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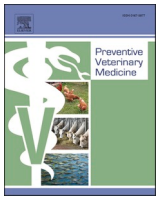

# Application of multiblock analysis to identify key areas and risk factors for dairy cow persistence

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ABSTRACT

The present study analysed the importance of individual variables and different thematic blocks of production areas, management, and herd infectious disease status on cow persistence, characterised by herd on-farm mortality rate (MR), culling rate (CR), and mean age of culled cows (MAofCC) applying multiblock partial least squares (mbPLS) analysis. This study included 120 free-stall dairy herds with ≥ 100 cows. Data on the previous year's predominant cow housing system and management practices were collected, and on-farm measurements and cow scoring were performed. Bulk tank milk (BTM) and heifer blood samples (10 samples per herd) were collected and analysed for antibodies against the selected pathogens. In total, 172 variables were aggregated into 14 thematic blocks. The annual CR, MR, and MAofCC values were calculated for each herd. Thematic blocks with significant impact on cow persistence (included herd MR, CR and MAofCC) were 'infectious diseases' (block importance index out of all blocks = 13.6%, 95% CI 10.3; 20.5), 'fertility management' (16.3%, 95% CI 6.8; 26.9), 'lactating cow management' (11.5%, 95% CI 6.4; 17.8), 'milking' (11.3%, 95% CI 3.2; 17.1), 'herd characteristics' (10.1%, 95% CI 6.3; 14.2), 'close-up period management' (9.7%, 95% CI 2.7; 15.7), 'calving management' (7.9%, 95% CI 3.1; 11.4) and 'disease management' (7.3%, 95% CI 0.2; 12.0). Variable categories with the highest importance in explaining composite outcome including herd MR, CR and MAofCC were rear-end and udder lesions in ≥ 20% of the cows, BTM and heifers seropositive to bovine respiratory syncytial virus, vaccination against bovine herpesvirus 1, twice daily milking and herd location in Northwest region. Larger herd size, higher levels of milk yield, and rearing predominantly Holstein breed cattle were herd factors associated with poorer cow persistency. Grazing cows and having semi-insulated barns were associated with lower CR and MR, respectively. Heat detection and farm pregnancy testing strategies were significant factors in the fertility block. Using disposable dry papers for teat cleaning and not using any wet teat-cleaning tools were risk factors for high MR. A robotic milking system was protective for increased herd MR and CR. A high pre-calving body condition score and poor rear body cleanliness of ≥ 30% of cows were associated with inferior herd persistency outcomes. Calving in group pens with deep litter bedding was associated with a lower CR. Multiblock PLS model is innovative tool that helped to identify most influential farming areas but also single risk factors associated with cow persistency described by multiple parameters.

## 1. Introduction

Globally increasing trends in dairy cow on-farm mortality over the last few decades (Thomsen et al., 2004; Alvåsen et al., 2012; Shahid et al., 2015; Compton et al., 2017) indicate deteriorating cow health and welfare (de Vries et al., 2011). Although cow culling rates have not increased considerably, the longevity of cows has decreased in most high-producing countries, and cows are more frequently culled for involuntary reasons, such as diseases, injuries, and infertility (Compton et al., 2017; Dallago et al., 2021; Mõtus and Niine, 2022). This impairs the economic profitability of dairy production, is not in accordance with the principles of sustainable animal use, and amplifies the environmental impacts of the sector (Grandl et al., 2019; Dallago et al., 2021). Societal expectations and trust in milk production contradict these

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prevailing trends (de Vries et al., 2011).

Extensive research has been conducted to examine animal- and herd-level risk factors for cow on-farm mortality, culling rates, and longevity (Bell et al., 2010; Alvåsen et al., 2012, 2018; Santman-Berends et al., 2014; Shahid et al., 2015; Sarjokari et al., 2018; Reimus et al., 2020). Despite the well-known animal level risk factors for cow persistency, e.g. assisted calving, abortion, presence of production-related diseases, indication of negative energy balance in early lactation period, increased somatic cell count etc. (Hadley et al., 2006; Alvåsen et al., 2014; Shahid et al., 2015; Reimus et al., 2017), these factors are mostly governed by herd management, housing and environmental conditions. Owing to the constant intensification of dairy production, dairy herds will continue to expand in the future (Barkema et al., 2015), whereas a larger herd size is often considered a limiting factor in achieving acceptable mortality and culling rates and superior longevity (Smith et al., 2000; Raboisson et al., 2011; Shahid et al., 2015; Strandberg and Emanuelson, 2016; Rilanto et al., 2020). Therefore, a better understanding of ensuring sufficient cow longevity and limiting involuntary culling of cows in large commercial farm settings is needed. Herds included in different risk-factor studies vary in size, production type, and housing system, and interactions between herd size and cow housing systems often occur (Simensen et al., 2010; Alvåsen et al., 2012; Shahid et al., 2015; Reimus et al., 2020). Therefore, it is challenging to apply the results of these studies to large-scale free-stall barns, which is the predominant farm type for commercial dairy production worldwide.

According to previous studies, a wide range of herd-level risk factors, such as housing, management, infectious diseases, and farmers' attitudes, affect dairy cow culling, mortality rates, and longevity (Raboisson et al., 2011; Alvasen et al., 2014; Shahid et al., 2015; Strandberg and Emanuelson, 2016; Sarjokari et al., 2018; Reimus et al., 2020; Mõtus et al., 2021; Rilanto et al., 2022). However, to date, studies on the overall importance and contribution of risk factor groups in explaining cattle loss and longevity are lacking. Culling includes all cow removal activities, independent of their reason, and determines herd longevity (Fetrow et al., 2006). Although cow persistence, including culling, mortality rates, and longevity, is one of the main concerns in dairy production (Schuster et al., 2020), these rates have mostly been analysed in different settings and studies. However, revealing the importance and contribution of each farming area in explaining cow persistence is valuable for developing improved herd health programs.

Common statistical analyses, such as generalised linear models, do not allow the explanation of a multivariate outcome or the aggregation of several explanatory variables into thematic blocks (Bougeard et al., 2012). An innovative statistical method called multiblock partial least squares (mbPLS) regression (Wold, 1984; Wangen and Kowalski, 1989) was recently introduced in veterinary epidemiology to describe datasets with a large number of variables organised into thematic blocks and to allow the use of composite outcomes (Lupo et al., 2010; Bougeard et al., 2012; Collineau et al., 2018). This method also identifies the associations between individual explanatory and dependent variables, in addition to identifying the contribution of each explanatory block to the studied composite outcome (Bougeard et al., 2012). The method is less sensitive to multicollinearity than standard regression methods such as (logistic) regression due to not involving any matrix inversion (i.e., for generalized linear models, such as standard regression or logistic regression, the variance-covariance explanatory matrix must be invertible, which is not possible when the explanatory variables are correlated). In addition, the mbPLS regression model is based on orthogonal components which also limits the method sensitivity to multicollinearity.

The objective of this study was to analyse the relative impact of different thematic blocks of production areas and herd status of selected infectious diseases on the overall persistence of cows characterised by herd on-farm mortality, culling rate, and age at culling using the innovative mbPLS analysis. We also aimed to reveal the individual risk factors within thematic blocks that had the highest contribution to any of the component outcomes describing cow persistence.

## 2. Materials and methods

### 2.1. Study design and herd recruitment

This historical single-cohort study aimed to include 120 dairy herds according to the following inclusion criteria: (1) having at least 100 dairy cows, (2) a loose-housed keeping system for cows, and (3) no intention to cease production in the coming years. A list of dairy herds with at least 100 cows was acquired from Estonian Livestock Performance Recording Ltd. (ELPR) in January 2019, including 182 holdings. Due to the lack of data on the last two inclusion criteria, herds were selected randomly from the sample frame using a random number generator in Stata® MP14.2 (StataCorp, Texas). The aims and methodology of the study were introduced to the farm manager over a phone call, specifying whether the herd met all inclusion criteria. In cases of non-fulfilment of criteria or no interest in participating in the study, a new herd was randomly selected from the sample frame. A total of 169 herds were contacted during the enrolment period. In total, 49 herds were excluded from the sample list because they either did not meet the inclusion criteria or were not interested in participating in the study. To obtain consent, a visit to the herd was arranged by one of the two veterinarians involved in the study between August 2019 and July 2020.

