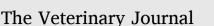
Contents lists available at ScienceDirect







journal homepage: www.elsevier.com/locate/tvjl

Risk factors for antimicrobial usage and diseases in Dutch veal calf farms: A cross-sectional study

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ARTICLE INFO

Keywords: Antibiotics Cattle Animal welfare Biosecurity Partial least squares

ABSTRACT

Antimicrobial use (AMU) is the main driver of antimicrobial resistance (AMR). In the Netherlands, the veal calf sector was among the largest consumers of antimicrobials in Defined Daily Doses Animal (DDDA) for the year of 2022. As preventive use in Dutch livestock farms is forbidden since 2011, most AMU is due to the herd health status which is affected by the farm environment in which the conditions for diseases to spread are created. The aim of this study was to determine which disease etiologies for group treatments are associated with AMU in rosé starter veal calves, and which modifiable technical risk factors on farm are associated with those diseases and with total AMU. Cross-sectional data were collected from 36 Dutch rosé starter veal calf farms in the Netherlands in 2021 using a digital survey. Linear regression analysis showed that the main indications for AMU were respiratory infections, for which mainly tetracyclines and macrolides were used. Partial least squares regression analysis (PLS) revealed 13 on-farm practices associated with the number of group treatments for respiratory diseases and 19 with total AMU. Overlapping variables in both PLS models were related to regrouping of calves, micro-climate conditions, water access and weaning strategies. Overall, these features focused on improving animal welfare and nutrition during production and enhancing a farm's internal and external biosecurity. This study identified opportunities for reducing AMU in rosé starter veal calf farms, which thereby could contribute to limiting AMR emergence and spread.

Introduction

Antimicrobial usage (AMU) promotes antimicrobial resistance (AMR) in bacterial populations (Gullberg et al., 2011; Larsson and Flach, 2022). Antimicrobials in livestock are used for therapeutic, metaphylactic and prophylactic purposes, and in some countries still as growth promoters. Currently in the European Union (EU), only therapeutic AMU is allowed (Simjee and Ippolito, 2022). In 2009, the Dutch government issued several policies for veterinarians and farmers to reduce AMU (Speksnijder, 2017), with a complete ban on preventive AMU since 2011 (Speksnijder et al., 2015).

Although AMU in Dutch veal calves has declined since 2009, they are among the largest antimicrobial consumers in the Netherlands (SDa,

2023). The Netherlands is also the largest producer of veal calf meat in the EU (Marcato, 2021), with only 10 % of total veal meat production being consumed within the country (Department of Trade and Industry, 2007). Overall, three veal calf production systems exist, namely white and rosé veal (both <8 months of age) and older rosé veal (8–12 months) (Pardon et al., 2014; Valgaeren, 2015). Calves stay in dairy farms for at least 14 days. Afterwards, they are transferred to collection centers and then to veal calf farms where they are typically housed individually ("calf boxes") for 2–3 weeks and subsequently sorted in groups (Marcato, 2021). This is the starter period, lasting approximately three months, during which calves are weaned to a solid diet. The final fattening stage before slaughter can take place in the same (i.e., combination farms) or other specialized farms. Due to various

https://doi.org/10.1016/j.tvjl.2024.106263

Received 28 August 2024; Received in revised form 30 October 2024; Accepted 31 October 2024 Available online 8 November 2024 1090-0233/© 2024 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

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environmental stressors and immunological immaturity (Chase et al., 2008), infections are common in veal calves (Pardon et al., 2013; Marcato, 2021).

Rosé veal starter farms in the Netherlands have the highest average AMU, with 69.2 defined daily dosages for animals per year (DDDA/Y) in 2022. DDDA is defined as the ratio of total kg of active substance divided by the total kg of animals present on the farm and disease-respective standardized daily authorized dosage (Lekagul et al., 2018; SDa, 2023).

