Estimates of dairy herd health indicators of mastitis, ketosis, inter-calving interval, and fresh cow replacement in the Piedmont region, Italy

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Estimates of dairy herd health indicators of mastitis, ketosis, inter-calving interval, and fresh cow replacement in the Piedmont region, Italy

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Conflict of interest

Alessia Tondo is employed as a statistician at the Italian Breeders Association.

Abstract

Test-day milk analysis has largely been used to study health and performance parameters in dairy cows. In this study, we estimated four health indicators of dairy cows using test-day data. Our purpose was to estimate (1) mastitis incidence rate, prevalence, and the probability of recovery; (2) the incidence proportion of ketosis; (3) the duration of inter-calving interval; and (4) the risk of a fresh cow being replaced, in a large cohort of dairy herds in the Piedmont region (Italy).

We retrospectively analysed test day records of 261,121 lactating cows and 1315 herds during five years (2015-2020). Mastitis was defined by somatic cell count and ketosis by fat-to-protein ratio. Calving dates were used to calculate ICI and to estimate the removal of a fresh cow from the herd. Mixed-effect generalized linear models were used to adjust for unmeasured herd-level risk factors.

The risk of mastitis increased by 120 % with parity (Odds ratio [OR] = 2.20, confidence interval [CI]: 2.17 – 2.23), by 7 % by months in milking (OR = 1.07, CI: 1.07 – 1.07), and even more if the cow was already affected during the same lactation (OR = 8.74, CI: 8.67 – 8.82). Lactose concentration on the previous test day was the best positive prognostic factor for mastitis recovery (OR = 1.12, CI: 1.08 – 1.17). Ketosis risk was the highest between 3rd and 4th lactations and itself increased the risk of having ICI longer than 440 days (OR = 1.12, CI: 1.02 – 1.22), and fresh-cow removal (OR = 1.75, CI: 1.58 – 1.93). Also, the removal of fresh cows was more likely when mastitis (OR = 1.31, CI: 1.19 – 1.45) or long ICI (OR = 1.34, CI: 1.22 – 1.48) occurred. For each health indicator, herd-level risk factors had an important role (18 to 56 % of within-herd covariance).

Our results indicate that milk analysis could be also useful for predicting mastitis, its cure rate, and ketosis. Cow-level risk factors are not enough to explain the risk of these issues. By studying a large population over a long period, this study provides an updated estimate of dairy cow health indicators in Piedmont (north-western Italy), useful for benchmarking dairy herds.

Introduction

Breeders’ associations usually monitor several diseases affecting the health of dairy cows by the means of dairy herd improvement programs (DHI) and test day records (TD). These are a source of information on individual cows' health and performance for a large number of the herd. Therefore, they are valuable data for epidemiologic purposes (Busanello et al., 2017; Dufour and Dohoo, 2013; Reyher et al., 2011; Torres et al., 2008).

Mastitis

To date, mastitis is the most common cause of morbidity in adult dairy cows, causing economic losses due to therapy, reduction of milk production, milk discard, reproduction losses, and increased culling probability (Rollin et al., 2015). Subclinical mastitis has been monitored for decades using somatic cells count (SCC) of individual composite milk from TD data (Bradley et al., 2007; Hiitiö et al., 2017; Østerås and Sølverød, 2009; USDA, 2009) which is an inexpensive yet useful method to estimate its prevalence and incidence (Barnum and Meek, 1982; Dohoo and Wayne Martin, 1984). Many studies provided an estimation of clinical mastitis incidence and prevalence, although subclinical mastitis is considered to have a greater economic impact on dairy herds (Aghamohammadi et al., 2018; Busanello et al., 2017). Moreover, for the evaluation of udder health, it is essential to estimate the mastitis incidence rate along with prevalence proportion, and the probability of recovery (Busanello et al., 2017).

Ketosis

Hyperketonaemia is another major concern in intensive dairy herds (Carvalho et al., 2019). The serum concentration of ketone bodies increases as the result of the negative energy balance when high-yielding cows face increased milk production at the same time as decreased feed intake. It occurs in the first weeks after calving and can result in severe damage to the cow's health, metritis, displaced abomasum, and eventually culling (Duffield et al., 2009; McArt et al., 2015). Both clinical and subclinical hyperketonaemia have a negative effect on cow health, but the latter often remains unnoticed (Denis-Robichaud et al., 2014; Tatone et al., 2017). From TD records it is generally possible to monitor fat to protein ratio (F:P), which serves as an indicator of subclinical ketosis (Atalay, 2019; Jenkins et al., 2015). Epidemiological studies that use large data sets of cows to monitor the incidence of ketosis are essential for benchmarking herds and comparing the risk of the disease in different geographic regions (Santschi et al., 2016; Tatone et al., 2017; van der Drift et al., 2012).

Inter-calving interval

For a long time, the cows' reproductive performance has been evaluated using the inter-calving interval (ICI) which mainly depends on the voluntary waiting period (VWP) for breeding after parturition (Crowe et al., 2014; Remnant et al., 2018) and the fertility of the cow. Cows with adequate body conditions and efficient immune systems tend to become pregnant earlier (Berry et al., 2006; Remnant et al., 2018), while long ICI could be due to many reproductive disorders (e.g., ovarian cysts, endometritis, failure to return to heat etc.) and has been associated to higher mortality rates (Crowe et al., 2014; Reimus et al., 2018). Being strongly dependent on herd management limited the usefulness of ICI. However, ICI estimation from TD records could provide information about the reproductive health of dairy cows.

Fresh-cow removal

Several health issues could lead to the sale or the culling of a cow, depending on many factors, e.g., the severity and the time of their occurrence (Carvalho et al., 2019). Replacing cows is a major cost of the dairy operation, and it is widely recognized that the sale or the culling of a fresh cow -within sixty days after calving- results in an even higher economic loss (Heinrichs and Heinrichs, 2011; McArt et al., 2012; McArt and Neves, 2020; Vergara et al., 2014). From TD records, it is not always possible to determine the fate of individuals, especially if they are sold to farms that do not participate in the DHI program. However, the removal rate can be used as a proxy for the occurrence of health issues in the postpartum that cause the sale or the culling of a fresh cow (Overton and Dhuyvetter, 2020; Rollin et al., 2015).

TD records are a comprehensive monitoring tool for dairy herds since several cow-level risk factors (e.g., parity, days in milk, milk yield, breed, etc.) can be studied using DHI data, but many herd-level risk factors cannot, even if they contribute to a large extent to the cows' health. Still, these factors, which are mainly related to management and the breeder's expertise, need to be taken into account when health indicators are estimated.

To benchmark dairy herds in the Piedmont region, this study aimed to estimate four health indicators (HIs), namely (1) the incidence rate, prevalence, and probability of recovery of mastitis; (2) the incidence proportion of ketosis; (3) the duration of inter-calving interval; and (4) the rate of fresh cow removal. The estimation would take into account both the cow-level and herd-level risk factors, providing risk estimates for the first and evaluating the weight of the second ones.