### 2.2. Data collection

For each herd, the data for the year preceding the farm visit were collected from the ELPR. This included the number of cows in the herd exactly a year before the farm visit and at the date of the visit, and the number and age of culled cows (including cows slaughtered for meat production, died, or euthanised on farm, and excluding cows that were sold to another farm for dairy purposes) during the year preceding the farm visit. The average number of cows in the herd over the year before the farm visit was obtained by calculating the average of the number of cows in the herd during the farm visit and one year prior. Herd culling rate (CR) was calculated as the proportion of culled cows among the average number of cows in the herd. The mean value was calculated from the ages of all culled cows (MAofCC) within the same period, excluding cows that were sold to continue the production in another herd and is referred to as 'longevity' hereafter. The Estonian Agricultural Registers and Information Board (EARIB) collects national cattle data, and animal owners are obliged to report all cattle births, movements, and deaths to the registry. For each herd, the cow on-farm mortality rate (MR) was calculated based on the EARIB data. The numerator included the number of cows that died (unassisted or euthanised) within the 12 months preceding the farm visit, and the denominator was the average number of cows present in the herd over the same period. The theoretical framework of the questionnaire and data collection protocol was to capture the data of the possible risk factors from different factor groups that affect dairy cow health, performance and persistency. After a thorough literature review and drafting the preliminary version of the questionnaire and data collection protocol, a group of herd health experts were consulted for the review of the questions and for possible additions. The questionnaire aimed to capture the information about cow management in different lactation stages: dry period, close-up period, at calving, during colostrum period (from calving to 4 days post-partum) and post-calving period (up to 3 months after calving) and during the rest of the lactation period. Also, special focus was on cow traffic and grouping principles, feeding management, milking procedures, overall disease prevention practices, fertility management and farm implementation of main external biosecurity practices. A protocol of animal testing for establishing herd status for main production-related infectious diseases was included. The manager of the farm or veterinarian working at the farm on a daily basis was interviewed and a farm questionnaire was completed by the interviewers.

On-farm measurements were performed to obtain more detailed information on housing factors. Farm measurements included the length of the feed front in the close-up period, colostrum period, and lactating cow groups; size of the pen/cubicle per animal during the colostrum period; cubicle dimensions (length, width, diagonal) for lactating cows; distance between the feed front and nearest rows of cubicles for lactating cows; number of cows per cubicle in the lactating cow group; length of the drinking front per cow in the lactating cow group; and farm-floor slipperiness in the group of cows 2–3 months after calving and in the lactating cow groups (Supplementary Table 1 and 2).

In addition, to assess the impact of the farm environment and management on animals, the respective animal scoring systems were used. Animal-based measurements (ABMs) included body condition scoring in the close-up group and 2–3 months after calving, animal cleanliness scoring in the close-up group, among calving animals, and 2–3 months after calving, rumen fill scoring 2–3 months after calving, presence of visible injuries in lactating cows, and visible damage by mites/lice and ringworm among lactating cows (Supplementary Table 1 and 2). ABMs were recorded with the intention to assess 25% of the animals in the respective group with a minimum of 10 records. In case of more than one lactating cow group on the farm, the one with the highest number of animals was chosen for animal assessments. Two veterinarians involved in data collection were pre-trained in three joint farm visits to harmonize the questioning and performing farm measurements and ABMs.

Information on the herd status of the selected endemic infectious diseases was also included. Authorisation for the animal testing project was obtained from the Estonian Ministry of Rural Affairs (Decision No. 147, date 03.07.2019). Ten blood samples from each herd were collected from heifers aged 8–16 months. Also, bulk tank milk (BTM) samples were collected from all milk tanks present in the farms. Specific details regarding sample size and sample collection procedures can be found in the report by Mõtus et al. (2021).

Bulk tank milk and heifer serum samples were tested for bovine herpesvirus 1 (BHV-1), bovine viral diarrhoea virus (BVDV), bovine respiratory syncytial virus (BRSV), *Mycoplasma bovis (M. bovis), Mycobacterium avium* spp. *paratuberculosis* (MAP), and *Salmonella* Dublin (*S.* Dublin) antibodies using the commercial enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Samples from herds that had vaccinated their cattle against BRSV or BVDV were not tested for antibodies. Serum and BTM samples were tested for BHV-1 glycoprotein E (gE) in herds that were vaccinated with bovine herpesvirus 1 gE-deleted marker vaccines. In BHV-1 non-vaccinated herds, BHV-1 glycoprotein B (gB) ELISA was used. All ELISA results were dichotomised as negative or positive based on the test cut-off values, and a herd was considered positive for certain pathogens when at least one serum or BTM sample showed a positive test result. More details about ELISA kits used, laboratory analysis, and result interpretation are provided in the report by Mõtus et al. (2021).

### 2.3. Data editing

The questionnaire data were digitalised using the electronic survey tool LimeSurvey (LimeSurvey GmbH) and farm data were exported in the Excel format. Data on farm variables were interpreted and answer categories were composed by aggregating identical answers. Rarely occurring answers were merged into one category (called 'Other') to avoid having variable categories with a low number of observations. The proportion of cows with visible lesions and empty rumen filling were calculated; cows with cleanliness scores of 3 and 4 were grouped together, and the proportion of those out of all assessed cows was used to calculate the proportion of dirty cows in the respective area. The average score was calculated for the body condition of the cows in each group.

Out of all 172 explanatory variables 136 were obtained by using farm employee questioning, 24 were ABMs including 6 variables established based on animal testing for infectious disease antibodies, and 12 variables were based on on-farm measurements. According to the discussion with herd health experts, explanatory variables were aggregated into 14 thematic ($X_1$, …, $X_{14}$) blocks, with several variables per block (11, 20, 4, 14, 13, 9, 8, 13, 27, 10, 7, 7, 21, and 8, respectively) collected from 120 dairy herds (Table 1, Supplementary Table 1 and 2). Continuous data were categorised when not normally distributed at roughly the median and quartile values to ensure balanced groups or at accepted threshold values when available. Out of 172 explanatory variables, 168 were categorical variables and four were continuous variables (Supplementary Table 1 and 2). The dataset including the recorded herd data was merged with one including herd MR, CR, and MAofCC data.

### 2.4. Statistical analysis

The composite outcome block Y, representing cow persistence, included three variables: CR, MR, and MAofCC. All three outcome variables were square-root transformed to ensure normal distribution in linear regression analysis.

First, variables were screened for univariate associations with the three outcome variables using linear regression analysis. Because of the substantial confounding effect of herd size, it was controlled for in the screening phase by including it as a categorical variable in the model. A liberal threshold p-value of 0.2 was chosen to decide whether the variable would be included in the further multiblock analysis. A variance inflation factor (VIF) was used to assess the strength of collinearity between the selected explanatory variables. A VIF threshold value of 10 was used to indicate substantial collinearity (Dohoo et al., 2009) in which the collinear variable with lowest significance was removed from further analysis.

The mbPLS regression (Wold, 1966; Wangen and Kowalski, 1989) was applied to achieve three objectives: (1) describe the relative contribution of the explanatory blocks to cow losses (Y block), (2) identify, within each block, the variables mostly contributing to cow persistence (Y block) and (3) to each response variable in the Y block (MR, CR and MAofCC). This method was designed as an extension of the standard PLS regression (Wold, 1966) to (K+1) datasets. It is a component-based method in which each of the (K+1) datasets, i.e., Y and ($X_1$,..., $X_K$), is summed with a component, a linear combination of the associated variables. Using components instead of datasets allows the handling of more explanatory variables compared to standard methods and could be applied in case of within-block multicollinearity. The mbPLS method derives a global component t related to all explanatory variables which is as close as possible to component u, which is a linear combination of the outcome variables. In addition, the global latent variable t sums up the partial variables ($t_1$,..., $t_K$) associated with the block datasets ($X_1$..., $X_K$). Equal weights were attributed to every variable in each X and Y blocks and all $X_K$ blocks in the model. All these constraints are summed in a single criterion to be maximised based on the squared covariance between the explanatory and outcome components (Fig. 1). Variables with a small contribution to explaining the variability in block Y (p-values of the regression coefficients >5% for the three dependent variables) were removed from the final model. For more details and interpretation tools, interested readers are referred to the study by Bougeard et al. (2011).

The data included numerical and categorical variables. Categorical variables were transformed into disjunctive variables to analyse the entire dataset. The data were then pre-processed. Due to being measured in different units, variables were centred and scaled so that they all had the same zero mean and unit variance. To assign equal weight to each block of variables in the analysis (regardless of the number of variables they held), they were weighted by their inertia (Westerhuis and Coenegracht, 1997).

