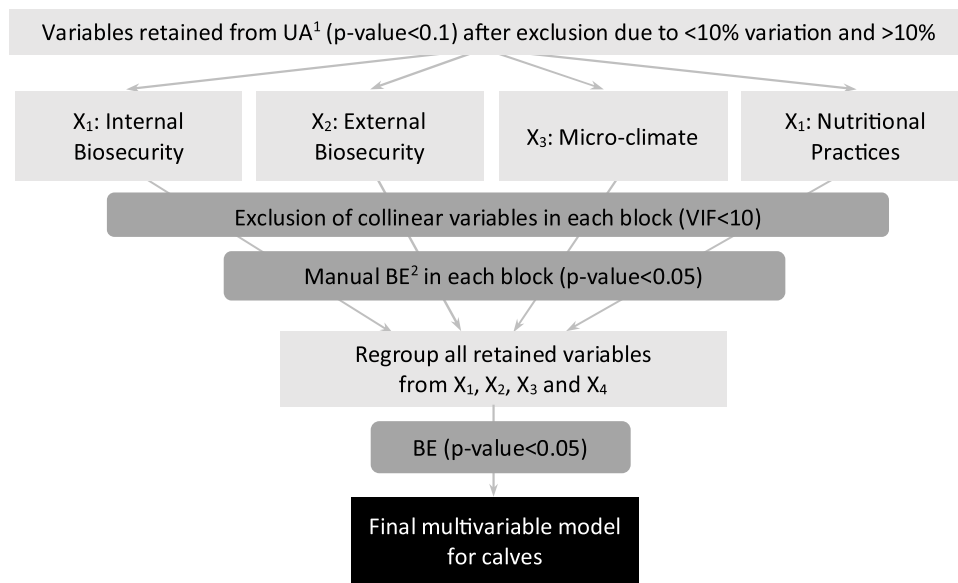
Fig. 3. Conceptual scheme of the risk factor analysis for antimicrobial use in the calf data set. Information about the variables present in the blocks can be found in Table S2 in supplementary material. UA[1]: Univariable analysis; BE[2]: Backward elimination.
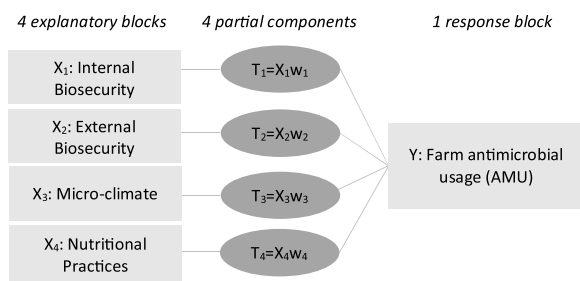

Fig. 4. Conceptual scheme of the relationships between explanatory blocks (X_1, …,X_4) and the response block Y in the calf dataset.

practices block and one from the nutrition block were found to be significant. In the internal biosecurity block, factors significantly associated with increased AMU were the mixing of slow growers when housed together after delivery of pigs, and the renewal of needles in sows once every week *vs.* at least once per day. Conversely, keeping stable groups of sows and applying 5-week farrowing system was significantly associated with decreased AMU. In the nutrition block, only drinking water-related variables were present and it was found that providing water from a private supply (water well) was significantly associated with increased AMU levels.

3.2. Risk factors for AMU in calves

The results from the risk factor analysis for calves are shown in Table 3. In total, four variables remained in the final multivariable model (two from the nutrition block, one from the micro-climate block and one from the internal biosecurity block). Specifically, the provision of pelleted feed was associated with reduced AMU while, the use of straw was associated with increased AMU. Furthermore, a lower AMU was observed when the temperature was not lower than 10 °C and when disinfection of the stables occurred every few rounds as compared to never. This association was not fully consistent, as the effect of disinfecting at every round although protective was not significant. This might be due to power since only a few farms applied that measure in both rounds.

Table 2
Factors significantly associated with antimicrobial use in the final multivariable model* for pig farms**.

Variable	Answer(s)	β-coefficient	p-value	95 % CI
- Piglets from various compartments are housed together after delivering ("leftover piglets") (Ref: No)	Yes	0.29	0.01*	0.07 / 0.5
- Needles for vaccination of sows are renewed (Ref: at least once per day)	Once per week	0.39	0.01*	0.09 / 0.69
	When needed	−0.13	0.62	−0.66 / 0.4
- The sows are housed in stable groups (Ref: No)	Yes	−0.31	0.01*	−0.55 / -0.07
- Animals get water from private mineral water source (Ref: No)	Yes	0.5	0.004*	0.16 / 0.8
	3-week	−0.33	0.38	−1.07 / 0.41
- Weekly system followed	4-week	0.16	0.66	−0.5 / 0.8
	5-week	−0.64	0.27*	−1.22 / - 0.07
- Open-close type (Ref: Open)	Close	−0.33	0.38	−1.08 / 0.42
- Production type (Ref: farrow-to-finish)	Farrowing	0.56	0.165	−0.22 / 1.36
- Number of animals (x100)		−0.005	0.02*	−0.01 / -0.001

* The used GEE model accounts for intercorrelation of the 4 repeated measurements in 36 farms with the following variables forced in the model: Open-close type, production type and number of animals.
** Data were obtained from 36 pig farms in the Netherlands during.2011–2013.