Materials and methods

Results of this study were reported following the "Strengthening the Reporting of Observational Studies in Epidemiology" (STROBE) statement (https://www.strobe-statement.org/). This research was designed as a retrospective longitudinal study to estimate the aforementioned HIs (mastitis, ketosis, ICI, and fresh-cow removal) using TD records from a large cohort of dairy cows in Piedmont, northwestern Italy. The five-year study period (from 2015 to 2020) secures the estimates from spurious fluctuations.

Study population and sampling

The population under study included all cows and all the dairy herds that participated in DHI programs, in Piedmont, from the 2nd of January 2015 to the 28th of April 2020. Test day analyses were performed by the Italian Breeders' Association (AIA) on individual milk samples every 20-40 days (median = 31 days, mean = 37.16 ± 30.97 days). During the months of August and December of each year, a smaller amount of milk samples were analyzed because of the reduced number of workdays due to national holidays.

During milking, the milk yield of each cow was measured, and a sample of composite milk was collected. Milk samples were taken to the nearest AIA laboratory to analyse milk concentration of fat, protein, lactose, urea, somatic cells, and casein. Milk analyses were performed by Fourier-transformed infrared (FTIR) spectrometry using FOSS MilkoScan FT+ (various models depending on the laboratory; FOSS Analytical A/S, Hillerød, DK). All laboratory methods and techniques have been certified by the national accreditation body designated by the Italian government (Accredia, https://www.accredia.it/) in compliance with the standards required by the UNI CEI EN ISO/IEC 1702. AIA is certified by the International Committee for Animal Recording (ICAR).

The largest data set breed was Holstein Friesian (n = 3,760,324 obs.) which was used as a reference for all breed comparisons. More details on the number of different breeds can be found in Supplementary Table 1.

Data management

The initial database contained 4,634,197 observations of 275,351 lactating cows from 1,408 different herds. Cows with only one test-day record were removed; subsequently, we excluded herds with less than five lactating cows. Out-of-range values of milk composition and SCC (e.g., fat < 1.5 g/100 g or > 10.0 g/100 g; protein < 1.5 g/100 g or > 7.0 g/100 g; lactose < 2.0 g/100 g or > 6.0 g/ 100g; SCC < 1,000 or > 15,000,000 cells/mL) were replaced with missing values. Observations with missing values of any relevant variable (herd ID, cow ID, breed, parity, date of birth, date of calving, SCC, fat, protein, lactose) were excluded. The remaining 4,483,057 observations formed the working database with 261,121 lactating cows and 1,315 herds (Figure 1).

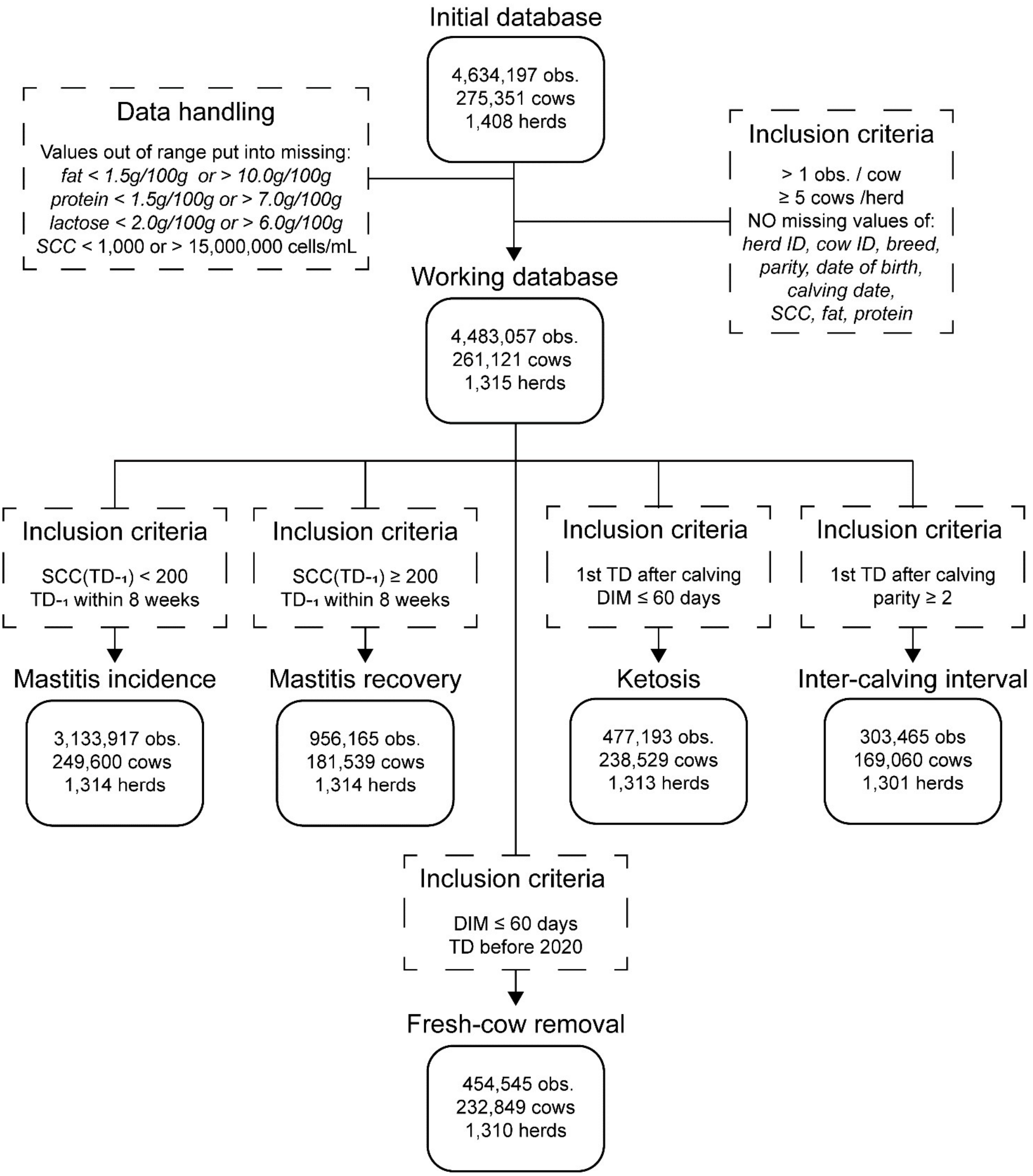

Figure 1. Flowchart of database clean-up and data handling before analysis. Abbreviations: DIM: days in milk; obs.: observations; SCC: somatic cells coun; TD: test-day; TD_{-1}*: previous test-day.*

All data used in this study were collected and managed by the AIA which owns the property and allowed their use for research purposes. All sensitive data were anonymized through the assignation of a dummy ID to each herd. Statistical analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). Graphs were made using the *ggplot2* package and base R language (R version 4.1.0, https://www.r-project.org/).

Herd health indicators

Mastitis. SCC was reported as 10^3 cells/mL; SCC ≥ 200,000 cells/mL was considered indicative of subclinical mastitis (Dufour and Dohoo, 2013). Mastitis prevalence was calculated as the number of cows with SCC ≥ 200 over the total number of cows at a given time.