Two interpretation tools, the 'variable importance index (VarImp)' and 'block importance index (BlockImp)', respectively describe the relative contribution of each explanatory variable and explanatory block to the explanation of cow persistence (Y block) (Bougeard et al., 2011). If K is the number of explanatory blocks and P is the total number of

**Table 1**
Overview of the created variable blocks, number and types of variables included.

| Block number | Name of the block | N of variables | Questionnaire data | Animal-based measurements | On-farm measurements |
|---|---|---|---|---|---|
| 1 | Herd characteristics | 11 | 11 | 0 | 0 |
| 2 | Management of lactating cows | 20 | 10 | 4 | 6 |
| 3 | Pre-calving period management | 4 | 4 | 0 | 0 |
| 4 | Close-up period management | 14 | 8 | 4 | 2 |
| 5 | Calving management | 13 | 10 | 3 | 0 |
| 6 | Colostrum period management | 9 | 6 | 0 | 3 |
| 7 | Milking cow traffic and grouping | 8 | 8 | 0 | 0 |
| 8 | Milking | 13 | 13 | 0 | 0 |
| 9 | Disease management | 27 | 27 | 0 | 0 |
| 10 | Fertility management | 10 | 10 | 0 | 0 |
| 11 | Feeding management | 7 | 7 | 0 | 0 |
| 12 | Post-calving period management | 7 | 1 | 5 | 1 |
| 13 | Biosecurity measures | 21 | 21 | 0 | 0 |
| 14 | Infectious disease status | 8 | 0 | 8 | 0 |

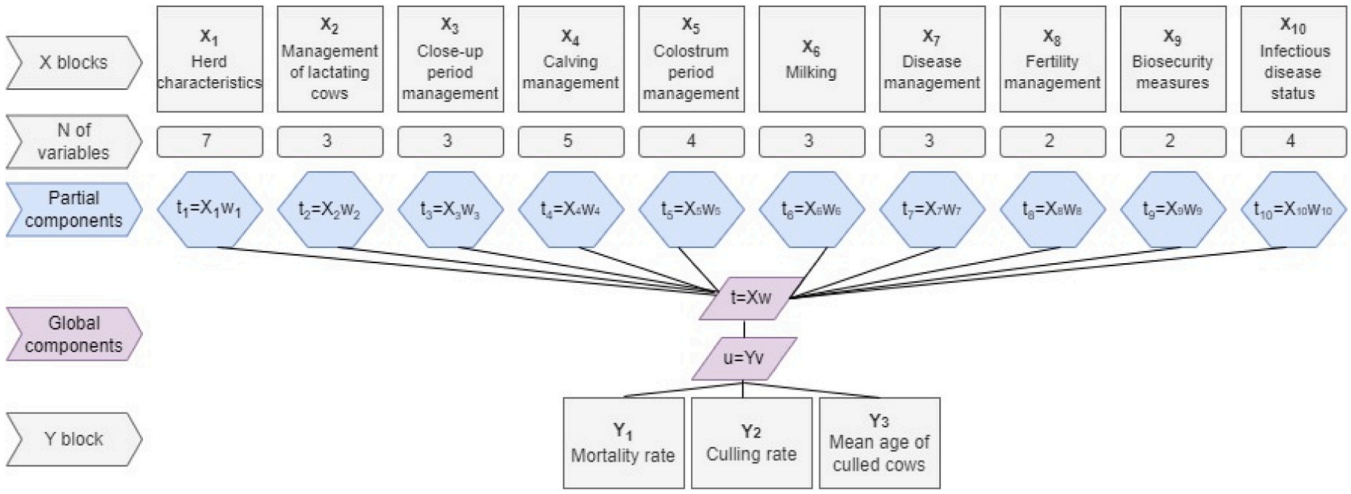


**Fig. 1.** Conceptual scheme for the components on the final multiblock PLS model. $X_1 \ldots X_K$ refer to the meaningful explanatory variable blocks, $t_1 \ldots t_K$ refer to partial components where w is the weight of the X block, u represents the linear combination of the Y variables with weights v.

explanatory variables in the model, VarImp and BlockImp verify the following properties: ΣpVarImp% = 100% and ΣkBlockImp% = 100%. The $X_K$ blocks verifying BlockImp> 100/k and the explanatory variables verifying VarImp> 100/p (i.e. contribution higher than the expected mean contribution) were considered to have strong significant association with cow persistence (Y block). Using bootstrap simulation with 500 repetitions, 95% confidence intervals (CI) were calculated and blocks/variables/variable categories whose estimates 95% CI did not include zero value were considered statistically significant. The mbPLS results were interpreted using a model with two components explaining certain amount of inertia which can be viewed as all the information contained in the data.

The results were obtained from the mbpls function of the ade4 R package (Bougeard and Dray, 2018) and then applied to centred and scaled data (scale = TRUE) and weighted blocks (option = 'uniform'). Figures were created using ggplot2 R package (Wickham, 2016) and RStudio (Posit team, 2023, version 2023.3.0.386). Software diagrams. net (JGraph Ltd., Northampton, England, 2022) was used to create illustrative figures.

## 3. Results

### 3.1. Study herds and cow persistency variables

A total of 120 dairy herds, with an annual average number of cows varying from 92 to 2275 (mean = 518) during herd visits, were included in the study. The average cow 305-day milk yield was 10,319 kg (range 5983–13,155 kg). In total, 34 farms had a robotic milking system, and 57 had milking parlours, including seven carousel milking parlours; combined milking systems were present in eight herds and robotic carousel on one farm. The majority of cow stables were semi-insulated barns (59.2%) and most were three-row barns (66.7%).

The average cow on-farm MR was 5.5% (median 5.1, range 0.3–13.3%). The mean annual cow CR of the herds was 33.0% (median 33.1, range 15.5–61.3%) and the herd MAofCC was on average 60.5 months (median 59.6, range 47.0–88.5 months) (Fig. 2).

The correlation between herd cow MR and CR was 0.47 ($p < 0.001$), between herd MR and MAofCC was –0.29 ($p = 0.001$), and between herd cow CR and MAofCC was –0.41 ($p < 0.001$).

### 3.2. Impact of variable blocks in explaining cow persistency

Overview of the farm variables including the descriptive statistics and their statistical association with three outcome variables (MR, CR and MAofCC) are presented in Supplementary Table 1 and 2. After the first univariate selection, 36 explanatory variables organised into 10 thematic blocks remained in the analysis (Fig. 1, Supplementary Table 1 and 2).

The mbPLS model explained 84.4% of the total variability of herd cow persistence (Y block). Fig. 3 provides an overview of the importance of the variable blocks remaining in the analysis after the variable selection phase. Thematic block that had the strongest significant impact on the outcome variables (block importance index and 95% CI > 10) was 'infectious diseases' (BlockImp index, 13.6%, 95% CI 10.3; 20.5). Blocks

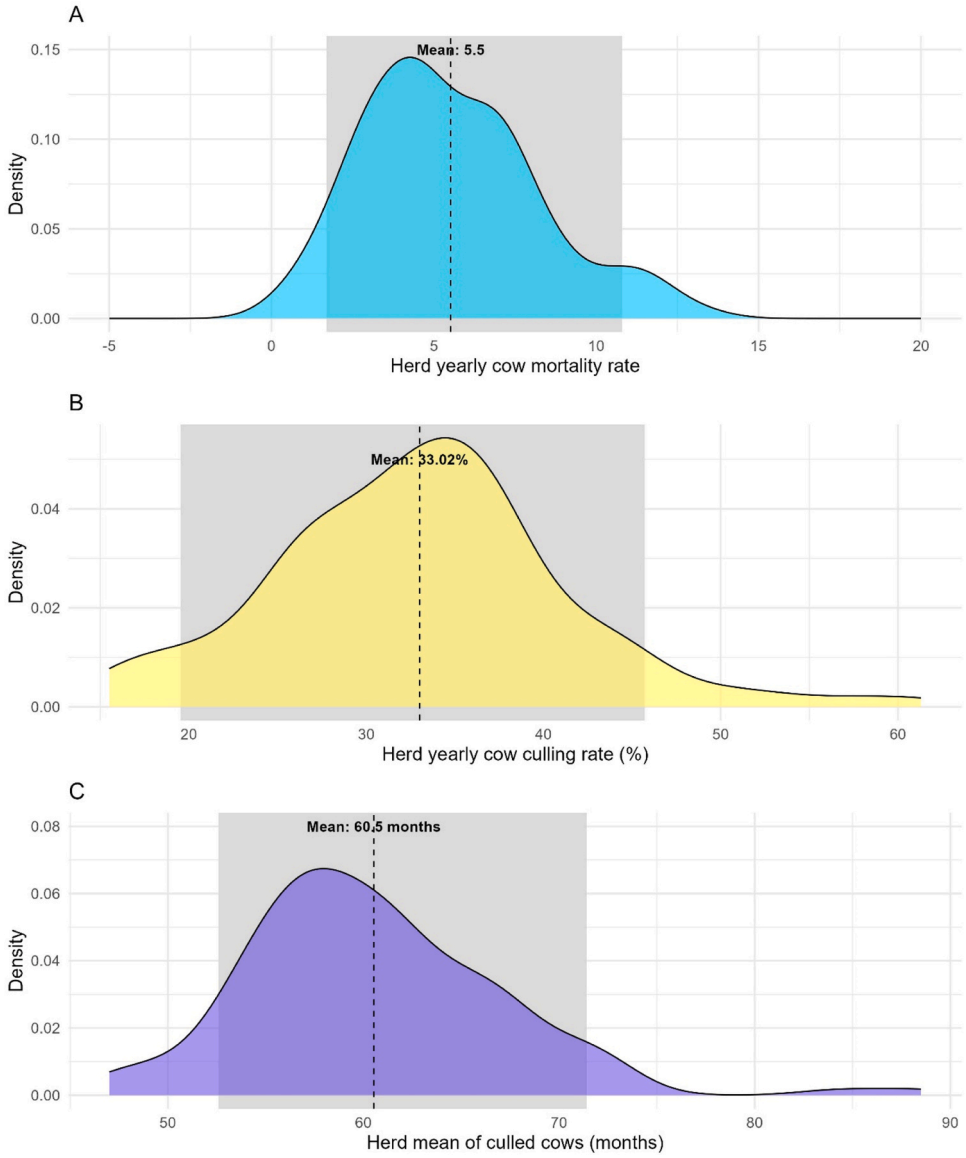


**Fig. 2.** Distribution of cow mortality rate (A), culling rate (B), and longevity (C) of 120 large commercial Estonian dairy herds.