Risk factors for AMU can be related to farm technical characteristics, including rearing practices and conditions, and to socio-economic and behavioral aspects (Bokma et al., 2018). Here, we mainly focused on the former. Variation in AMU among veal calf farms reflects their potential for improvement regarding on-farm practices/conditions for further AMU reduction (SDa, 2023). This study aimed to determine: i) which diseases are associated with AMU in Dutch veal calf farms, ii) which on-farm practices/conditions are associated with total AMU in these farms.

Materials and methods

Study design and data collection

In 2022, a cross-sectional study was conducted on 36 conventional rosé veal calf farms with starters (i.e., calves at the starting period) in the Netherlands (N=249, so 14.5 % of total starter and combination Dutch farms (SDa, 2022)). Enrollment of more farms was limited by the COVID-19 pandemic and farmers' protests for the national policies on nitrogen emissions. However, a Monte Carlo simulation based power analysis indicated that our sample had sufficient power to estimate the global effect size of the final model (Peugh, 2010), as measured by Cohen's f^2 (Supplementary Material 1: Figures S2 and S3).

Farms were enrolled and surveyed through their veterinarians: 14 veterinarians working in 8 different practices specialized in bovine medicine participated in the study. These practices are among the largest ones in the Netherlands. The number of participating veterinarians reflected their availability during their daily working schedule. Veterinarians were asked to randomly enroll farms from those under their care: the number of farms enrolled depended on veterinarians' workload and farmer's willingness to participate in the study. Inclusion criteria were being a conventional rosé starter veal calf farm and having complete knowledge of the practices applied there by the veterinarian to avoid information bias. Collected data reflected the situation of farms' last production cycle of starters (approximately 3 months).

A digital survey was used to gather information on various aspects of on-farm characteristics, including husbandry practices, external and internal biosecurity, animal care, calf nutrition, health management, microclimate and housing conditions, along with disease etiologies for group treatments and AMU. Veterinarians could answer the survey offline, in the office or on farm during routine visits, with input from the farmer if needed. The survey is available in Supplementary Material 2 and Table S2. The survey was constructed based on other surveys, such as Biocheck-UGent (Damiaans et al., 2020), as well as literature reviews (Bokma et al., 2018) and previous survey-based studies (Bokma-Bakker et al., 2017). The survey was tailored to the specifics of the Dutch calf sector through consultations with veal calf farming experts and participating veterinarians. The survey was also piloted in three farms before use. Questions contained a mixture of continuous, counts, binary or categorical (mutually exclusive) variables. Some questions used Likert scale measurements with five levels. Total cattle, dairy cattle and veal calf densities in the provinces of the enrolled farms were obtained from 2021 data from Statistics Netherlands (CBS, 2023). All participants were informed about the objectives of the study and agreed to participate. Data were analyzed anonymously. Financial compensation was provided to the participating veterinarians and farmers for the time invested in completing the questionnaires.

The AMU per farm was calculated based on a national database of farm-level delivery sales data. Due to current regulations (Speksnijder, 2017) and national AMU monitoring systems, veterinarian-registered antimicrobial delivery records represent the most robust measure of antimicrobial quantities administered on farm (SDa, 2023). The classes of antimicrobials recorded are aminoglycosides, amphenicols, macrolides. penicillins, quinolones, tetracyclines and trimethoprim-sulphonamides. Antimicrobial deliveries were converted to DDDA values and their total sum represented the total DDDA of the farm for starters' last production cycle. Total active substance for DDDA calculation was estimated based on quantities and concentrations of each delivered product. Standardized authorized dosages for each product were retrieved by the "DG Standard" database (SDa, 2021; Moura et al., 2022). Total number of animals was recorded at each delivery time and the weight was estimated based on a standard growth table for rosé starters of 50 kg at arrival on farm and 140 kg at day 91.