A new case of mastitis was observed when SCC increased from SCC < 200 to SCC ≥ 200 over two consecutive TDs if they did not take place more than 8 weeks apart (56 days). Cow-days at risk were calculated assuming that both new cases and recoveries occurred halfway between two consecutive test-days, thus multiplying the days between TDs for 0.5. When the previous mastitis status was unknown, such as for the very first record of each cow or the observations that occurred more than 56 days after the previous one, data were excluded. If SCC was above 200 cells/mL for two consecutive test-days (i.e. for two consecutive months) it was considered chronic mastitis and therefore censored. After data clean-up, the dataset for mastitis incidence contained 3,133,917 observations of 249,600 cows from 1,314 herds. The incidence rate of mastitis was defined as the number of new cases divided by the sum of cow-days at risk. IR was reported as cases/cow-month, in reference to a standard month of 30.5 days (Dufour and Dohoo, 2013). When a new case occurred, the individual rate was calculated as 1 divided by the days elapsed from the previous negative TD.

Recovery was observed when SCC went from ≥ 200 to < 200 over two consecutive TDs within 56 days. The database for mastitis recovery was 956,165 observations of 181,539 cows from 1,314 herds. The recovery rate from mastitis was computed as the number of recoveries over the number of mastitis cases.

Ketosis. Ketosis was defined as F:P > 1.42 (Jenkins et al., 2015) at the first record within 60 days from calving. We did not differentiate between subclinical and clinical cases. The ketosis database included 477,193 observations of 238,529 cows from 1,313 herds. Incidence proportion was calculated as the number of new ketosis cases over the total of cow-lactations at risk.

Inter-calving interval. The inter-calving interval was calculated as the difference between the date of calving and the date of the previous calving, thus it can only be measured after the first lactation. Since the events that occurred in the previous lactation are crucial for breeding success and for the fate of the pregnancy, some variables (milk yield, ketosis, and mastitis), were referred to the previous lactation. For this analysis, only one record per cow per lactation was used, thus the database consisted of 303,465 observations of 169,060 cows from 1,301 herds.

Fresh-cow removal. Since we were unable to differentiate cows' fate based on the TD data, any disappearance of a cow from the database was considered a removal. When it occurred within 60 days from calving, it was considered a fresh-cow removal. To avoid bias due to right censoring at the end of the study, data from 2020 were excluded. Therefore, the database for the analysis of the fresh-cow removal consisted of 454,545 observations of 232,849 cows from 1,310 herds. The incidence proportion was calculated as the number of cows removed over the total number of cows within 60 DIM.

Data analysis

Unless otherwise specified, the analyses were performed on the working database. We accounted for the following cow-level risk factors: breed, parity, days elapsed from calving (days in milk, DIM), milkings per day, daily milk production (milk yield), and age at first calving. Milk yield was not analysed as a possible risk factor for mastitis. However, their association was evaluated by Pearson's correlation. Other outcomes were analysed as possible risk factors as well.

Continuous variables are reported as mean ± the standard deviation. The milk yield was estimated by AIA as L/day according to AM/PM methods mentioned in ICAR's guidelines (ICAR, 2020). Estimates, odds, prevalence proportion (PP), incidence rate (IR), incidence proportion (IP) and all measures of association (odds ratio, relative risk, and incidence rate ratio; OR, RR, and IRR, respectively) are reported along with their 99 % confidence intervals (CI).

Milk components. Univariate analysis (PROC UNIVARIATE) of milk components was performed to explore the consistency of data throughout the study period. The normality of data distribution was visually assessed. The correlation among milk components was computed by Pearson's coefficient (PROC CORR).

Bivariate analysis. Annual and seasonal trends were studied for each HI. The association with cow-level **risk factors were analysed separately for each HI by the means of bivariate analysis** (PROC FREQ, PROC MEANS)**. Regression models were used to estimate the unitary effect of risk factors.** Local and/or less-represented breeds were aggregated (Supplementary Table 1). Parity was recorded by aggregating cows with five or more lactations. **For data exploration and visualization, the following continuous variables were split into discrete categories.** DIM were aggregated into four phases of the lactation curve, assuming a Legendre-shaped ideal curve (Macciotta et al., 2005): (1) early lactation, DIM ≤ 60; (2) production peak: 60 < DIM ≤ 120; (3) late lactation: 120 < DIM ≤ 305; (4) over-lactation: DIM > 305. For ketosis, DIM were aggregated in weeks not only for data exploration and visualization but also in the regression model. Milk yield and lactose were divided into quintiles.

Generalized regression model. **Binary outcomes (mastitis, recovery, ketosis, fresh-cow removal) were studied by logistic regression (PROC GLIMMIX, DIST=BINARY, LINK=LOGIT). Continuous outcome (ICI) was studied by linear regression (PROC GLIMMIX, DIST=GAUSSIAN, LINK=IDENTITY). All biological relevant cow-level risk factors were included in the model and then evaluated by manual stepwise forward selection. The gain in terms of goodness of fit (GOF) was assessed by comparing the log-likelihood of new models with that of the saturated model. To adjust for the unmeasured herd-level risk factors, random effect mixed models (PROC GLIMMIX) were fitted, with the herds as random intercepts. Where appropriate to mimic the seasonal variation, the calendar period was introduced into the model with an empirically produced sinusoid function. The function was designed *ad hoc* to span from -1 to 1 following the trend of the certain outcome. The final model was a generalized linear model, family and link function depending on the outcome, with a random intercept for each herd, fitted to estimate the effect of the selected covariates, as follows:**

$$Y_{ij} = \beta_0 + \beta_1 X_{i,1} + \dots + \beta_k X_{i,k} + \beta_S S(i) + \varepsilon + u_j$$

where Y_{ij} is alternatively the outcome or its natural logarithm (depending on the outcome, binary or not), β_0 is the intercept, β_{1-k} are the *k* coefficients of each k^{th} covariate, $S(i)$ is the empirical function for calendar-period correction, ε is the random error, and u_j is the random intercept of each j^{th} herd with $u_j \sim N(0, \sigma^2)$.

Confidence intervals for proportions were calculated, using the formula proposed by Agresti and Coull (1998). Coefficients estimated by logistic regression were exponentiated into odds ratios, and their confidence intervals were calculated based on the observed frequency (Rothman et al., 2008). For incidence rates, the exact confidence interval was calculated from the χ^2 distribution (Garwood, 1936). We selected some of the estimates from the body of the text to be presented in the abstract. Since confidence intervals do not account for the additional uncertainty due to the selection process, we corrected the intervals as described by Benjamini and Yekutieli (2005).

Results

Study population

On average, each herd had an average of 7.53 ± 3.18 TD per year. The size of the herds ranged from 5 to 2,143 lactating cows (mean = 195.93 ± 207.53). On average, each cow was sampled 17.16 ± 11.12 times during the study period, having an average of 7.94 ± 3.89 TD per lactation.

Within the study period, more than one-third of the cows (35.26 %) were observed during only one lactation, 32.01 % for two, and 32.73 % for three or more lactations. The 37.28 % of observations were of primiparous cows, 27.25 % of 2nd lactation, 17.11 % of 3rd lactation, and 9.54 % of the 4th one; 8.82 % were from the 5th lactation.