that had a significant impact on Y were: 'fertility management' (16.3%, 95% CI 6.8; 26.9), 'lactating cow management' (11.5%, 95% CI 6.4; 17.8), 'milking' (11.3%, 95% CI 3.2; 17.1), 'herd characteristics' (10.1%, 95% CI 6.3; 14.2), 'close-up period management' (9.7%, 95% CI 2.7; 15.7), 'calving management' (7.9%, 95% CI 3.1; 11.4) and 'disease management' (7.3%, 95% CI 0.2; 12.0) (Fig. 3).

Variables and variable categories with the highest importance in explaining composite outcome including herd MR, CR and MAofCC were Northwest (vs Northeast) region, proportion of lactating cows with rear end/udder lesions, twice daily (vs robot) milking, BRSV positive BTM and youngstock (vs negative) and vaccinating against BHV-1 (vs negative) (Figs. 4–5, Supplementary Table 3).

### 3.3. Risk factors for each cow persistency variable

Variables significantly associated with any of the three outcome variables (MR, CR and MAofCC) are presented in Fig. 4. Detailed overview about the associations between individual explanatory and cow persistency variables is provided in Figs. 6–8 and Supplementary Table 3.

Farms located in the northeastern region had a significantly higher cow MR and CR than those in the northwestern region. In general, the smallest herds (<250 cows) had the highest MAofCC and lowest CR compared to medium-sized herds (460–629 cows). Predominantly, Holstein-breed herds had higher MR and CR than herds comprising of Estonian Red breed cattle. Farms on which cows were reared in uninsulated barns had higher cow MR than those in which cows were reared in semi-insulated barns. In general, grazing cows was associated with lower herd CR and higher MAofCC. Higher milk yield entailed a higher CR and lower MAofCC.

Herds in which ≥ 20% of lactating cows had rear-end or udder lesions had significantly higher on-farm MR and CR and lower MAofCC. Housing cows and heifers in the same close-up group was protective against high MR. Higher cow body condition scores during the close-up period increased the herd cow MR. Higher proportion of cattle (≥30%) with dirty upper hind limbs and flanks resulted in lower MAofCC and higher MR and CR.

Calving in group pens and using deep litter bedding resulted in the lowest herd CR. Calving pens with concrete flooring were associated with increased cow mortality. The use of sawdust bedding in calving pens was a risk factor for high culling probabilities and lower MAofCC. Placing a cow or heifer in the calving pen only for foetus ejection was

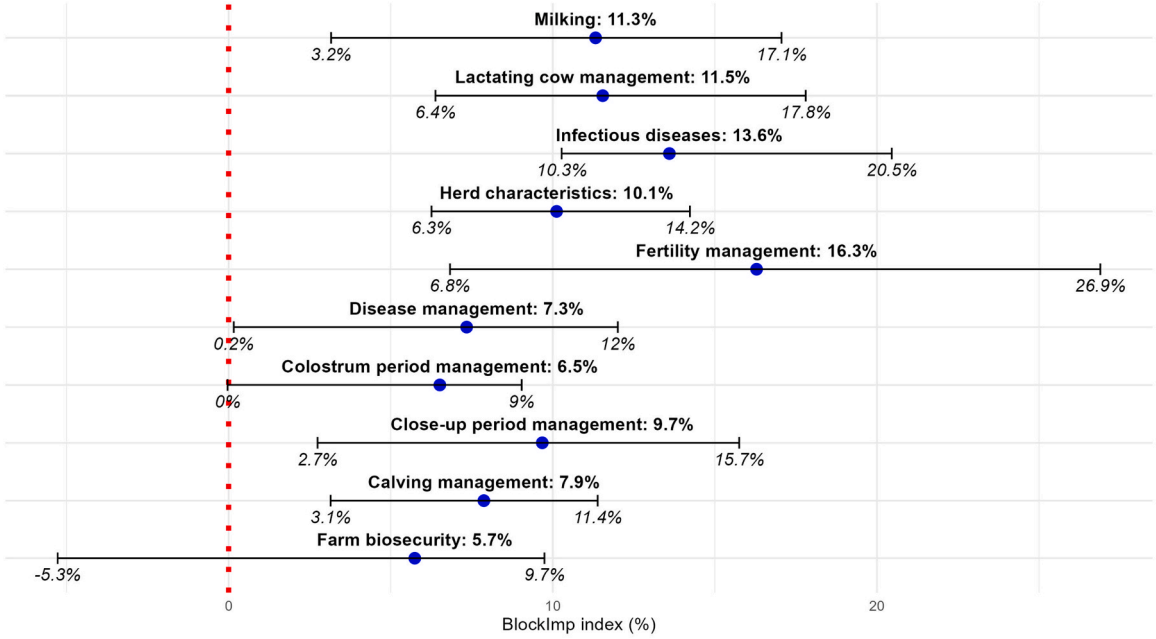


**Fig. 3.** Block importance index in explaining dairy cow persistence expressed as herd annual on-farm mortality rate, culling rate, and mean age at culling.

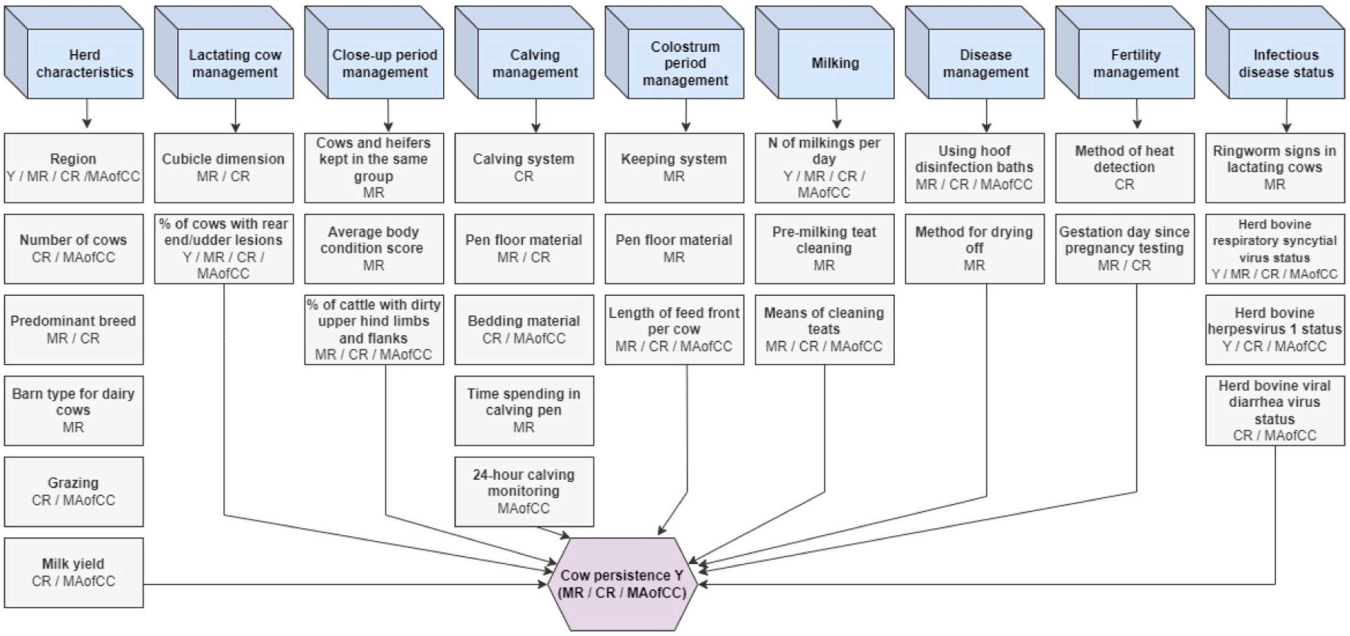


**Fig. 4.** Variable blocks significantly associated with herd cow persistence (Y) including cow on-farm mortality rate (MR), culling rate (CR), and mean age at culling (MAofCC), and variables' associations with Y, MR, CR, MAofCC in multiblock PLS model.

associated with lower MR.