Determining from national AMU monitoring data the DDDA for group and individual treatments was not possible. However, the number and types of group treatments are recorded by each veterinary practice. Therefore, veterinarians were asked to report each group treatment in the survey with the corresponding disease etiology and antimicrobial used. The diagnosis was categorized into predetermined disease groups based on guidelines of the Royal Dutch Veterinary Association (KNMvD), including digestive, locomotive, respiratory, systemic diseases, wound/naval infections and otitis (KNMvD, 2017); Table S3 summarizes the conditions and pathogens involved. Individual treatments could be identified from the remaining deliveries (after removing the provided group treatments), but those had no known diagnosis available. Therefore, antimicrobial deliveries other than group treatments represented a 7th "unknown disease etiology" category.

Statistical analysis

Diseases associated with AMU

Associations between calf diseases and AMU were explored using linear mixed-effects regression with backward variable selection. The initial set of fixed effects included farm size (i.e., number of calf places/100), the number of group treatments for respiratory conditions and the number of individual treatments in the last production cycle and their interaction. The other disease indications did not meet the variation criterion of >30 % farms. The initial set of random effects were two nested random intercepts, accounting for potential clustering of veterinarians within veterinary practices. Backward stepwise variable selection was performed on both random- and fixed-effects (apart from farm size which was always forced) based on Akaike's information criterion (AIC). Diagnostics were performed on all models to examine whether residuals were normally distributed and homoscedastic.

Farm characteristics associated with AMU and diseases

Data was characterized by multicollinearity and high dimensionality; thus, the dimension reduction technique of partial least squares (PLS) regression without random effects was used, as clustering was not observed in previous backward selection. Overfitting was prevented by k-fold cross-validation (CV). Given the low number of observations resulting in more manageable computational time, we used multiple kfolds, including leave-one-out (LOO), 18, 12, 9, 6, and 4 folds. The applied algorithm is visualized in Fig. 1 and used two criteria: root mean squared error (RMSE) and Variable Importance in Projection (VIP) (Fernández Pierna et al., 2009; Akarachantachote et al., 2014; Dong and Ma, 2019; Mendez et al., 2020). Step 1 in Fig. 1 was repeated 150 times for each k, except LOO, defining 751 candidate subsets. Step 2 included additional candidate subsets based on frequencies of each variable across Step 1 models. In Step 3, a new k-fold CV was applied to define the final model based on the candidate models from Step 2 with the lowest average RMSE. Twenty iterations were applied for each k, except LOO, and the model most frequently selected for its minimum average RMSE

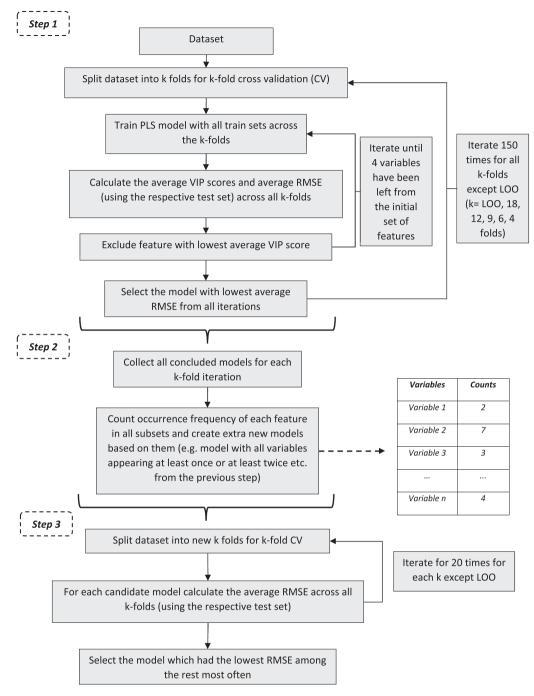


Fig. 1. Visualization of the developed variable selection algorithm within a PLS framework. Variable importance in projection (VIP) and the cross-validated root mean square error (RMSE) were used to achieve convergence towards the best set of factors based on minimizing the cross-validated prediction error.

across all iterations was the final one (Fig. 1). In the final model, selection of principal components (PC) was done based on BIC corrected for degrees of freedom (BIC.dof). PLS for normally distributed outcomes was used for AMU. For disease occurrence, PLS for Poisson distributed outcomes was used. As before, farm size was forced as a control covariate in all models.