Milk components

The average values of milk production, fat, and protein, measured on the entire study population, are shown in Figure 2. All milk components were quite normally distributed.

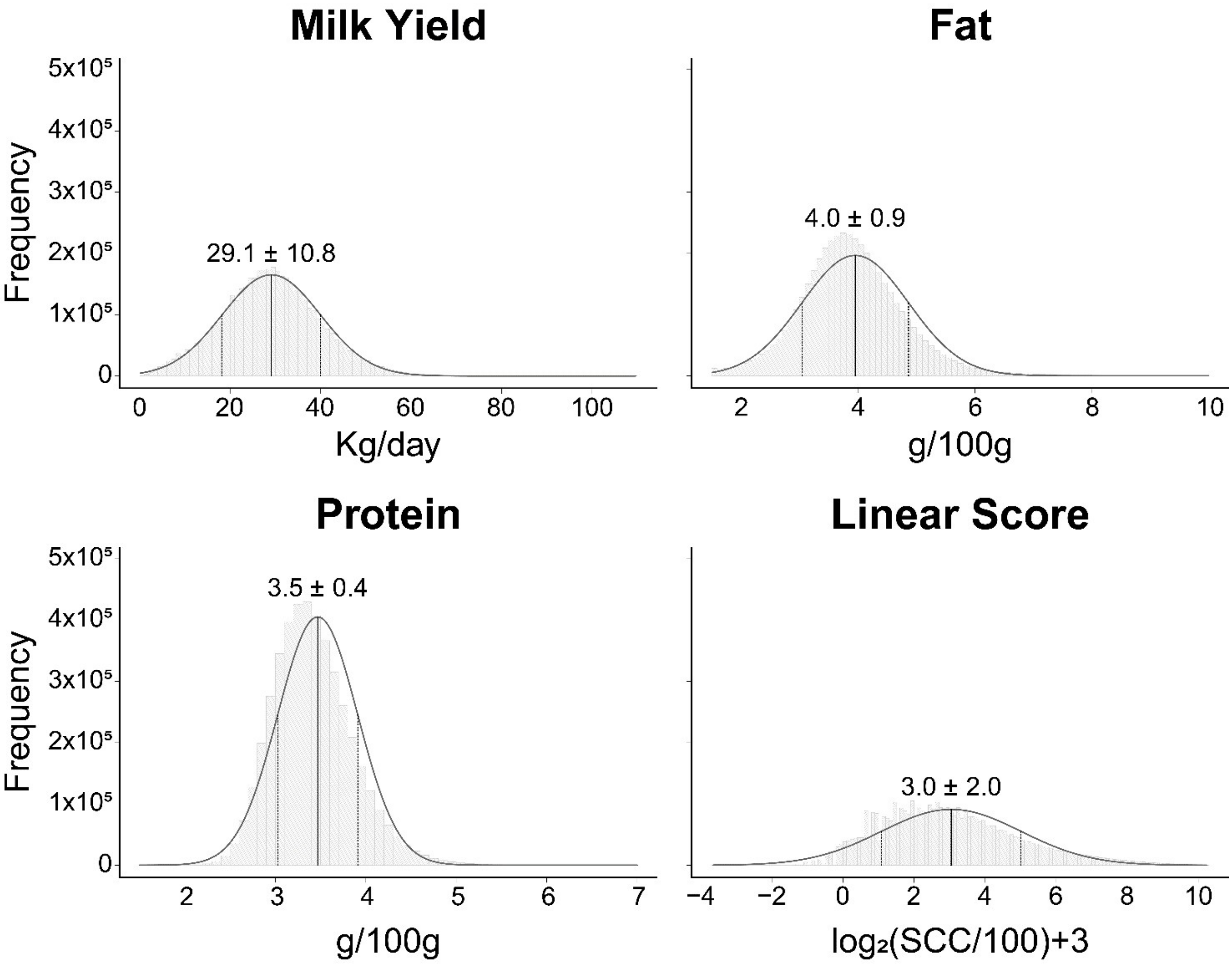

Figure 2. Histograms of milk yield and milk component values. Milk yield, lactose, fat, and protein are reported as raw values, with mean and standard deviation (solid and dashed line, respectively). Somatic cells count (SCC) is reported as the linear score [LS=log$_2$(SCC/100)+3] to improve data visualization.

Milk yield was positively correlated with lactose (ρ = 0.24), while negatively with fat (ρ = -0.21, protein (ρ = -0.42), and SCC (ρ = -0.12). Accordingly, a negative correlation was observed between lactose and fat (ρ = -0.22), protein (ρ = -0.24), and SCC (ρ = -0.26), whereas a positive one was observed between fat and protein (ρ = 0.42). Their correlation with SCC was very low (ρ = 0.04 and ρ = 0.05, respectively).

Milk yield varied by breed, and the Holstein-Friesian was the only breed whose daily production exceeded 30 Kg (30.80 ± 10.08 Kg). It increased monotonically from below 21 Kg/day in 2015 to over 31 Kg/day in 2020. During the year, it recorded minimum values in summer (July to September) and maximums in spring (February to April). Also, fat and protein increased during the years of study, both peaking in autumn (November and December, respectively) and reaching minimum concentrations in July. Lactose did not show a seasonal pattern or a monotonic trend during the study period. The average SCC decreased during the study period by more than 50,000 cells/mL. Annually, it followed an oscillation from minimum values in March to maximums in August. The average SCC of mastitic and non-mastitic cows remained constant throughout the study period, indicating that its decrease from 2015 to 2020 was due to the reduction of mastitis cases.

Mastitis

Mastitis prevalence. Out of 261,121 cows, 23 % (n = 60,937) never had SCC ≥ 200 during the study period. The average prevalence proportion of mastitis was 0.29 (CI: 0.28 – 0.29). It decreased linearly from 0.30 in 2015 to 0.27 in 2020. The seasonal pattern repeated every year and ranged from 0.26 in March to 0.32 in August. Mastitis prevalence increased by 22 % (RR = 1.22, CI: 1.22 – 1.23) between early and late lactation (Figure 3A). Among breeds, only Holstein Friesians have a prevalence lower than the population average (data not shown).

From the bivariate analysis, we observed that the prevalence of mastitis increased quite linearly by 27 % after each calving, ranging from 0.19 in primiparous cows to 0.47 over the 5th delivery (Figure 3B).