3.3. Multiblock variable importance for antimicrobial use in pigs

With 60.14 % variance explained in the AMU levels, internal biosecurity was the main block, with factors contributing significantly to the total adjusted R^2 (mean bootstrapped adjusted R^2 = 0.288, with CI = 0.286 / 0.290), along with the null model. The three factors included in the null model (i.e. type of farm, type of production, and number of animals) explained altogether 20.6 % of the variation (mean

Table 3

Factors significantly associated with antimicrobial use in the final multivariable model* for veal calves**.

Description	Answer(s)	β-coefficient	p-value	95 % CI
- Are pellets provided to the calves (Ref: No)	Yes	−0.2	0.01*	−0.34 / -0.04
- Is straw provided to the calves (Ref: No)	Yes	0.31	<0.001*	0.16 / 0.45
- What is the minimum temperature inside the stables (Ref: Less than 10 °C)	Between 10−15 °C	−0.35	<0.001*	−0.53 / -0.16
	Between 15−20 °C	−0.43	<0.001*	−0.64 / -0.21
- When do you generally disinfect the stables? (Ref: Never)	Not after every round	−0.3	<0.001*	−0.45 / -0.15
	After every round	−0.07	0.27	−0.20 / 0.05
- Number of animals (x100)		0.003	0.53	−0.006 / 0.01

* The used mixed-effects model included two random intercepts for two nested clusters, i.e. 17 farms per each study arm with 2 repeated measurements per farm, with the number of animals as control variable forced in the model.

** Data were obtained from 51 veal calf farms in the Netherlands in 2012.

bootstrapped adjusted $R^2 = 0.083$, with CI = 0.081 / 0.086). This was followed by nutrition (8.74 %), husbandry practices (7.07 %), vaccination (5.14 %), and external biosecurity (1.79 %). Fig. 5A summarizes the results of block importance to AMU.

As shown in Fig. 5B, the individual factors within the internal biosecurity and nutrition blocks had the largest importance in explaining the AMU levels. More specifically, within the internal biosecurity block, cleaning of the farrowing passage with cold water and mixing of slow growers after delivery of pigs displayed the highest risk effect sizes (10.6 % and 6.7 % respectively), followed by keeping manure on the farm during winter and summer less than three months compared to 3–6 months (3.4 % and 1.8 %, respectively), having a group size of gestating sows more than 30 compared to less than 10 (1.9 %) and changing the needles for piglets and fatteners per pen instead of not (1.6 %). On the other hand, cleaning the farrowing passage by sweeping and supplying water mainly via a nipple in the fattener section had a significant protective effect on AMU with variable importance of 8.5 % and 7% respectively. Similar protective effects were observed for: keeping piglets within the same litter (4.4 %), having at least 24 h drying period after cleaning (3%), keeping manure during winter 6–9 months compared to 3–6 months (2.1 %), using soaking agents for cleaning the fattener section (2.1%), placing the carcass cooler on the dirty road (i.e. passage where the animals have access) (1.7 %) and having a group size of gestating sows between 25 and 30 compared to less than 10 (1.7 %). Although the blocks of nutrition, vaccination and husbandry practices as a whole were not significant, two similar variables in the latter were (i.e. their importance was higher than 1/K*1/P). That was application of a 5-week system compared to 1-week which was associated with reduced AMU (2.7 %). Within the nutrition block, providing tap water had a protective effect (3.8 %), whereas providing water from a private source was a risk factor for AMU (3.8 %) but both were not significant. Lastly, in the vaccination block, vaccinating piglets and fatteners and implementing PCV2 vaccination were not significant but positively associated with AMU (1.2 % and 1.0 % respectively). As in the risk factor analysis here we also found that "closed" farms (mean of bootstrapped coefficient
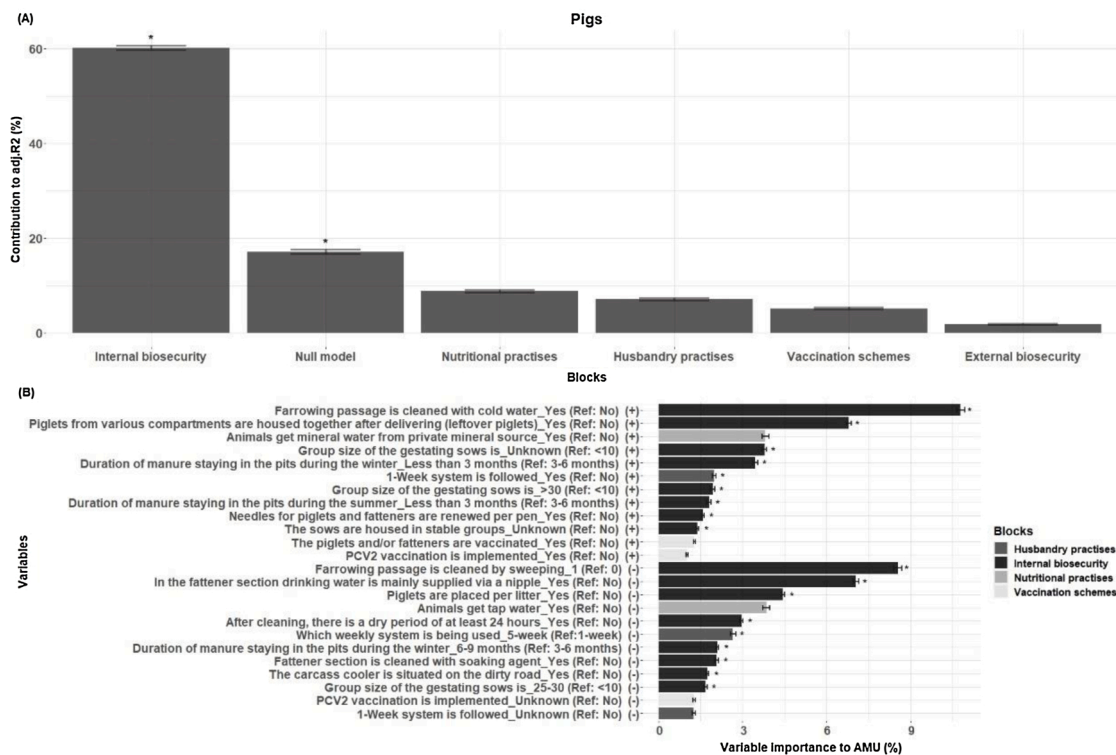