After fitting the final PLS model and selecting the respective optimal number of PCs, normal bootstrap confidence intervals **(CI)** of standardized beta-coefficients were calculated with Sidak adjustment for family wise-error within each PLS model. We performed 100,000 iterations with a bootstrap sample size equivalent to the original dataset. A variable was considered statistically significant if the standardized bootstrapped CI did not include zero.

The R packages used for the mixed effects model was "lme4" version 1.1–28 (Bates et al., 2015) and for the PLS models were "plsRglm" version 1.5.0 (Meyer et al., 2010; Bertrand and Maumy-Bertrand, 2014; Bertrand and Maumy-Bertrand, 2022) and "boot" version 1.3–28 (Davison and Hinkley, 1997; Canty and Ripley, 2021). All analyses were performed using the R language and the environment version 4.0.3 (R Core Team, 2020).

Results

Diseases and AMU

The number of enrolled veterinary practices, veterinarians and farms

was 8, 14, and 36, respectively. Farms were located in 4 of the 12 Dutch provinces, mainly in the South and East of the country and their average size was 767 heads. Mean farm AMU was 35.64 DDDA (SD=12.03). Fig. 2 shows the AMU per antimicrobial class and disease etiology. Respiratory infections accounted for 63.7 % of total AMU, and the main classes used were tetracyclines and macrolides, which were also the most used classes overall. Tetracyclines in particular accounted for 62.3 % of total AMU, and were used to treat respiratory infections and individual treatments (72.1 % and 27.9 % of total tetracycline use, respectively). Macrolides accounted for 63.9 % of total AMU, of which respiratory infections accounted for 63.9 % of total macrolides used, and the rest was attributed to otitis and individual treatments.

After backward variable selection, the random effects were removed and the final fixed-effects included only the number of group treatments for respiratory infections. Beta-coefficient was 3.96 DDDA/Y, i.e., the expected change in AMU for every unit increase in these counts (P=0.026; 95 %CI: 0.50–7.41).

Farm characteristics and diseases

600

As group treatments counts for respiratory infections had the greatest impact on AMU, this was the only indication for which a PLS model with Poisson distributed outcome was built (hereinafter called 'respiratory-PLS model'). The variable selection algorithm determined that 13 variables and 2 PCs were optimal based on BIC, explaining 49.1 % and 15.4 % of the Y variation, respectively. Fig. 3 shows the bootstrapped CI of standardized coefficients, and Fig. 4 the score plot. Table 1 summarizes coefficients in the real scale and Table S1 (in Supplementary Material 1) their descriptive statistics. The frequency of cleaning milkproviding equipment in calf boxes was the only variable for which the standardized CI did not include zero and could therefore be considered statistically significant (coefficient: -0.04 log counts, meaning that farms cleaning more frequently this equipment had 4.2 % less respiratory disease group treatments). In terms of average effect size, this factor ranked fifth, with the top four factors being farmer's better knowledge on ventilation (22 % more group treatments), sorting calves by moving them only within the same compartment (17.9 % less group treatments), higher density of dairy cattle in the province in which the farm is located (55 % more group treatments) and using portable gas forced air heaters (in Dutch "Warmtekanon") to warm up the stable (19 % more group treatments).

Farm characteristics and AMU

The PLS model for total farm AMU (hereinafter called 'AMU-PLS model') included 19 factors. Fig. 5 shows the bootstrapped CI of standardized coefficients of the AMU-PLS model, while Fig. 6 depicts the score plot. Table 2 contains the coefficients in the real DDDA scale and Table S1 their descriptive statistics. The number of selected PCs was two. The first PC explained 69.4% of total Y variation, while the second PC explained 13.6%. Out of the 19 factors, seven had statistically significant effect on AMU. Regrouping/mixing of starter calves for teat access (5.8 DDDA), use of smoke to check air circulation (5.1 DDDA) and dairy cattle density per province (10.3 DDDA) were the strongest risks. Conversely, statistically significant protective factors included housing veal calf starters in the same pen as calf boxes (at the beginning of the starter period) (-5.1 DDDA), having the finishing phase on the same farm (-3.8 DDDA), better cleaning and disinfection of water pipes (-2.0 DDDA) and longer periods in which milk was given once a day (-1.2 DDDA).