Housing cows in deep litter bedding group pen during colostrum period was associated with substantially higher herd MR. Also, concrete pen floor material was associated with increased MR during the colostrum period. Farms milking cows twice a day had generally higher MAofCC compared to farms using robot milking system. Milking cows three times a day resulted in lower MAofCC and higher CR than in farms who milk their cows with a robotic milking system. Wet cleaning of teats before milking was associated with a lower herd MR than dry cleaning or no cleaning. Herds that used water rinsing and air teat-cleaning methods before milking had significantly lower MR and CR and increased MAofCC.

Using hoof disinfection baths for cows was associated with increased cow MR and CR, and reduced MAofCC. Farms that used only visual heat observation had reduced CR compared to farms using other or combined methods. Starting pregnancy testing since 60 days after insemination was associated with lower cow MR and CR. The herd status of BRSV-seropositive cows and heifers was associated with significantly higher cow MR and CR and smaller MAofCC. Herds that vaccinated against BHV-1 had significantly higher CR and reduced MAofCC than BHV-1 negative herds. In addition, the herd's positive BVDV status concurred with higher culling probabilities and lower MAofCC compared to the herd's negative BVDV status (Figs. 6–8, Supplementary Table 3).

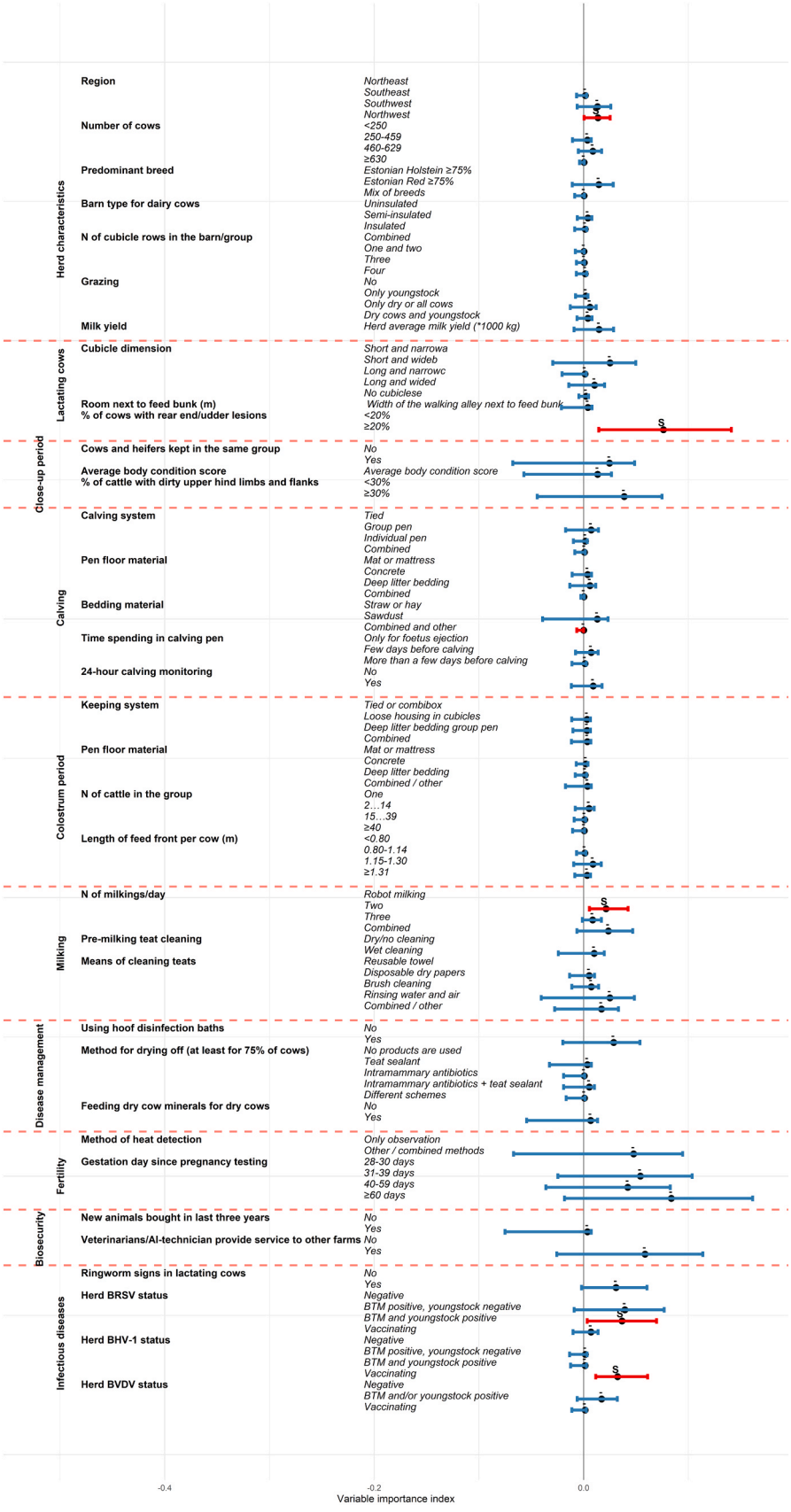


**Fig. 5.** Variable importance index of the selected variables in explaining the composite outcome Y representing cow persistence based on three underlying variables (cow mortality rate, culling rate and mean age of culled cows) according to multiblock partial least squares regression analysis. Red colour 95% CI indicates statistically significant difference compared to the first variable category. S+ refers to statistically significant importance of the variable category in explaining cow persistence.

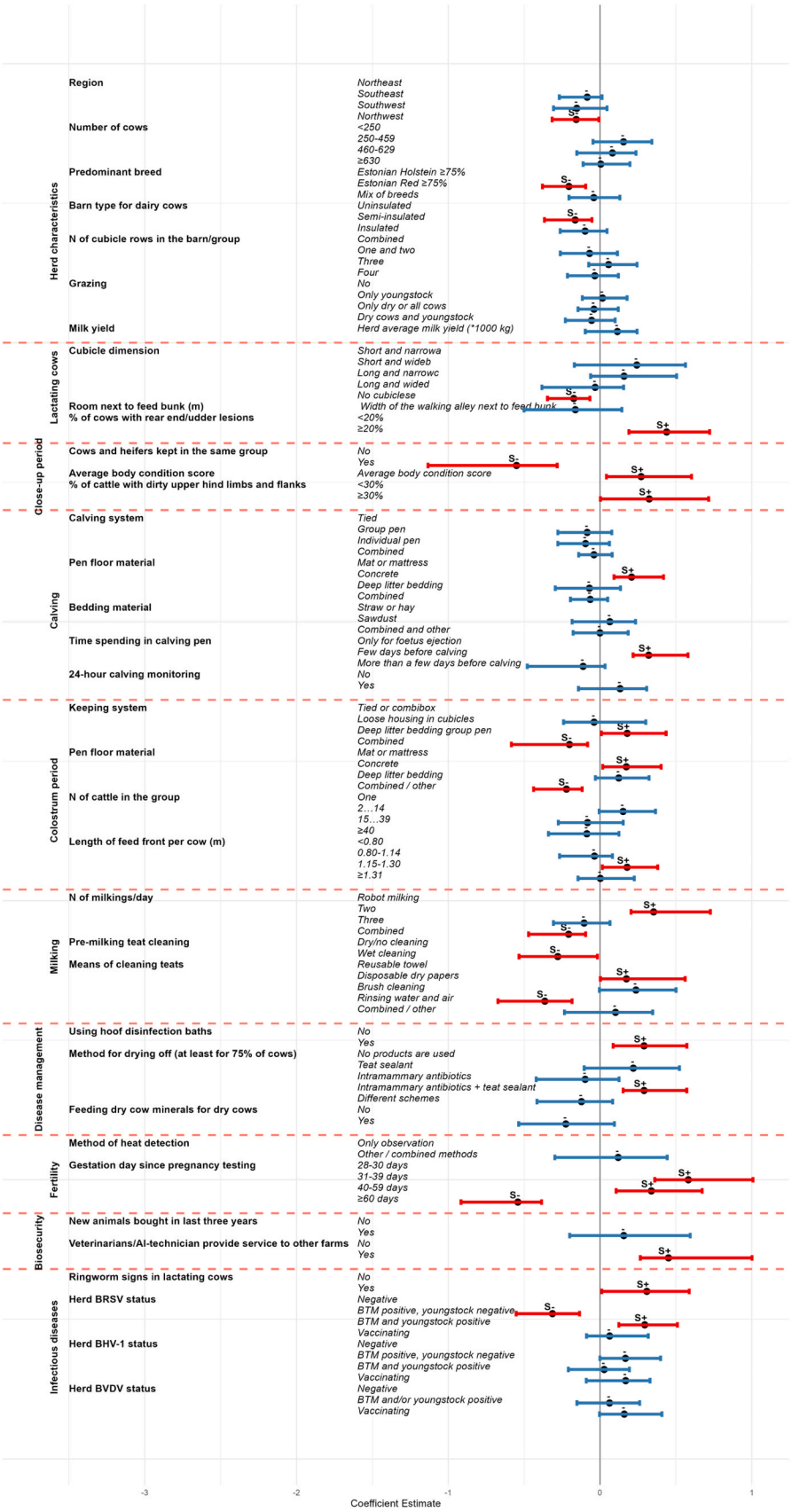


**Fig. 6.** Association between the selected variables with herd annual cow on-farm mortality rate according to multiblock partial least squares regression analysis. Regression coefficient estimates are presented as dots and 95% confidence intervals (CI) are given as horizontal lines. Red colour 95% CI indicates statistically significant difference compared to the first variable category. S+ refers to statistically significant increase and S- to decreased effect to herd annual cow on-farm mortality rate.