Fig. 5. (A & B); A: Relative contributions of each block of variables to the overall antimicrobial use levels (i.e. block importance) of 36 pig farms in the Netherlands based on the dominance analysis in the GEE model of the first partial component of each block which was extracted after applying the PLS method. The asterisk * denotes whether the block was contributing significantly. Bars display 95% confidence intervals. The null model was composed by three original variables which were: i) the number of animals; ii) the type of production, which was a binary variable defined as "open" or "closed"; and iii) the type of farm, which was a binary variable defined as "farrowing" or "farrow-to-finish" farms. **B:** Variable importance based on the PLS weights for all variables in all blocks except null model (variables with variable importance <1% are not shown). The asterisk * denotes whether the variable was contributing significantly. Signs in parentheses display the direction of the regression coefficients β showing a protective (-) (i.e. last twelve entries) or risk (+) (i.e. first twelve entries) effect of the variable. Bars display 95% confidence intervals.

= -0.802, with CI = -0.811 / -0.793) and higher number of animals were negatively associated with AMU (mean of bootstrapped coefficient = -0.0064, with CI = -0.0064 / -0.0063), while, "farrow-to-finish" farms were positively associated (mean of bootstrapped coefficient = 0.52, with CI = 0.51 / 0.53).

3.4. Multiblock variable importance for antimicrobial use in calves

Block and variable importance for AMU in veal calves are displayed in Fig. 6A and B, respectively. Micro-climate had the biggest importance in explaining AMU (55.4 %; mean bootstrapped marginal R^2 = 0.38, with CI = 0.37 / 0.38), followed by the number of animals (i.e. null model) with 28.9 % block importance (mean bootstrapped marginal R^2 = 0.2, with CI = 0.19 / 0.2). The nutrition block had an importance of 10.4 % but it was not significant in itself. Internal and external biosecurity had minor importance to the marginal R^2 (3% and 2.2 % respectively).

Within the micro-climate block, several variables displayed significant associations with AMU, as shown in Fig. 6B. In particular, mechanical ventilation compared to natural ventilation (32.7 %), more days for drying after cleaning of stables (20 %) and minimum temperature of stables at 15−20 °C compared to 10 °C (11 %) had the largest significant protective effects. Although the nutrition block as a whole and the individual variables within had no significant importance towards AMU, the provision of straw was still apparent as a risk factor. From the internal biosecurity block, disinfecting the stables every few rounds as compared to never (1.5 %) was negatively associated with AMU.