Discussion

This study identified disease etiologies associated with AMU in Dutch rosé veal calf farms, as well as risk factors associated with group treatments for those diseases and total AMU. As both disease and AMU occur in the same causal pathway (see Figure S1), the two models were built separately. Group treatments for respiratory infections were the main etiology for AMU compared to other disease etiologies, with tetracyclines and macrolides being the main antimicrobial classes used.

Group treatment Etiologies [Counts | % of total AMU] 400 Respiratory [109 | 63.7%] Individual treatments of unknown etiology [591 | 31.4%] DDDA Digestive [9 | 2.3%] Otitis [4 | 1.3%] Systemic [1 | 1.2%] Locomotion [4 | 0%] Wound/Naval infection [1 | 0%] 200 0 Trimethoprim-Sulfonamides Aminoglycosides etracyclines Amphenicols Quinolones Macrolides Penicillins **Antimicrobial Class** Fig. 2. Sum of AMU in DDDA per antimicrobial class within the last production cycle of 36 rosé veal starter farms in 2021 with the respective etiology. Within

Fig. 2. Sum of AMU in DDDA per antimicrobial class within the last production cycle of 36 rosé veal starter farms in 2021 with the respective etiology. Within brackets counts stand for the number of antimicrobial deliveries from the national registry and the percentage shows how much each etiology accounted for in the total AMU.

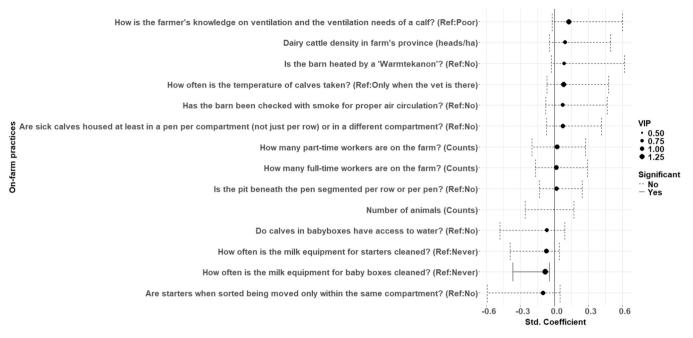


Fig. 3. Normal bootstrapped CI of the standardized coefficients of the respiratory-PLS model and their variable importance in projection (VIP) scores. At the end of each variable the parenthesis indicates the reference category or the units in which it was measured and significance is defined by whether the confidence intervals cross zero or not.

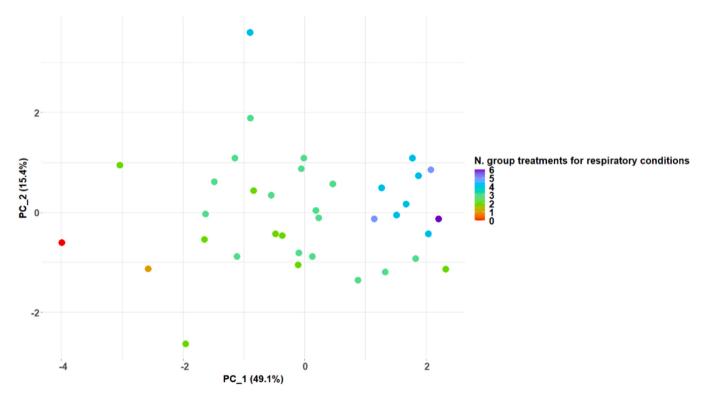


Fig. 4. Score plot of the respiratory-PLS model. Each dot represents a farm (N=36) and the color represents the number of respiratory group treatments. Principal component 1 explained 49.1 % of Y variation and from left to right the orientation towards higher counts of respiratory group treatments can be seen.