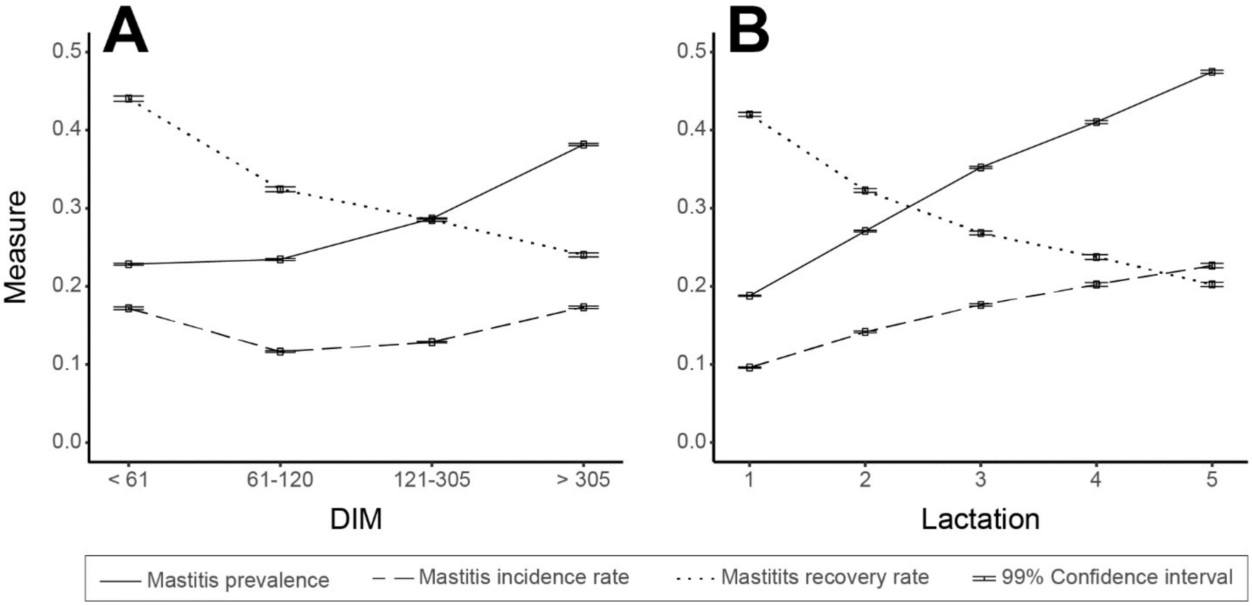

Figure 3A-B. Mastitis prevalence according to days in milk and parity. A: prevalence of mastitis, its incidence rate, and the recovery proportion by days in milk; lactation phases were divided based on a Legendre-shaped lactation curve. B: prevalence of mastitis, its incidence rate, and the recovery proportion by parity; observations over the 4th lactation were aggregated.

Only 9 % (n = 392,488) of the observations were from cows milked three times a day, which had 22% less risk of mastitis (RR = 0.78, CI: 0.77 – 0.79). Ketosis increased the risk of mastitis (RR = 1.03, CI: 1.03 – 1.04) by 3 %. On average, the cows with mastitis had an average 4.81 Kg lower milk yield (25.66 ± 11.12 Kg) than healthy ones (30.46 ± 10.40 Kg).

Breed, parity, DIM, and the milkings per day were included in the final model along with the mastitis status at the previous TD. A sinusoid function with a six-month period, from March (-1) to August (1), was included in the model to adjust for seasonality. The estimated effect of each relevant variable was adjusted for unmeasured herd-level risk factors. The model formula was:

$$\log\left(Y_{ij}\right) = \beta_0 + \beta_1 X_{i_{breed}} + \beta_2 X_{i_{parity}} + \beta_3 X_{i_{DIM}} + \beta_4 X_{i\frac{milkings}{day}} + \beta_5 S(i) + \beta_6 X_{i,TD-1\ mastitis} + \varepsilon + u_j$$

where $\log\left(Y_{ij}\right)$ is the natural logarithm of mastitis prevalence, β_0 is the intercept, β_{1-6} are the coefficients of each covariate, $S(i)$ is the calendar-period correction, ε is the random error, and u_j is the random intercept of each j^{th} herd and $u_j \sim N(0, \sigma^2)$. The results of the model are reported in Table 1.