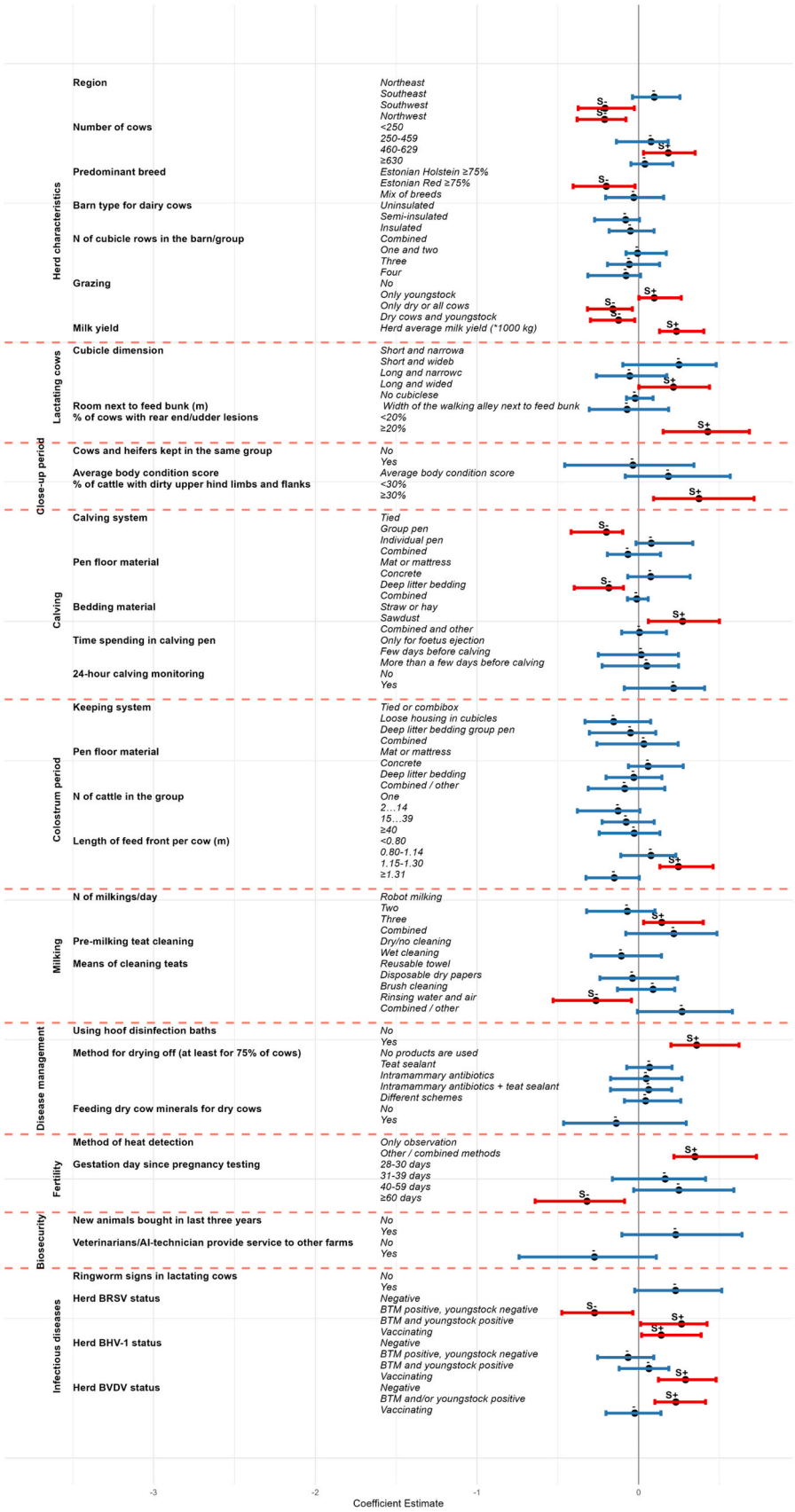


**Fig. 7.** Association between the selected variables with herd annual cow culling rate according to multiblock partial least squares regression analysis. Regression coefficient estimates are presented as dots and 95% confidence intervals (CI) are given as horizontal lines. Red colour 95% CI indicates statistically significant difference compared to the first variable category. S+ refers to statistically significant increase and S- to decreased effect to herd annual cow culling rate.

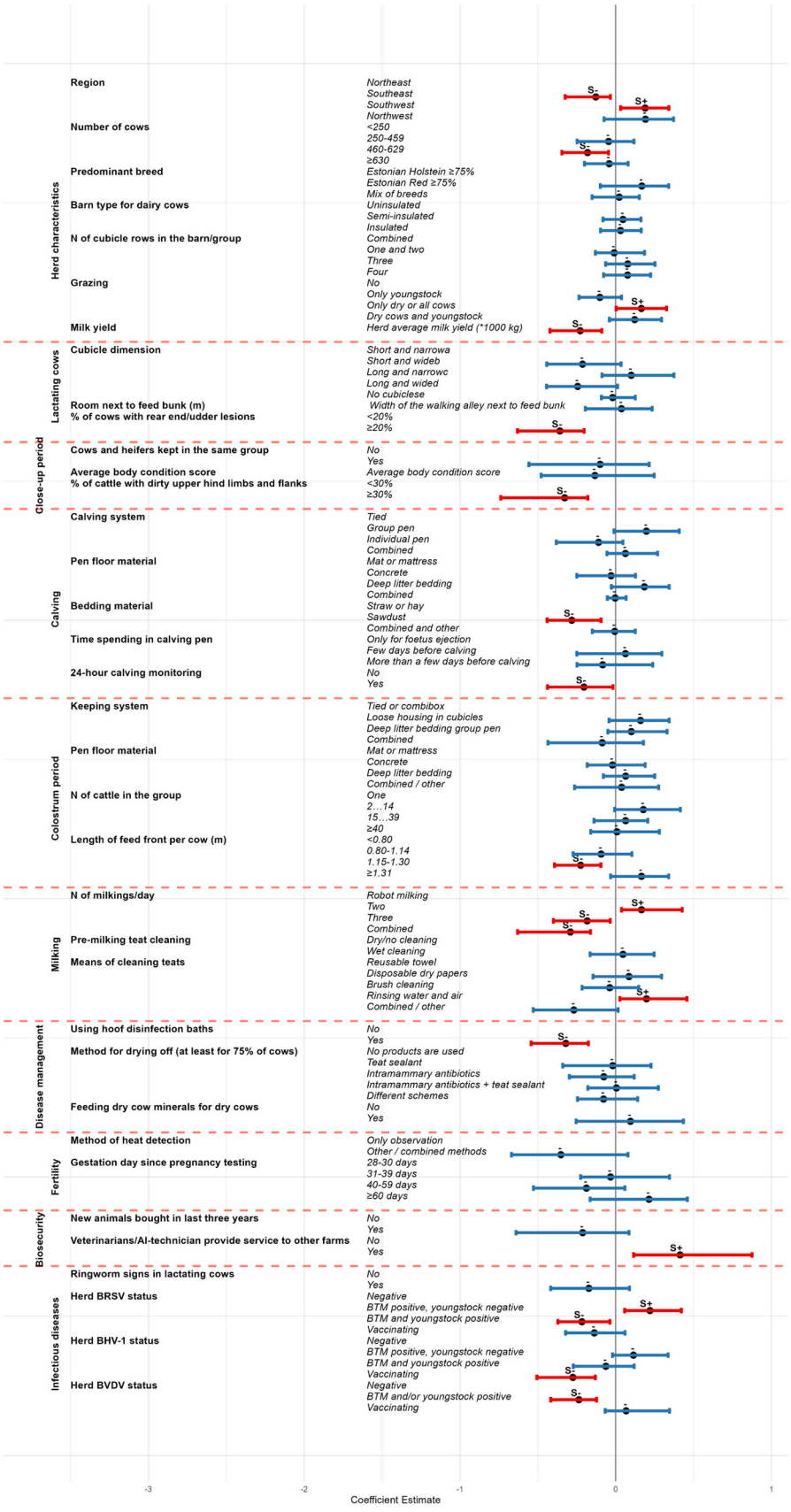


**Fig. 8.** Association between the selected variables with herd annual mean age of culled cows according to multiblock partial least squares regression analysis. Regression coefficient estimates are presented as dots and 95% confidence intervals (CI) are given as horizontal lines. Red colour 95% CI indicates statistically significant difference compared to the first variable category. S+ refers to statistically significant increase and S- to decreased effect to herd annual mean age of culled cows.