4. Discussion

In this study, risk factors for AMU in pig and veal calf farms were inferred using two different analytical approaches applied to two longitudinal data sets. These data sets were generated in previous studies on risk factors for AMR, but were not used to study AMU per se, so no comparison could be done with them. The importance of blocks of variables, describing certain characteristics of the farms, regarding AMU and their individual effects were also assessed. Although several studies exist on risk factors for AMU in both pigs and veal calves, only one study so far has used a multiblock approach to assess variable importance in a cross-sectional study design (Collineau et al., 2018), whilst this method is also valuable in identifying risk factors for diseases (Bougeard et al., 2011b). The advantage of this approach is particularly evident when multiple and seemingly related risk factors are analyzed in blocks, as this is epidemiologically more informative and reflects better the complexity of the farm. Livestock farming is such a case since there is a handful of different aspects that a farmer has to pay attention to. Indeed, the analysis can be done also in the "classical" way where all factors are included as one group, but then the information regarding those different aspects of the farm would be limited to only those factors concluded in the final multivariable model. Consequently, one would not be able to identify which factors are the most important within each block (e.g. internal biosecurity or others) and what is the relative importance of the different blocks. This approach therefore provides an original perspective to the study of risk factors that are, in fact, better addressed altogether as a more general entity. As the backward variable elimination method is quite drastic in nature (i.e. variables are excluded from the model completely until it is final), the PLS approach provides a more "gentle" exploration of the variables and their blocks altogether with regard to AMU.
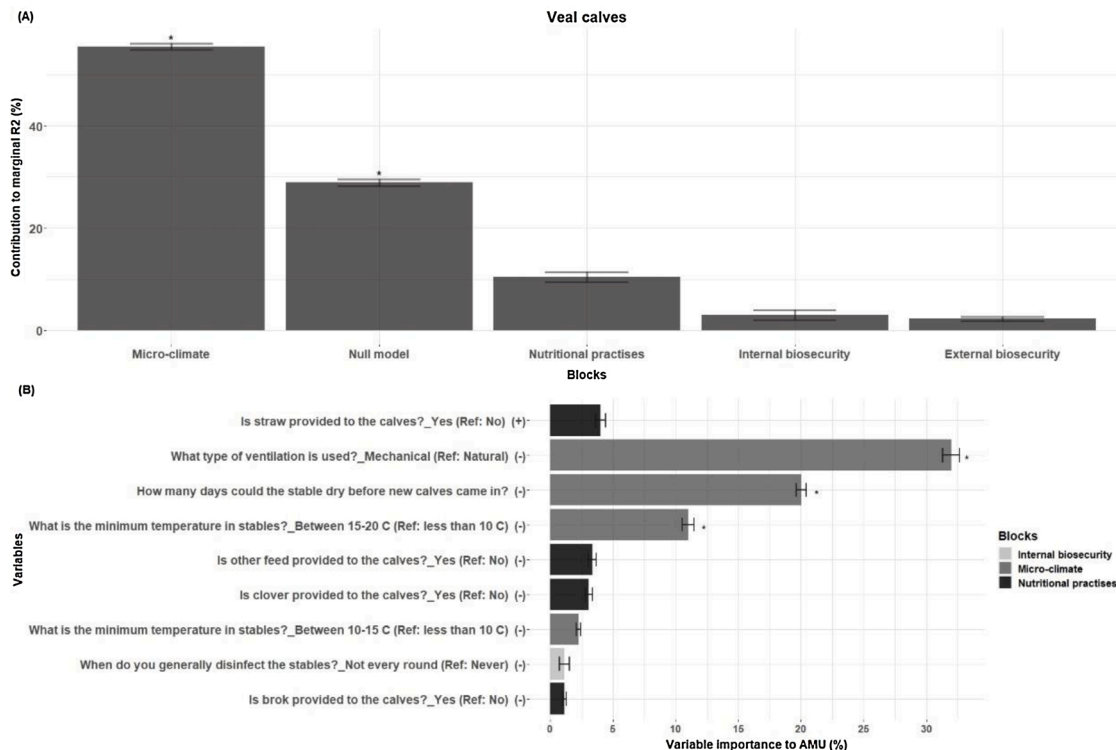

Fig. 6. (A & B); A: Relative contributions of each block of variables to the overall antimicrobial use levels (i.e. block importance) of 51 veal calf farms in the Netherlands based on the dominance analysis in the mixed-effects model of the first partial component of each block, which was extracted after applying the PLS method. The asterisk (*) denotes whether the block was contributing significantly. Bars display 95% confidence intervals. The null model was composed by one original variable which was the number of animals. **B:** Variable importance based on the PLS weights for all variables in all blocks except null model (variables with variable importance <1% are not shown). The asterisk (*) denotes whether the variable was contributing significantly. Signs in parentheses display the direction of the regression coefficients β showing a protective (-) (i.e. last eight entries) or risk (+) (i.e. first entry) effect of the variable. Bars display 95% confidence intervals.

Here, we found that internal biosecurity as a whole had the largest impact on AMU in pig farms. This is in line with the initial risk factor analysis, as 3 out of the 5 significant factors belonged to that block. Previously identified risk factors for AMU in pigs are limited and studies did not look at different farm characteristics altogether, but rather to a certain selection of factors (e.g. only biosecurity). In the study of Laanen et al. (Laanen et al., 2013) in which the Biocheck® questionnaire (Anon, n.d.) was used, it was also found that improved internal biosecurity was highly likely to reduce prophylactic AMU, but the impact of similar factors is expected to vary among countries (Collineau et al., 2018). Regarding cleaning and disinfection practices, three factors appeared to be significant: sweeping instead of cleaning with just cold water, the usage of soaking agents and a drying period of at least 24 h. The last two have also been observed elsewhere (Hancox et al., 2013). Besides cleaning and disinfection procedures in pig farms, another important risk factor observed here and in other studies is the mixing of pigs. This may manifest in multiple ways across the different production stages. Such cases, for example, are the mixing of gilts when originate from different suppliers without proper quarantine, absence of an all-in/all-out system or as in our case was the mixing of slow growers when placed together after delivery of the properly fattened pigs (i.e. mixing of 'leftover' pigs). In the study of (Arnold et al., 2016), increased oral AMU in Swiss fattening pig farms was associated with mixing of pigs from different suppliers, as well as the absence of an all-in/all-out system, the latter factor though was not significant in our final model. In another Swiss study, however, the all-in/all-out system was identified again as a significant risk factor (Kreuzer, 2014). Another important topic is the needle management, which has been found to be associated with animal welfare, carcass quality and on-farm accidents; hence, needle-free vaccines are increasingly being promoted (Technical and Unit, n.d.).