Both have been observed before (Jarrige et al., 2017; Antonis et al., 2022), and it is generally recognized that young calves are particularly susceptible to respiratory diseases, which are exacerbated by early and lengthy transportation to veal calf farms (Sanderson et al., 2008). This analysis allowed us to prioritize disease etiologies and estimate how much AMU reduction can theoretically be expected if such group treatments would be reduced in occurrence.

Regarding farm characteristics associated with group treatments for respiratory infections, 13 variables were selected, and 19 for total AMU. In the two PLS models four variables overlapped, and overall the selected variables were related to calf sorting/regrouping, barn ventilation and temperature management, milk and water provision management and equipment hygiene. Drawing a hard line between statistically significant and non-significant factors was not necessarily

Table 1

Coefficients of the respiratory-PLS model at normal scale; the coefficients show the expected % change in the number of group treatments per unit increase of the predictors for the last production cycle.

Predictors in respiratory-PLS model	%
Dairy cattle density in farm's province (heads/ha)	55
How is the farmer's knowledge of ventilation and the ventilation needs of a calf? (Ref:Poor)	22
Is the barn heated by a 'Warmtekanon'? (Ref:No)	19
Are sick calves housed in a pen per compartment or in a different compartment? (Ref:No)	16
Has the barn been checked with smoke for proper air circulation? (Ref:No)	16
How often is the temperature of calves taken?	11
Is the pit beneath the pen segmented per row or per pen? (Ref:No)	4
How many full-time workers are on the farm?	3
How many part-time workers are on the farm?	2
How often is the milk equipment for starters cleaned?	-3
How often is the milk equipment for calf boxes cleaned?	-4
Do calves in calf boxes have access to water? (Ref:No)	$^{-13}$
Are starters when sorted being moved only within the same compartment? (Ref:No)	-18

constructive in this analysis given the relatively small sample size. Therefore, also the inclusion of factors in the final model was seen as an indication of importance on the association, given that they were selected here by the variable selection algorithm used.

The calf box period which has been shown to reduce respiratory problems (Brscic et al., 2012) is followed by sorting and regrouping the calves in various ways to harmonize the new groups in the pens in terms of weight, sex, drink speed and health problems, including ruminal drinking (Brscic et al., 2012; Damiaans et al., 2019). This reduces competition among calves for access to feed (Damiaans et al., 2019). At the same time, regrouping introduces a disease transmission risk (Perttu et al., 2023), as well as stress (Lyu et al., 2023). We found that the primary risk factor for AMU was regrouping of calves to be placed on teats, usually due to ruminal drinking (Herrli-Gygi et al., 2006). This is probably related to reverse causation, as the study design was

cross-sectional and those calves placed on teats usually already face problems. For AMU, two protective factors were the frequency of calves being sorted by weight (not statically significant) and starters being placed in the same pen as their calf boxes when the starter period starts (statistically significant). Interestingly, (Brscic et al., 2012) reported that calf boxes at the beginning of fattening were able to reduce prevalence of some respiratory symptoms, possibly due to fewer contacts but they should not be prolonged for too long for socialization. The most protective effect for respiratory infections was seen for sorting calves without moving them outside their initial compartment, a practice that is usually applied for isolation of diseased animals (Damiaans et al., 2019).

In all PLS models, checking the barn with smoke for proper air circulation was associated with increased risk for AMU and respiratory group treatments. Additionally, for AMU, the need for improvement in ventilation (as indicated by the farmer) and having adjustable inlets (regardless of mechanical/natural ventilation), were risk factors, and so was a higher score on farmer's ventilation knowledge (based on veterinarian's assessment) for respiratory infections. While checking ventilation can be a proxy for better farm management, it could also be a proxy for ventilation problems. Suboptimal farm ventilation is a common risk factor for calves, as they are particularly sensitive to air drafts (Brscic et al., 2012). To avoid this, inlets should be automatically and continuously regulated. Linked to ventilation is also barn temperature regulation, and in both models warming the barn with a portable gas forced air heater was a risk factor. This machine has the disadvantage that heat is only locally dispersed and that exhaust fumes/pollution are released in the stable. This has also been observed in broiler farming (Antwerpen Provinci, 2018) although it might be more critical in closed air systems.