## 4. Discussion

The present study aimed to provide a comprehensive map of factors and factor groups belonging to a widened list of areas revealing the limiting factors of large commercial dairy herds affecting dairy cow persistence. Previous studies aimed to identify the effect of different herd factors, e.g. herd characteristics, management routines, housing conditions and herd performance indicators on different cow persistency outcomes (Weigel et al., 2003; Burow et al., 2011; Alvåsen et al., 2012, 2018; Shahid et al., 2015; Sarjokari et al., 2018; Bugueiro et al., 2019; Reimus et al., 2020; Han et al., 2022). All the cited studies used somewhat different data (e.g. limiting to the use of available herd performance indicators, or applying questionnaires or on-farm measurements capturing factors hypothesised to be related to cow persistency), statistical approaches (e.g. survival analysis or negative binomial models using cow or lactation level data, or linear regression models for herd level persistency indicators) and outcomes (e.g. age at culling, on-farm mortality or culling risk or lifetime milk production). Also, these studies were applied in herds with different sizes, production and milking systems but also had temporal differences. Nevertheless, all previous studies identified several risk factors but improving single factors might not be sufficient to achieve improved outcomes. Disclosing influential factor groups provides important but neglected pieces of information that would help relevant stakeholders prioritise areas that have the most substantial impact on cow resilience. To achieve this goal, a relatively novel statistical method in veterinary epidemiology was employed, which has the advantage of including and analysing an extended list of factors from several factor groups without losing information on their meaningful relationships (Bougeard et al., 2012). This method allowed the use of composite outcomes that provided a more comprehensive description of dairy cow persistence compared to using single measures (e.g. mortality or culling rate) analysed individually using conventional statistical approaches. Therefore, the method provides a wider scope of information about the multifactorial problem that is characterised by a complex outcome (Bougeard et al., 2012).

The MR, CR, and MAofCC values determined for the herds were roughly comparable to those reported in many countries with high-yielding intensive rearing systems (Hadley et al., 2006; Haine et al., 2017a; Alvåsen et al., 2018; Sarjokari et al., 2018; Dallago et al., 2021). Large between-herd variations in these metrics were observed in the present study, as well as studies in other countries (Dechow and Goodling, 2008; Strandberg and Emanuelson, 2016; De Vries, 2017; Haine et al., 2017b; Sarjokari et al., 2018) likely due to differences in the underlying risk factors for diseases or disorders associated with culling and longevity.

The lifespan of a dairy cow is mostly determined by either the farmer's decision to remove the cow from the herd or its death (Fetrow et al., 2006). According to a recent Estonian study (Mõtus and Niine, 2022), the majority (98%) of cows sent for slaughter were culled because of diseases, disorders, or infertility. Although the discrimination between voluntary and involuntary culling is aggravated by the absence of clear and exclusive criteria, on-farm mortality is solely attributable to involuntary culling (Fetrow et al., 2006). This indicates that, at least in large Estonian dairy herds, cow longevity is mostly determined by involuntary culling, and the factors identified in the present study provide an overview of measures aiding to avoid or reduce the undesired and premature removal of cows in large commercial dairy herds.

### 4.1. Impact of thematic blocks and variables in explaining cow persistency

Regarding the contribution of the variable blocks in explaining overall cow persistence, the most pronounced impact could be attributed to herd infectious disease status. In addition to variable clinical manifestations possibly affecting cow survival, endemic circulation of the pathogens in the herd might encumber the immunity of the cows thus making cows more prone to suffer from several disorders. Therefore, control and eradication of the main production-related infectious diseases might entail more resilient herd. The next most important blocks of variables were fertility management, lactating cow management, cow milking practices, herd characteristics, close-up period and calving management. Improvements in these areas could result in most favourable trends in cow persistence. All these blocks include the influence of several factors. The risk factors forming these important blocks should be considered as ones needing particular attention and interventions to improve cow persistence.

Among all tested variables, rear-end or udder lesions present in a higher proportion of cows had the highest contribution to inferior cow persistency. This indicates that cubicle dimensions, bed surface, and bedding type possibly associated with leg injuries (Weary and Taszkun, 2000; Wechsler et al., 2000) have far-reaching implications for cow health and welfare.

In the present study, the active spread of BRSV in a herd resulted in decreased cow persistence. As previously identified, the incursion and spread of BRSV in a herd can cause severe respiratory diseases in adult cows, sometimes resulting in mortality (Elvander, 1996; Giammarioli et al., 2020). In total, 31 herds vaccinated their cattle with BHV-1 gE-deletion marker vaccines to eradicate the disease. Most of these vaccinated herds had seronegative young stock and BTM samples (Mõtus et al., 2021) meaning that these farms were in the final stages of the eradication program. Therefore, the reduced herd level persistence may to some extent be the result of more active elimination of BHV-1 carrier cows.

Robotic milking system farms had overall improved cow persistence. This finding is in agreement with previous studies (Alvåsen et al., 2012; Bugueiro et al., 2019) suggesting that more flexible and individual milking times, avoidance of traffic, reduced forced standing time, and precision feeding that accompanies robotic milking systems (Bach and Cabrera, 2017) may respond better to the needs of high-performing dairy cows.

Herds located in the northeastern region had most unfavourable cow persistence being in line with previous Estonian studies (Reimus et al., 2017; Rilanto et al., 2020). Most possibly, this could be explained by differences in regional herd specifics that should be examined in more detail in future studies.

### 4.2. Impact of herd factors on cow persistency variables

Association of possible risk factors with three separate persistency outcomes differed in some instances. Variables of the blocks 'close-up period management' and 'colostrum period management' had higher impact in explaining herd MR than CR or MAofCC. Possibly, this can be explained by highly right-skewed distribution of cow mortality risk compared to somewhat more diffusely distributed cow culling hazards throughout the lactation (Reimus et al., 2018; Rilanto et al., 2020). Some variables reflecting rather herd management strategies, e.g. 'method of heat detection' and 'herd vaccination status against BHV-1' were related to herd CR and variables that could be directly related to disease risk, e.g. 'body condition score of close-up period cows' and 'pre-milking teat cleaning' were related to herd MR.

The active spread of BVDV in a herd, indicated by antibody-positive heifers and BTM samples, was associated with increased culling rates and reduced longevity, likely because of the adverse health and fertility outcomes caused by the virus (Lanyon et al., 2014). Visible ringworm skin lesions in lactating cows identified in 15 herds included in this study were associated with increased on-farm mortality. As susceptibility to ringworm is largely determined by the immunological status of the animal and the presence of disease-related risk factors, such as stressful climatic conditions, poor nutrition, concurrent disease, and overcrowding (Radostits et al., 2000; Tartor et al., 2020), identifying ringworm lesions in older cattle may reflect the higher stress level and presence of the ascertained risk factors predisposing cows to other diseases.

The method of oestrous detection and the time for pregnancy testing were found to be important in determining herd CR and MR. Farms that used only visual oestrus detection had lower cow CRs than farms that used other methods or combined visual oestrus detection with other methods. According to an earlier study, if performed twice daily for 30 min, visual oestrus detection is a good method for detecting cows in heat (Van Eerdenburg et al., 1996). At the same time, thorough and regular visual observation of cows during the first third of the lactation period, which overlaps with the highest risk period for mortality and culling (Reimus et al., 2017; Rilanto et al., 2020), could allow for more efficient detection of diseased cows. Therefore, the identified association may indicate the important role of human supervision of cows in early lactation period in terms of assuring cow persistence.

Testing pregnancy 60 days after breeding was associated with lower culling and mortality risk than with early testing (<30 days). We speculate that farms where cow pregnancy was diagnosed at later times may also have relatively fair breeding outcomes, contrary to farms where early identification of non-pregnant cows could be considered important due to overall poor reproductive performance. Therefore, these associations may reflect different policies in herds based on health status and reproductive success. Also, the variable is somewhat confounded with herd size as 31.3% of the herds that started pregnancy testing since 60 days from breeding belonged to the smallest herd size category with the best cow persistence.

Milking cows three times a day was associated with shorter cow longevity possibly due to increased demands on metabolism and foot health, and shorter resting and eating times. Not using wet pre-milking teat cleaning or using disposable papers for cleaning teats were risk factors for increased herd cow mortality. According to previous studies, wet teat cleaning resulted in the lowest bacterial counts in milk and teats, indicating its effectiveness against environmental bacteria, such as *Escherichia coli* and *Streptococcus uberis* (Gibson et al., 2008; Gleeson et al., 2009, 2018). However, the latter can cause severe clinical mastitis in cows, sometimes resulting in fatalities (Cebra et al., 1996; Verbeke et al., 2014). In terms of herd cow persistence, water rinsing and air teat cleaning, which are used in robotic milking systems, were the most favourable practices.