Vaccination itself tends to have variable effects on AMU among studies. Here a non-significant positive association was observed by the mbPLS method for PCV2 vaccination and vaccination of weaners and/or fatteners in general. Overall, vaccinations are proven strategies for preventing and controlling infectious diseases (or secondary infections) that require antimicrobial treatment, thereby limiting the need of AMU. To achieve this consistently, a vaccine should be safe, easily adapted to mass-application, inexpensive to produce and use, easy to register across key jurisdictions, and should generate durable protection (ideally after a single administration and against a broad range of pathogens), but existing vaccines still fall short of these ideals. In fact, many current vaccines for food-producing animals have a number of shortcomings with regard to safety, efficacy and/or user-friendliness that limit their efficiency and thus their relationship with AMU is not clear-cut (Hoelzer et al., 2018a; Hoelzer et al., 2018b). In addition, farmers tend to use vaccines when they face a problem, mostly due to their high costs and labor intensity, making this cause and effect relationship difficult to achieve. Furthermore, manure and water management have shown to affect AMU, as they are among the main determinants of several swine diseases (Filippitzi et al., 2017). With regard to those factors, in pigs it was found that keeping manure on farms for longer periods is significantly associated with lower AMU, while for water, its source and means of provision are important thus hygienic and bio-secure management of those two should be a priority for the farmer. The effects of proper grouping of sows and lactating piglets need further investigation, as other on-farm characteristics are most likely to influence their impact (e. g. hygiene, protocol for placing piglets on different litters, density). Nevertheless, group-housing of litters and multi-litter systems are more promising as compared to the conventional single-litter system with a confined sow (van Nieuwamerongen et al., 2015; Nieuwamerongen, 2017). Finally, the farrowing rhythm seems also to have an effect, with the 5-week system associated with lower AMU. This can be possibly explained with farms operating in a 1- or 2-week system having a less strict separation of piglets of different age groups; therefore, a higher chance of within-herd transmission of pathogens. In farms with a 3-, 4-

or 5-week rhythm, the older group is already or nearly weaned, when new piglets are born, thereby preventing a transfer of pathogens by staff or other vectors between the different farrowing compartments for the corresponding farrowing group (Nathues et al., 2018).

Regarding veal calves, few studies have been conducted to identify on-farm factors associated with AMU (Bokma et al., 2019; Holstege et al., 2018; Lava et al., 2016; Schnyder et al., 2019). We found that the use of pelleted feed was associated with lower AMU, whereas the use of straw was associated with higher. Indeed, pelleted feed is regarded as being of high quality and more hygienic compared to other feed forms due to its thermal treatment (Ghassemi Nejad et al., 2012) and possible additives (e.g. acidifiers). Straw on the other hand, providing it as is without any treatment, is more prone to molding if not properly stored, creating health risks due to mycotoxins and dust (Bennett and Klich, 2003). However, this hypothesis is unlikely because the straw given to calves is inspected and certified under the IKB regulations (IKB, 2008) and therefore unlikely to pose a health risk as such. An alternative explanation is that this association is actually the result of reverse causality, as from discussions with calf farmers it appeared that it is common practice to provide (extra) straw as supportive therapy to calves with health problems. So the straw would be a consequence and not a cause of the disease requiring AMU.

Air quality-related factors are not extensively examined, however, in one case (Lava et al., 2016) the researchers found that using the same air space for different groups of calves was positively associated with increased treatment incidence in Swiss farms and in another (Schnyder et al., 2019) elevated ammonia levels >10 ppm were positively associated with AMU, most likely due to its influence on calf's vulnerability to bovine respiratory disease (BRD) (Assié et al., 2009). Overall, there are several predisposing factors ("stressors") for respiratory diseases among bovines, and specifically BRD, which is among the main diseases in white veal operations along with digestive disorders and arthritis (Pardon et al., 2012; Bähler et al., 2012). These pertain to transportation, commingling with other cattle, dust, cold, sudden and extreme weather changes, dehydration, hypoxia, exposure to endotoxin, cold coupled with wetness, and acute metabolic disturbances. In accordance to these stressors, here we found that low temperature (<10 °C), natural *vs.* mechanical ventilation and short dry periods after cleaning were risk factors for AMU. Large temperature variation was associated with increased mortality when tested univariably in a Swiss study (Schnyder et al., 2019). In the same study, mechanical ventilation was also associated with increased AMU, while natural ventilation in another study had no clear effect (Jarrige et al., 2017). These varying results regarding ventilation may be due to the general acceptance that the matter at hand here is the susceptibility of calves to air drafts (Schnyder et al., 2019; Brscic et al., 2012) either through mechanical or natural ventilation.

Overall, the risk factor analysis was in line with the multiblock analysis, with the latter providing a broader overview of the relations between all the variables and the outcome. Interestingly, with regard to the control covariates forced into the models (i.e. null model), we saw that they accounted for a large portion of the variance, especially in the calf data set, in which only one factor was present (i.e. number of animals).