		Mastitis prevalence		**Mastitis incidence**		**Mastitis recovery**		**Ketosis incidence**		**Inter-calving interval**		**Fresh-cow removal**	
	Effect	**OR**	**99 % C. I.**	**OR**	**99 % C. I.**	**OR**	**99 % C. I.**	**OR**	**99 % C. I.**	**β**	**99 % C. I.**	**OR**	**99 % C. I.**
	Intercept	0.09	0.08 – 0.09	0.07	0.07 – 0.08	0.17	0.14 – 0.20	0.30	0.28 – 0.32	424.44	419.56 – 429.32	0.10	0.08 – 0.13
Breed	Holstein Friesian	1 (ref.)	-	1 (ref.)	-	1 (ref.)	-	1 (ref.)		1 (ref.)	-	1 (ref.)	-
	Cross-bred	0.99	0.97 – 1.00	1.00	0.98 – 1.03	0.98	0.95 – 1.01	**0.80**	**0.76 – 0.84**	**-18.71**	**-21.28 – -16.14**	**0.58**	**0.50 – 0.67**
	Brown Swiss	0.97	0.93 – 1.01	0.99	0.93 – 1.05	0.95	0.88 – 1.03	**0.70**	**0.61 – 0.79**	-3.48	-10.58 – 3.61	**0.29**	**0.19 – 0.43**
	Italian red roan	**0.88**	**0.85 – 0.90**	**0.92**	**0.89 – 0.96**	**1.06**	**1.01 – 1.12**	**0.63**	**0.58 – 0.68**	**-19.53**	**-23.87 – -15.18**	**0.36**	**0.29 – 0.45**
	Piedmontese	**0.88**	**0.77 – 0.99**	1.15	0.99 – 1.34	1.11	0.88 – 1.39	**0.34**	**0.24 – 0.49**	**-29.36**	**-50.44 – -8.29**	**0.19**	**0.08 – 0.44**
	Oropa red roan	**1.12**	**1.02 – 1.22**	**1.30**	**1.18 – 1.45**	**0.87**	**0.77 – 0.99**	**0.24**	**0.19 – 0.30**	**-12.18**	**-23.14 – -1.21**	**0.11**	**0.07 – 0.16**
	Grauvieh	1.07	0.97 – 1.18	1.09	0.96 – 1.24	**0.69**	**0.58 – 0.82**	**0.45**	**0.34 – 0.61**	**-41.74**	**-57.82 – -25.66**	**0.17**	**0.08 – 0.38**
	Jersey	1.02	0.94 – 1.10	1.02	0.92 – 1.12	1.01	0.87 – 1.16	1.02	0.83 – 1.25	**-17.68**	**-29.63 – -5.73**	**0.53**	**0.28 – 0.99**
	Abondance	0.91	0.79 – 1.06	1.00	0.82 – 1.23	0.87	0.64 – 1.19	**0.62**	**0.39 – 0.99**	27.58	-5.00 – 60.16	1.92	0.67 – 5.49
	Brown	1.05	0.91 – 1.22	**1.37**	**1.14 – 1.65**	0.98	0.74 – 1.29	**0.34**	**0.22 – 0.53**	**-36.79**	**-58.2 – -15.39**	**0.13**	**0.05 – 0.39**
	Pustertaler	0.94	0.86 – 1.02	1.06	0.94 – 1.18	1.04	0.89 – 1.21	**0.44**	**0.33 – 0.58**	**-21.75**	**-34.74 – -8.76**	**0.15**	**0.07 – 0.30**
	Valdostana	0.96	0.90 – 1.02	**1.10**	**1.02 – 1.19**	1.02	0.91 – 1.13	**0.31**	**0.26 – 0.38**	**-35.56**	**-44.14 – -26.98**	**0.16**	**0.11 – 0.24**
	Other	**1.17**	**1.04 – 1.30**	1.15	0.98 – 1.35	**0.73**	**0.58 – 0.91**	1.05	0.79 – 1.41	-16.98	-37.05 – 3.09	0.44	0.16 – 1.23
Parity	1st lactation	1 (ref.)	-	1 (ref.)	-	1 (ref.)	-	1 (ref.)	-	1 (ref.)	-	1 (ref.)	-
	2nd lactation	**1.56**	**1.55 – 1.58**	**1.53**	**1.51 – 1.55**	**0.68**	**0.67 – 0.69**	**1.21**	**1.18 – 1.25**	**1.70**	**0.49 – 2.90**	**6.36**	**5.83 – 6.93**
	3rd lactation	**2.08**	**2.06 – 2.10**	**1.94**	**1.91 – 1.96**	**0.52**	**0.51 – 0.53**	**1.76**	**1.71 – 1.81**	-1.56	-3.27 – 0.14	**10.15**	**9.22 – 11.18**
	4th lactation	**2.47**	**2.44 – 2.50**	**2.24**	**2.20 – 2.27**	**0.44**	**0.43 – 0.45**	**1.85**	**1.78 – 1.91**	**-8.87**	**-11.73 – -6.02**	**12.22**	**10.78 – 13.84**
	5th lactation	**2.86**	**2.82 – 2.90**	**2.45**	**2.40 – 2.49**	**0.37**	**0.36 – 0.38**	**1.70**	**1.64 – 1.76**	**-24.76**	**-31.75 – -17.78**	**13.99**	**11.13 – 17.59**
Days since calving	DIM (30.5 days)	**1.07**	**1.07 – 1.07**	**1.05**	**1.04 – 1.05**	**0.94**	**0.93 – 0.94**	-	-	-	-	-	-
	1st week after calving	-	-	-	-	-	-	1 (ref.)	-	-	-	-	-
	2nd week after calving	-	-	-	-	-	-	0.99	0.95 – 1.02	-	-	-	-
	3rd week after calving	-	-	-	-	-	-	0.96	0.93 – 1.00	-	-	-	-
	4th week after calving	-	-	-	-	-	-	0.97	0.93 – 1.00	-	-	-	-
	5th week after calving	-	-	-	-	-	-	**0.84**	**0.81 – 0.88**	-	-	-	-
	6th week after calving	-	-	-	-	-	-	**0.74**	**0.71 – 0.78**	-	-	-	-
	7th week after calving	-	-	-	-	-	-	**0.67**	**0.63 – 0.71**	-	-	-	-
	8th week after calving	-	-	-	-	-	-	**0.62**	**0.58 – 0.66**	-	-	-	-
	9th week after calving	-	-	-	-	-	-	**0.54**	**0.49 – 0.59**	-	-	-	-
Milkings per day	2 milkings/day	1 (ref.)	-	1 (ref.)	-	1 (ref.)	-	-	-	-	-	-	-
	3 milkings/day	**0.88**	**0.85 – 0.91**	**0.88**	**0.82 – 0.94**	**1.12**	**1.18 – 1.06**	-	-	-	-	-	-
	Milk yield (5 Kg)	-	-	-	-	-	-	**0.95**	**0.94 – 0.95**	**-0.69**	**-0.76 – -0.62**	**0.59**	**0.58 – 0.60**
	Age at first calving	-	-	-	-	-	-	-	-	**0.76**	**0.64 – 0.89**	1.00	0.99 – 1.01
Contingent problems	Ketosis	**1.22**	**1.20 – 1.23**	**1.43**	**1.41 – 1.45**	-	-	-	-	**7.27**	**5.93 – 8.61**	**1.75**	**1.61 – 1.90**
	Mastitis	-	-	-	-	-	-	**1.16**	**1.13 – 1.18**	**7.09**	**5.72 – 8.46**	**1.31**	**1.21 – 1.42**
	Long ICI (> 440 days)	-	-	-	-	-	-	-	-	-	-	**1.34**	**1.24 – 1.45**
Previous parameters	Previous mastitis	**8.76**	**8.70 – 8.82**	-	-	-	-	-	-	-	-	-	-
	Previous milk yield	-	-	-	-	1.00	0.99 – 1.00	-	-	-	-	-	-
	Previous lactose	-	-	-	-	**1.59**	**1.54 – 1.65**	-	-	-	-	-	-

	Previous SCC	-	-	-	-	1.00	1.00 – 1.00	-	-	-	-	-	-

Table 1. Estimates of odds ratio and their 99% confidence intervals adjusted for other covariates and herd-level unmeasured risk factors. The effect of each relevant variable is reported for mastitis prevalence, mastitis incidence rate, mastitis recovery, ketosis prevalence, the prevalence of reproductive disorders, and fresh-cow removal prevalence.

We observed a covariance of 0.20 ± 0.01 within herds. After adjusting for other risk factors, Holstein Friesian was no longer the breed with the lowest prevalence of mastitis, while the effect of other risk factors was confirmed. The effect of ICI was nullified by the herd-level risk factors.

Mastitis incidence. The overall incidence rate (IR) was 0.14 new cases per cow-month at risk (CI: 0.14 – 0.14), while the monthly individual rate was 0.27. The incidence rate decreased from 2015 (IR = 0.15, CI: 0.15 – 0.15) to 2020 (IR = 0.13, CI: 0.12 – 0.13). The burden of new cases reached the maximum in July (IR = 0.17, CI: 0.17 – 0.17) and the minimum in January (IR = 0.12, CI: 0.11 – 0.12), with an annual oscillation of 47.2 %. The individual rate peaked in September (0.41) and reached minimum values in January (0.23). The incidence rate varied among breeds, from Holstein Friesian (IR = 0.13, CI: 0.13 – 0.13) to Brown cows (IR = 0.23, CI: 0.20 – 0.27). It increased from the first lactation (IR = 0.10, CI: 0.09 – 0.10), reaching the highest values after the fourth lactation (IR =0.23, CI: 0.22 – 0.23). The incidence rate of mastitis was high in the first 60 days after calving (IR = 0.17, CI: 0.17 – 0.17), then it decreased in mid-lactation (IR = 0.12, CI: 0.11 – 0.12) to increment again towards the end of the lactation (IR = 0.13, CI: 0.13 – 0.13). Over 305 DIM, the incidence rate reached the same values observed in fresh cows (IR = 0.17, CI: 0.17 – 0.17). Milking three times a day decreased the risk of new mastitis cases by 15 %, while ketosis at the beginning of the lactation increased the risk of new cases by 38 % (IRR = 1.38).