Among the blocks containing the overall herd characteristics, the smallest herds had the best cow longevity and smallest CR. A larger herd size is a proxy for many herd-related factors and is often associated with increased incidence of diseases (Stengärde et al., 2012; Chapinal et al., 2013; Persson Waller et al., 2020). The present study also revealed breed differences in cow persistence. Herds with Holstein cows had significantly higher CRs and MRs, which is consistent with the findings of other studies (Alvåsen et al., 2012; Shahid et al., 2015; Sarjokari et al., 2018; Rilanto et al., 2020). Holstein cows have substantially higher milk yields and lower resistance to several production-related diseases (Washburn et al., 2002; Nyman et al., 2007; Alvåsen et al., 2012; Estonian Livestock Performance Recording Ltd, 2021). As suggested by Sarjokari et al. (2018), Holsteins are generally larger and heavier than many other dairy breeds, and therefore suffer more easily from suboptimal free-stall environments. A higher milk yield entailed higher herd CRs and reduced longevity, which is consistent with the findings of previous studies (Smith et al., 2000; Weigel et al., 2003; Nor et al., 2014). As suggested by Weigel et al. (2003), high-yielding cows have increased demands for husbandry conditions, nutrition, and health care practices (Gröhn et al., 1995; Heuer et al., 1999; Koeck et al., 2014).

The on-farm cow MR was higher in herds with uninsulated barns than in those with semi-insulated barns, may be because of more stable climatic conditions in the latter system and differences in the milking system entailing distinct cow management practices. Our study found that grazing cows was associated with improved cow persistence, whereas sending young stock to pastures had the opposite effect. This result is confounded by herd size, as young stock grazing was more often practiced by larger herds, as opposed to cow grazing, which is more common in smaller herds. Still, possible beneficial effects of grazing include the ability to express natural behaviour, less competition for resources, lower animal density, more natural surfaces for walking and lying, and better hoof health (Somers et al., 2005).

During the close-up period, maintaining cows and heifers in the same group was associated with lower on-farm mortality. Sarjokari et al. (2018) also concluded that increased regrouping before parturition is stressful for cows, particularly for subordinate individuals. Mingling cattle of different hierarchical levels far before parturition may avoid the stress induced by mixing cattle during the sensitive post-partum period, and therefore entail favourable health effects. Pre-calving over-conditioning is an important risk factor for dystocia and postpartum metabolic diseases (Pires et al., 2013; Roche et al., 2015), explaining the higher on-farm MR among herds with cattle having higher average pre-calving body condition scores. Inferences about stall hygiene could be made on the basis of cow cleanliness (Robles et al., 2021), and a higher proportion of dirty cows in herds was associated with inferior herd persistency outcomes. Previous studies showed that cows housed on farms with dirtier stalls had decreased lying times, greater odds of being lame, and higher somatic cell scores (Reneau et al., 2005; Robles et al., 2021).

Our results indicate the beneficial effects of calving conditions resembling natural calving environments (i.e. calving in group pens and using deep litter bedding) in achieving improved cow persistency. In terms of mortality, moving cows to calving pens a few days before expected delivery, rather than moving them for only foetal ejection, resulted in a higher herd-level MR. Farms that employ this methodology are more likely to have less intensive calving surveillance, which could entail delayed intervention in cases of dystocia, a relevant cause of cow mortality (Alvåsen et al., 2014; Reimus et al., 2017). In terms of herd cow longevity, day-around calving supervision in place was identified as a risk factor. Still, this study did not reveal the real efforts and exact policies present in those farms.

Housing postpartum cows in deep litter bedding group pen was a risk factor for increased on-farm MR. We can assume that ensuring sufficient hygiene in these systems is challenging potentially predisposing cows to mastitis and uterine diseases (Cheong et al., 2011; Zigo et al., 2021). Flexibility in rearing cows in different keeping systems during the colostrum period resulted in a lower herd cow MR. A similar association has been found for calving environments (Sarjokari et al., 2018; Reimus et al., 2020). After calving, cows are most susceptible to post-partum diseases, and their individual needs could be met with the availability of different environments. The length of the feed front per cow somewhat correlated with the cow keeping system due to which the category associated with the reduced persistency outcomes (feed front of 1.15–1.30 m per cow) mostly included tie-stall keeping systems (72%), therefore confounding the identified association.

### 4.3. Limitations of the study

During the farm interviews, we collected information about the prevailing housing systems and management in the preceding year. This timeframe was specified because the data describing herd cow persistence (herd MR, CR, and MAofCC) were also obtained for the period of 12 months preceding the farm visit. In gathering risk factor data, we aimed to go beyond ordinary analysis and obtain more precise data on less-studied but possibly important parameters. Additional farm measurements (e.g. length of the feed front and dimensions of the cubicles) and cow scoring data (e.g. cow body condition, rumen filling, cleanliness, and presence of injuries) were included to capture more specific information about relevant risk factors. Overall, this resulted in a model with high explanatory capacity regarding cow persistence. As a limitation, these measurements represented the risk factor status at specific time points. However, as most of the related factors were static (e.g. cubicle dimensions, feed rails, and stall designs), their effect is presumed be constant over a long period, allowing us to make presumptions about their impact on cow culling, mortality, and longevity.

Owing to the study design (exposure and outcome factor data were collected for the same period), we were hesitant to make causal inferences about the identified associations. For example, as determined in the analyses, herds that used regular hoof baths or antibiotics at dry-off had higher cow losses and reduced longevity. Here, we were unsure whether the practice used was insufficient to entail improvements or just a compensatory practice more often used in herds with more pronounced animal health issues.

The biosecurity block consisted of variables that described the external biosecurity measures used to prevent the introduction of a pathogen to a farm. Although longitudinal herd-based data was missing, it is likely that the infected herds had an endemic disease status. Registered biosecurity measures did not have a substantial impact on cow persistence, contrary to the block that included herd infection status. Although it can be argued whether some important biosecurity measures remained unregistered, or whether registering biosecurity measures at a single time point reflected their long-lasting effect, the infectious disease block indirectly incorporated the effects of herd biosecurity practices (Ortiz-Pelaez and Pfeiffer, 2008).

To examine the spread of pathogens in the herd, testing for the presence of antibodies in young heifers was included to BTM antibody testing (Mõtus et al., 2021). Detecting antibodies in BTM may indicate a historical infection, whereas detecting antibodies in young replacement heifers may indicate a recent active spread of the pathogen (Lindberg and Alenius, 1999; Sayers et al., 2015). This would entail better compliance in terms of examining disease dynamics in the period during which the outcome variable data were collected.

As multiblock analyses are recent methods currently under further development, it holds some limitations. Currently, it is not possible to adjust for confounding or interaction in a mbPLS regression model (Collineau et al., 2018). Based on the causal diagram, herd size was considered as important confounder and was therefore controlled in the variable screening phase by eliminating variables with no independent effect to the outcomes. Due to the limited sample size and high number of categories in most of the variables, it would have been impossible to test for interactions.

To date, there are no simulation studies available on how sensitive mbPLS approach is for the defined blocks and the number of variables within blocks. Nevertheless, merging variables from different blocks and attributing them equal weights, mathematically reduces their importance. Therefore, the method is sensitive to block composition and variable weight allocation. Other component analysis methods, as well as PLS regression are methods devoted to single explanatory blocks. The only other (K+1) method available is multiblock redundancy analysis, but this method is sensitive to within-block multicollinearity. Regardless of the existing deficiencies, the mbPLS method could be applied for the more complex datasets opening the opportunities to analyse, visualize and interpret data in a new and more comprehensive way.

## 5. Conclusions

The present study applied multiblock PLS model to gain a better overview of the importance of specific farming areas, and identify individual factors that contribute to cow persistency described by three parameters, i.e. cow on-farm mortality, culling rates and mean age of culled cows in large commercial high-yielding dairy herds. According to the findings of this study, herd infectious disease status, cow fertility management, lactating cow management, milking routines, herd characteristics, close-up period and calving management were the most influential areas that contributed to dairy cow persistency. High proportion of lactating cows with rear end/udder lesions stood out as most influential factor counteracting cow persistency meaning that more emphasis should be placed on barn design and installation to ensure acceptable cow comfort and hygiene and to avoid injuries. Farms with robotic milking systems showed an overall better cow persistence, whereas milking cows three times a day counteracted in achieving fair longevity. Herd BHV-1 vaccination programs had influence on the overall cow persistence possibly due to more exaggerated replacement policies. Active spread of BRSV in a herd entailed inferior cow persistence motivating its control. Regardless of the few existing deficiencies of the mbPLS method, this innovative approach opens new perspectives in risk factor data analysis that could be important to make broader conclusions at multiple data dimension levels.

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## 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.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2023.106081.

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