5. Conclusions

Reducing AMU in livestock is a priority for controlling the emergence and spread of AMR. To do so in a sustainable and pragmatic manner, a good understanding of the factors that determine the need for AMU is crucial. Here we showed that a multiblock approach can be of help in the analysis when the goal is to summarize and compare the effects of several (groups of seemingly related) factors. In doing so, we were able to show that internal biosecurity and micro-climate conditions influence AMU the most among pig and veal calf farms, respectively. This also highlights the variability of targets to be pursued in the

different livestock sectors to reduce AMU.

Declaration of Competing Interest

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prevetmed.2021.105563.

References

Anon, n.d. Biocheck.ugent®. Available at http://www.biocheck.ugent.be/.
Arnold, C., Schüpbach-Regula, G., Hirsiger, P., Malik, J., Scheer, P., Sidler, X., Spring, P., Peter-Egli, J., Harisberger, M., 2016. Risk factors for oral antimicrobial consumption in Swiss fattening pig farms - A case-control study. Porc. Heal. Manag. 2, 1–9. https://doi.org/10.1186/s40813-016-0024-3.
Assié, S., Bareille, N., Beaudeau, F., Seegers, H., 2009. Management- and housing-related risk factors of respiratory disorders in non-weaned French Charolais calves. Prev. Vet. Med. 91, 218–225. https://doi.org/10.1016/j.prevetmed.2009.06.003.
Azen, R., Budescu, D., 2003. The dominance analysis approach for comparing predictors in multiple regression. Psychol. Methods 8, 129–148. https://doi.org/10.1037/1082-989X.8.2.129.
Bähler, C., Steiner, A., Luginbühl, A., Ewy, A., Posthaus, H., Strabel, D., Kaufmann, T., Regula, G., 2012. Risk factors for death and unwanted early slaughter in Swiss veal calves kept at a specific animal welfare standard. Res. Vet. Sci. 92, 162–168. https://doi.org/10.1016/j.rvsc.2010.10.009.
Bennett, J.W., Klich, M., 2003. Mycotoxins. Clin. Microbiol. Rev. 16, 497–516. https://doi.org/10.1128/cmr.16.3.497-516.2003.
Bjorn-Helge, Mevik, Wehrens, Ron, Liland, Kristian Hovde, 2019. pls: Partial Least Squares and Principal Component Regression.
Bokma, J., Boone, R., Deprez, P., Pardon, B., 2019. Risk factors for antimicrobial use in veal calves and the association with mortality. J. Dairy Sci. 102, 607–618. https://doi.org/10.3168/jds.2018-15211.
Boruff, B.J., Nathan, A., Nijënsten, S., 2012. Using GPS technology to (re)-examine operational definitions of "neighbourhood" in place-based health research. Int. J. Health Geogr. 11, 1–14. https://doi.org/10.1186/1476-072X-11-22.
Bougeard, S., Dray, S., 2018. Supervised multiblock analysis in R with the ade4 package. J. Stat. Softw. 86, 1–17. https://doi.org/10.18637/jss.v086.i01.
Bougeard, S., Qannari, E.M., Lupo, C., Hanafi, M., 2011a. From multiblock partial least squares to multiblock redundancy analysis. A continuum approach. Informatica 22, 11–26. https://doi.org/10.15388/informatica.2011.311.
Bougeard, S., Qannari, E.M., Rose, N., 2011b. Multiblock redundancy analysis: interpretation tools and application in epidemiology. J. Chemom. 25, 467–475. https://doi.org/10.1002/cem.1392.
Brscic, M., Leruste, H., Heutinck, L.F.M., Bokkers, E.A.M., Wolthuis-Fillerup, M., Stockhofe, N., Gottardo, F., Lensink, B.J., Cozzi, G., Van Reenen, C.G., 2012. Prevalence of respiratory disorders in veal calves and potential risk factors. J. Dairy Sci. 95, 2753–2764. https://doi.org/10.3168/jds.2011-4699.
Busse, M., Hefeker, C., 2007. Political risk, institutions and foreign direct investment. Eur. J. Polit. Econ. 23, 397–415. https://doi.org/10.1016/j.ejpoleco.2006.02.003.
Cassini, A., Högberg, L.D., Plachouras, D., Quattrocchi, A., et al., 2019. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet Infect. Dis. 19, 56–66. https://doi.org/10.1016/S1473-3099(18)30605-4.
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.
De Boer, E., Zwartkruis-Nahuis, A., Heuvelink, A.E., Harmanus, C., Kuijper, E.J., 2011. Prevalence of Clostridium difficile in retailed meat in the Netherlands. Int. J. Food Microbiol. 144, 561–564. https://doi.org/10.1016/j.ijfoodmicro.2010.11.007.