Breed, parity, DIM, and milkings per day were included in the model along with ketosis, and adjusting for herd-level risk factors as random intercepts. A six-month period function, spanning from -1 in January and 1 in July was included to adjust for the calendar period. The model formula was:

$$\log(Y_{ij}) = \beta_0 + \beta_1 X_{i_{breed}} + \beta_2 X_{i_{parity}} + \beta_3 X_{i_{DIM}} + \beta_4 X_{i_{\frac{milkings}{day}}} + \beta_5 S(i) + \beta_6 X_{i_{ketosis}} + \varepsilon + u_j$$

where $\log(Y_{ij})$ is the natural logarithm of mastitis incidence, β_0 is the intercept, β_{1-6} are the coefficients of each covariate, $S(i)$ is the calendar-period correction, ε is the random error, and u_j is the random intercept of each j^{th} herd and $u_j \sim N(0, \sigma^2)$.

As for the prevalence model, Holstein Friesian had no longer the lowest mastitis incidence rate, while all other risk factors were confirmed (Table 1). The average covariance among observations of the same herd was lower than for mastitis prevalence (0.18 ± 0.01).

Recovery from mastitis. On average, 31 % of mastitis cases recovered. The recovery proportion followed a seasonal pattern opposite to mastitis incidence, from 0.28 in July to 0.33 in December. Jersey and Holstein Friesian cows had the best chance of recovery (0.33 and 0.32, respectively) and were the sole breeds whose recovery proportion exceeded 30%. The probability of cure was the highest in primiparous (0.44) and fresh (0.44) cows. It decreased with parity, reaching 0.20 after the fourth lactation, and with DIM, to 0.29 in late lactation and even lower beyond 305 DIM (0.24). Milking three times a day ameliorated the probability of recovery by 20 % (RR = 1.20, CI: 1.19 – 1.21).

Some variables measured at the previous TD were useful for predicting the outcome of mastitis (Figure 4). The chance of recovery increased by 9 % per 5 Kg increase in milk production (RR = 1.09, CI: 1.09 – 1.09). Lactose was the best predictor of recovery, as a 0.25 g / 100 g increment in its concentration led to a 29 % increase in the chances of recovery (RR = 1.29, CI: 1.27 – 1.31), while SCC was not a predictor at all (RR = 1.00, CI: 1.00 – 1.00).

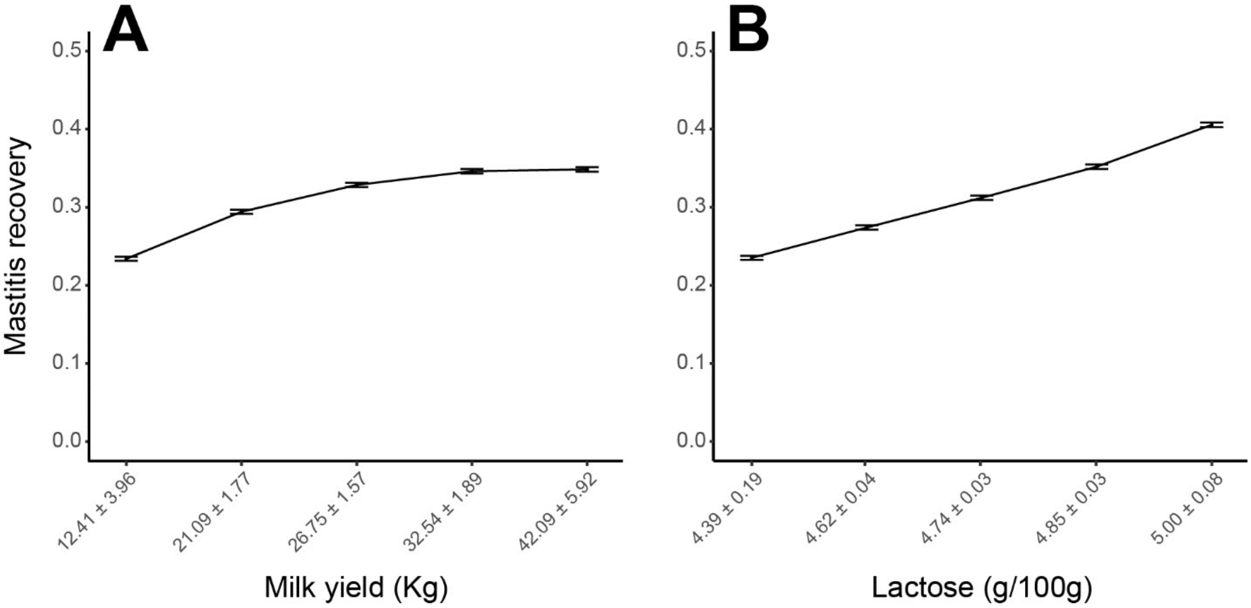

Figure 4A-B. Most important predictors of mastitis recovery. The figure reports the observed recovery proportion with 99 % confidence intervals, by milk yield (A), and lactose concentration (B).

Breed, parity, DIM, milkings per day, milk yield at TD-1, lactose at TD-1, and SCC at TD-1 were included as a covariate in the model, along with the season and herd-level corrections. The final model formula was:

$$\log(Y_{ij}) = \beta_0 + \beta_1 X_{i_{breed}} + \beta_2 X_{i_{parity}} + \beta_3 X_{i_{DIM}} + \beta_4 X_{i\frac{milkings}{day}} + \beta_5 S(i) + \beta_6 X_{i,TD-1\,{milk\ yield}} + \beta_7 X_{i,TD-1\,[lactose]} + \beta_8 X_{i,TD-1_{SCC}} + \varepsilon + u_j$$

where $\log(Y_{ij})$ is the natural logarithm of mastitis prevalence, β_0 is the intercept, β_{1-8} are the coefficients of each covariate, $S(i)$ is the calendar-period correction, ε is the random error, and u_j is the random intercept of each j^{th} herd and $u_j \sim N(0, \sigma^2)$. The sinusoid function for the season ranged from -1 in July to 1 in December. We observed 0.21 ± 0.01 covariance among observations of the same herd.

Ketosis

The first TD occurred on average 22.91 ± 12.84 days after calving. The mean F:P was 1.25 ± 0.34, and the overall incidence proportion of ketosis was 0.23. We did not observe an annual trend, but a seasonal pattern opposite to mastitis repeated every year, with the minimum number of cases in September (IP = 0.19, CI: 0.18 – 0.19) and the maximum in March (IP = 0.26, CI: 0.26 – 0.27). Ketosis varied with the breed, reaching the highest values in Jersey, and Holstein Friesian cows. Based on bivariate analysis, the risk of ketosis increased from the first (IP = 0.20, CI: 0.20 – 0.21) to the fourth lactation (PP = 0.28, CI: 0.27 – 0.28), then decreased over the fifth lactation (PP = 0.23, CI: 0.23 – 0.24).