Access to water in the calf box period appeared to protect (not statically significant) against group treatments for respiratory infections and AMU. Although this is not unexpected, a common misconception is that milk covers the water needs of a calf. The EU Council Directive 2008/119/EC states that calves of \geq 2 weeks of age should have access to

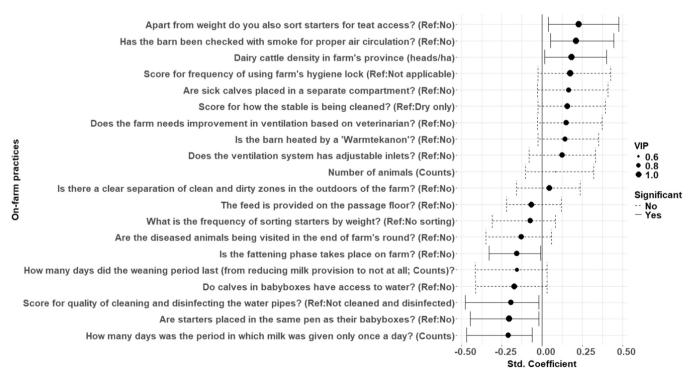


Fig. 5. Normal bootstrapped confidence intervals of the standardized coefficients of the AMU-PLS model and their variable importance in projection (VIP) scores. At the end of each variable the parentheses indicates the reference category or the units in which it was measured and significance is defined by whether the confidence intervals cross zero or not.

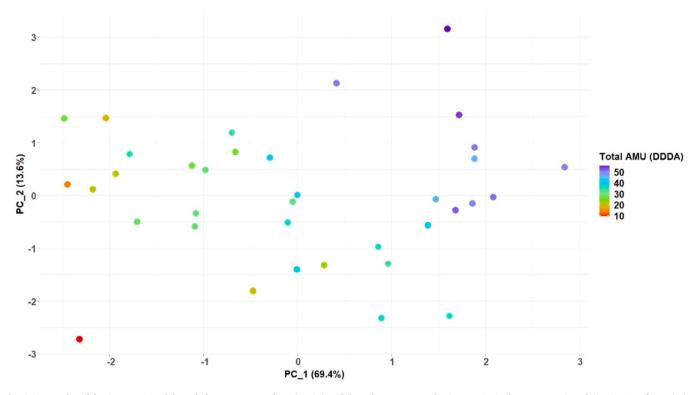


Fig. 6. Score plot of the AMU-PLS model. Each dot represents a farm (N=36) and the color represents the AMU. Principal component 1 explains 69.4 % of Y variation and from left to right the orientation towards higher usage can be seen.

Table 2

Coefficients of the AMU-PLS model at normal scale; the coefficients show the expected change in DDDA per unit increase of the predictors for the last production cycle.

Predictors in AMU-PLS model	DDDA
Dairy cattle density in farm's province (heads/ha)	10.3
Apart from weight do you also sort starters for teat access? (Ref:No)	5.8
Has the barn been checked with smoke for proper air circulation? (Ref:No)	5.1
Are sick calves placed in a separate compartment? (Ref:No)	3.9
The farm needs improvement in ventilation based on veterinarian	3.5
Is the barn heated by a 'Warmtekanon'? (Ref:No)	3.5
Does the ventilation system has adjustable inlets? (Ref:No)	3.0
Score for how the stable is being cleaned?	1.8
Score for frequency of using farm's hygiene lock	1.7
Is there a clear separation of clean and dirty zones in the outdoors of the	1.1
farm? (Ref:No)	
How many days did the weaning period last (from reducing milk provision	-0.5
to not at all)?	
What is the frequency of sorting starters by weight?	-0.7
How many days was the period in which milk was given only once a day?	$^{-1.2}$
The feed is provided on the passage floor? (Ref:No)	-1.7
Score for quality of cleaning and disinfecting the water pipes?	-2.0
Are the diseased animals being visited in the end of farm's round? (Ref:No)	-3.1
Is the fattening phase takes place on farm? (Ref:No)	-3.8
Do calves in calf boxes have access to water? (Ref:No)	-4.3
Are starters placed in the same pen as their calf boxes? (Ref:No)	-5.1