Dohmen, W., Dorado-García, A., Bonten, M.J.M., Wagenaar, J.A., Mevius, D., Heederik, D.J.J., 2017. Risk factors for ESBL-producing Escherichia coli on pig farms: a longitudinal study in the context of reduced use of antimicrobials. PLoS One 12, 1–14. https://doi.org/10.1371/journal.pone.0174094.
Dorado-García, A., Dohmen, W., Bos, M.E.H., Verstappen, K.M., Houben, M., Wagenaar, J.A., Heederik, D.J.J., 2015a. Dose-response relationship between antimicrobial drugs and livestock-associated MRSA in pig farming. Emerg. Infect. Dis. 21, 950–959. https://doi.org/10.3201/eid2106.140706.
Dorado-García, A., Graveland, H., Bos, M.E.H., Verstappen, K.M., Van Cleef, B.A.G.L., Kluytmans, J.A.J.W., Wagenaar, J.A., Heederik, D.J.J., 2015b. Effects of reducing antimicrobial use and applying a cleaning and disinfection program in veal calf farming: experiences from an intervention study to control livestock-associated MRSA. PLoS One 10, 1–21. https://doi.org/10.1371/journal.pone.0135826.
Dorado-García, A., Mevius, D.J., Jacobs, J.J.H., Van Geijlswijk, I.M., Mouton, J.W., Wagenaar, J.A., Heederik, D.J., 2016. Quantitative assessment of antimicrobial resistance in livestock during the course of a nationwide antimicrobial use reduction in the Netherlands. J. Antimicrob. Chemother. 71, 3607–3619. https://doi.org/10.1093/jac/dkw308.
Filippitzi, M.E., Goumperis, T., Robinson, T., Saegerman, C., 2017. Microbiological zoonotic emerging risks, transmitted between livestock animals and humans (2007–2015). Transbound. Emerg. Dis. 64, 1059–1070. https://doi.org/10.1111/tbed.12484.
Ghassemi Nejad, J., Torbatinejad, N., Naserian, A.A., Kumar, S., Kim, J.D., Song, Y.H., Ra, C.S., Sung, K.I., 2012. Effects of processing of starter diets on performance, nutrient digestibility, rumen biochemical parameters and body measurements of Brown Swiss dairy calves. Asian-Australasian J. Anim. Sci. 25, 980–987. https://doi.org/10.5713/ajas.2011.11457.
Hancox, L.R., Le Bon, M., Dodd, C.E.R., Mellits, K.H., 2013. Inclusion of detergent in a cleaning regime and effect on microbial load in livestock housing. Vet. Rec. 173, 167. https://doi.org/10.1136/vr.101392.
Hoelzer, K., Bielke, L., Blake, D.P., Cox, E., Cutting, S.M., Devriendt, B., Erlacher-Vindel, E., Goossens, E., Karaca, K., Lemiere, S., Metzner, M., Raicek, M., Collell Suriñach, M., Wong, N.M., Gay, C., Van Immerseel, F., 2018a. Vaccines as alternatives to antibiotics for food producing animals. Part 1: challenges and needs. Vet. Res. 49, 1–10. https://doi.org/10.1186/s13567-018-0560-8.
Hoelzer, K., Bielke, L., Blake, D.P., Cox, E., Cutting, S.M., Devriendt, B., Erlacher-Vindel, E., Goossens, E., Karaca, K., Lemiere, S., Metzner, M., Raicek, M., Collell Suriñach, M., Wong, N.M., Gay, C., Van Immerseel, F., 2018b. Vaccines as alternatives to antibiotics for food producing animals. Part 2: new approaches and potential solutions. Vet. Res. 49, 1–15. https://doi.org/10.1186/s13567-018-0561-7.
Holstege, M.M.C., de Bont-Smolenaars, A.J.G., Santman-Berends, I.M.G.A., van der Linde-Witteveen, G.M., van Schaik, G., Velthuis, A.G.J., Lam, T.J.G.M., 2018. Factors associated with high antimicrobial use in young calves on Dutch dairy farms: a case-control study. J. Dairy Sci. 101, 9259–9265. https://doi.org/10.3168/jds.2017-14252.
IKB, 2008. BIJLAGE I : Voorschriften Kalverhouderij (Behorende Bij BIJLAGE 1 Voorschriften IKB Vleeskalveren 2008) Norm Voorschrift Administratie / Materialen, pp. 1–21.
Iwu, C.D., Korsten, L., Okoh, A.I., 2020. The incidence of antibiotic resistance within and beyond the agricultural ecosystem: a concern for public health. Microbiologyopen 9, 1–28. https://doi.org/10.1002/mbo3.1035.
Jarrige, N., Cazeau, G., Morignat, E., Chanteperdrix, M., Gay, E., 2017. Quantitative and qualitative analysis of antimicrobial usage in white veal calves in France. Prev. Vet. Med. 144, 158–166. https://doi.org/10.1016/j.prevetmed.2017.05.018.
Kreuzer, M., 2014. Brauchen Nutztiere Antibiotika?. In: 15 Jahre AML Verbot : Tagungsbericht, 6. Mai 2014, ETH-Schriftenreihe zur Tierernährung. ETH Zürich, Institut für Agrarwissenschaften, Zürich.
Laanen, M., Persoons, D., Ribbens, S., de Jong, E., Callens, B., Strubbe, M., Maes, D., Dewulf, J., 2013. Relationship between biosecurity and production/antimicrobial treatment characteristics in pig herds. Vet. J. 198, 508–512. https://doi.org/10.1016/j.tvjl.2013.08.029.
Lava, M., Schüpbach-Regula, G., Steiner, A., Meylan, M., 2016. Antimicrobial drug use and risk factors associated with treatment incidence and mortality in Swiss veal calves reared under improved welfare conditions. Prev. Vet. Med. 126, 121–130. https://doi.org/10.1016/j.prevetmed.2016.02.002.
Lee, Y., Nelder, J.A., 2004. Conditional and marginal models: another view. Stat. Sci. 19, 219–238. https://doi.org/10.1214/088342304000000305.
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.
Neill, J.O.', 2014. Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations the Review on Antimicrobial Resistance Chaired.
Nieuwamerongen, S.Evan, 2017. Development of Pigs Raised in a Group Housing System for Lactating Sows and Their Litters.
O'Brien, R.M., 2007. A caution regarding rules of thumb for variance inflation factors. Qual. Quant. 41, 673–690. https://doi.org/10.1007/s11135-006-9018-6.
Pardon, B., De Bleecker, K., Hostens, M., Callens, J., Dewulf, J., Deprez, P., 2012. Longitudinal study on morbidity and mortality in white veal calves in Belgium. BMC Vet. Res. 8, 26. https://doi.org/10.1186/1746-6148-8-26.
Pikkemaat, M.G., Yassin, H., van der Fels-Klerx, H.J., Berendsen, B.J.A., 2016. Antibiotic residues and resistance in the environment. RIKILT Rep. https://doi.org/10.18174/388253, 2016.009. 32.
Policy on the use of antibiotics in food-producing, 2015. 1–8.
R Core Team, 2020. R: A Language and Environment for Statistical Computing.