water constantly regardless of milk intake (Nielsen et al., 2023). Moreover, we found that extending the period in which milk is given only once a day was statistically significantly protective towards AMU, as was the prolongation of the period when milk provision starts to decrease until complete weaning. These findings agree with the literature and the aforementioned EU directive, which also discusses the benefits of later weaning (8–12 *vs* 4–6 weeks). Linked to these variables, higher hygiene status of the milk-providing equipment protected against group treatments for respiratory infections, and cleanliness of water pipes against total AMU. Dairy cattle density in the province where the farm was located was also a risk factor in both PLS models. The reasons are unclear, but it can be speculated that a higher dairy cattle density nearby influences pathogen circulation and act as a proxy for shared infrastructure, spaces, personnel and tools between farms, affecting their external biosecurity. Nevertheless, as our sample contained farms from 4 of the 12 Dutch provinces, sampling bias could also have occurred.

Limitations of this study are mainly due to the sample size. Although this was partly compensated by the PLS approach (Jia et al., 2022), large uncertainty in the estimates could not be avoided. Another disadvantage of small sample sizes is the increased probability of sampling bias. Here, CV with multiple k-folds was applied to evade inclusion of noisy variables and prevent overfitting. Moreover, the cross-sectional study design is known to be prone to reverse causation, as it fails to capture temporality. Finally, certain variables have either no variation or cannot be assessed/controlled for in observational studies. These limitations influence the signal-to-noise ratio intrinsic to the data (Andrade, 2013). As such, findings should be interpreted with caution.

Conclusions

Reducing AMU is vital to prevent AMR. Prioritizing control of the risk factors identified in this study would essentially mean to enhance animal welfare, nutrition, micro-climate and biosecurity in veal calf farms. The observed impact of respiratory infections on AMU appeared to be mainly related to sorting/regrouping of veal calf starters, ventilation and temperature regulation of the barn, weaning strategies and hygiene of feed, milk and water equipment. This study provided a basis for future epidemiological studies looking at associations of interest in more detail using larger samples. This will provide insights to farmers, veterinarians and other stakeholders into how improvements in farm management may assist AMU reduction even further.

Ethics statement

This study was based on anonymized farm registry data. No sampling of animals or human subjects was performed, so no ethical approval was needed.

Funding

This research was supported by the Netherlands Organization for Health Research and Development (ZonMw) with the project "CIAO-CIAO! Comparative Impact Assessment of Options to Curtail Inessential Antimicrobials On-farm" (Grant number 541002002).

CRediT authorship contribution statement

Panagiotis Mallioris: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Lapo Mughini-Gras:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Arjan Stegeman:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization. **Jaap A. Wagenaar:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization. **Jaap A. Wagenaar:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization. **Peter Theeuwes:** Writing – review & editing, Resources, Investigation. **Effrosyni Kritsi:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank all the veterinary practices and farmers participated in the project, all the veterinarians assisting with the data collection and Niels Geurts (DAP Thewi B.V.), Koen D' Hoe (DAP Thewi B.V.) and Henk Bekman for their valuable feedback.

Consent for publication

Not applicable.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2024.106263.

Data Availability

The anonymized data of this study are available from authors on reasonable request and the R code is available here https://github. com/forestiy/CIAOCIAO_calves.

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