Schnyder, P., Schönecker, L., Schüpbach-Regula, G., Meylan, M., 2019. Effects of management practices, animal transport and barn climate on animal health and antimicrobial use in Swiss veal calf operations. Prev. Vet. Med. 167, 146–157. https://doi.org/10.1016/j.prevetmed.2019.03.007.

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

Sjölund, M., Backhans, A., Greko, C., Emanuelson, U., Lindberg, A., 2015. Antimicrobial usage in 60 Swedish farrow-to-finish pig herds. Prev. Vet. Med. 121, 257–264. https://doi.org/10.1016/j.prevetmed.2015.07.005.

Speksnijder, D., 2017. Antibiotic Use in Farm Animals : Supporting Behavioural Change of Veterinarians and Farmers.

Tang, K.L., Caffrey, N.P., Nóbrega, D.B., Cork, S.C., Ronksley, P.E., Barkema, H.W., Polachek, A.J., Ganshorn, H., Sharma, N., Kellner, J.D., Ghali, W.A., 2017. Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. Lancet Planet. Heal. 1, e316–e327. https://doi.org/10.1016/S2542-5196(17)30141-9.

Technical, G., Unit, S.B., n.d. Intradermal Vaccination of Pigs – a Perfect Match 31.

Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillant, A., Laxminarayan, R., 2015. Global trends in antimicrobial use in food animals. Proc. Natl. Acad. Sci. U. S. A. 112, 5649–5654. https://doi.org/10.1073/pnas.1503141112.

van Bunnik, B.A.D., Woolhouse, M.E.J., 2017. Modelling the impact of curtailing antibiotic usage in food animals on antibiotic resistance in humans. R. Soc. Open Sci. 4 https://doi.org/10.1098/rsos.161067.

Van Cleef, B.A.G.L., Van Benthem, B.H.B., Verkade, E.J.M., Van Rijen, M.M.L., Kluytmans-Van Den Bergh, M.F.Q., Graveland, H., Bosch, T., Verstappen, K.M.H.W., Wagenaar, J.A., Bos, M.E.H., Heederik, D., Kluytmans, J.A.J.W., 2015. Livestock-associated MRSA in household members of pig farmers: Transmission and dynamics of carriage, a prospective cohort study. PLoS One 10, 1–13. https://doi.org/10.1371/journal.pone.0127190.

van Nieuwamerongen, S.E., Soede, N.M., van der Peet-Schwering, C.M.C., Kemp, B., Bolhuis, J.E., 2015. Development of piglets raised in a new multi-litter housing system vs. Conventional single-litter housing until 9 weeks of age. J. Anim. Sci. 93, 5442–5454. https://doi.org/10.2527/jas.2015-9460.

Walker, D.B., Mächler, Martin, Bolker, Ben, Walker, Steve, 2015. Fitting Linear Mixed-Effects Models Using {lme4}. https://doi.org/10.18637/jss.v067.i01.

Wold, S., 1984. Three PLS algorithms according to SW. In: Wold, S. (Ed.), Report from the Symposium MULTDAST (Multivariate Data Analysis in Science and Technology), pp. 26–30.

Yan, J., 2002. geepack: Yet Another Package for Generalized Estimating Equations.

Ziegler, A., 2011. Generalized estimating equations, antimicrobial agents and chemotherapy. Lecture Notes in Statistics. Springer, New York, New York, NY. https://doi.org/10.1007/978-1-4614-0499-6.