In the first four weeks after calving, it remained permanently above 0.24 then declined linearly until the ninth week (PP = 0.14, CI: 0.13 – 0.15). The risk of ketosis increased by 5 % for every 5 Kg increase in milk yield (RR = 1.05, CI: 1.05 – 1.05), and by 15 % with mastitis (RR = 1.15, CI: 1.13 – 1.17), while milking the cows three times a day did not ameliorate nor worsen their metabolic condition (RR = 0.99, CI: 0.96 – 1.01). Breed, milk yield, parity, and DIM were included in the regression model. Their effects were estimated by adjusting for herd-level risk factors and season. The season function followed the pattern of ketosis cases, with a six-month period from September (-1) to March (1).

$$\log(Y_{ij}) = \beta_0 + \beta_1 X_{i_{breed}} + \beta_2 X_{i_{parity}} + \beta_3 X_{i_{DIM}} + \beta_4 X_{i_{milk\ yield}} + \beta_5 S(i) + \beta_6 X_{i_{mastitis}} + \varepsilon + u_j$$

where $\log(Y_{ij})$ is the natural logarithm of the ketosis incidence proportion, β_0 is the intercept, β_{1-6} are the coefficients of each covariate, $S(i)$ is the calendar-period correction, ε is the random error, and u_j is the random intercept of each j^{th} herd and $u_j \sim N(0, \sigma^2)$. The observed covariance within the same herd was 0.52 ± 0.02. All the estimated odds ratios are reported in Table 1.

Inter-calving interval

The mean inter-calving interval was 428.92 ± 104.18 days, and it was longer than 500 days in more than 10 % of the cows. The average duration of ICI decreased during the study period from 448.19 ± 129.09 in 2015 to 409.06 ± 76.02 in 2019. The mean ICI of 2020 (ICI = 367.03 ± 39.81) could be underestimated due to the study end. The ICI of Holstein Friesians was 429.70 ± 101.07 days long, a middle ground between 411.91 ± 115.96 days of Piedmontese and 473.87 ± 181.97 days of Abondance cows. The ICI decreased from the first (429.33 ± 109.89) to the second calving (427.71 ± 98.37), then increased monotonously to the fifth one (431.13 ± 104.53). Also, the higher the milk yield the shorter the ICI, and the decrement was monotonous. The ICI duration slightly decreased in cows milked three times a day (424.07 ± 92.07) in reference to those milked twice (429.36 ±105.20), while it increased with the age at first calving. We considered ages at first calving that ranged from 20 to 37 months. Ketosis and mastitis during pregnancy determined an extension of ICI, which came from 419.75 ± 93.01 to 427.31 ± 94.61 in the case of ketosis, and from 424.50 ± 100.23 to 443.64 ± 115.19 in the case of mastitis.

Breed, parity, milk yield, age at first calving, mastitis, and ketosis were included in the final model, which was adjusted for unmeasured herd-level risk factors including herds as random intercepts (Table 1). The model formula was:

$$ICI = \beta_0 + \beta_1 X_{i_{breed}} + \beta_2 X_{i_{parity}} + \beta_3 X_{i_{milk\ yield}} + \beta_4 X_{i_{calving\ age}} + \beta_5 X_{i_{mastitis}} + \beta_6 X_{i_{ketosis}} + \varepsilon + u_j$$

where ICI is the predicted inter-calving interval, β_0 is the intercept, β_{1-6} are the coefficients of each covariate, ε is the random error, and u_j is the random intercept of each j^{th} herd and $u_j \sim N(0, \sigma^2)$.

Nine breeds had ICI shorter than Holstein Friesian. All other estimates confirmed what was observed by bivariate analysis. The number of milkings per day was removed since it worsened the GOF of the model.

Fresh-cow removal

The overall proportion of fresh cows removed was 0.03, and no trend was observed over years. Fresh-cow removal followed a seasonal pattern which peaked in August (IP = 0.05, CI: 0.04 – 0.05) and reached the minimum in April (IP = 0.03, CI: 0.02 – 0.03).

The bivariate analysis showed that the risk of removal increased with parity, from about 1 % in primiparous cows to 7 % (0.06 – 0.07) in the fifth lactation. Holstein Friesians had the fourth to lowest prevalence (IP = 0.03): lower values were recorded for Brown Swiss, Italian red roan, and "other" breeds

For each 5 Kg increment in milk production, the risk of fresh cow removal decreased by 24 % (RR = 0.76, CI: 0.76 – 0.76). The risk of being removed raised by 37 % if the cow had an ICI ≥ 440 days (RR = 1.37, CI: 1.32 – 1.41), almost doubled in the case of ketosis (RR = 1.96, CI: 1.91 – 2.00), and it grew even higher in case of mastitis (RR = 2.36, CI: 2.32 – 2.40). Based on the stepwise selection, breed, parity, milk yield, age at first calving, ketosis, mastitis and excessively long ICI were included in the regression model. The estimates were adjusted for unmeasured herd-level risk factors including herds as random intercepts, and for the calendar period including a function that ranged from -1 in April to 1 in August. Estimates are reported in Table 1.

The final model formula was:

$$\log(Y_{ij}) = \beta_0 + \beta_1 X_{i_{breed}} + \beta_2 X_{i_{parity}} + \beta_3 X_{i_{milk\ yield}} + \beta_4 X_{i_{age\ at\ 1s\ \ calving}} + \beta_5 S(i) + \beta_6 X_{i_{mastitis}} + \beta_7 X_{i_{ketosis}} + \beta_8 X_{i_{ICI>4}} + \varepsilon + u_j$$

where $\log(Y_{ij})$ is the natural logarithm of the incidence proportion of fresh-cow removal, β_0 is the intercept, β_{1-8} are the coefficients of each covariate, $S(i)$ is the calendar-period correction, ε is the random error, and u_j is the random intercept of each j^{th} herd and $u_j \sim N(0, \sigma^2)$.

Based on the results of the model, many breeds had a lower prevalence than Holstein Friesian, which recorded the second-highest value. Adjusting for herd-level risk factors, the age at first calving was no longer significant; however, keeping it into the model ensures a better fit. The covariance among observations of the same herd was 0.56 ± 0.04.

Discussion

This study estimated four herd health indicators using TD data from a large cohort of dairy cows and herds in the Piedmont region. The estimates were calculated considering cow-level risk factors, as previously described, and herd-level risk factors. The latter were accounted for as random effects, whose covariance was the measure of the variability that occurred within the same herd, but not analysed in detail since from TD data many of them remained occult. To reduce the computational load, we chose to limit the hierarchy of the model at the herd level, and no auto-correlation factor was included for the cows, although some authors suggested that the herd performance depends on a single cow's resilience (Poppe et al., 2021).

Mastitis

The mastitis prevalence observed in Piedmont was 29 %. It was consistent with the prevalence reported in other European countries (Krishnamoorthy et al., 2021, Bradley et al., 2007) and fell inside the interval estimation by Shook et al. (2017) for intramammary infections, but it was lower than what was reported in Italy by Ceniti et al. in 2017, and in other geographical regions (Fesseha et al., 2021; Krishnamoorthy et al., 2021) In this work, the underestimation of mastitis prevalence could be due to the chosen threshold of 200,000 cells/mL on composite milk samples. In fact, it has been reported that 44 % of the cows with at least one infected quarter in composite milk do not exceed this threshold in composite milk (Petzer et al., 2017). On the other hand, the mastitis incidence rate was similar to that reported by Busanello et al. (2017), where the same threshold was used, but lower than in other studies (Aghamohammadi et al., 2018; Olde Riekerink et al., 2008; Reyher et al., 2011). More in general, the lack of recent studies in the same geographical area and the difference in the methods, threshold selection, and definition of incident cases make results difficult to compare. The optimal SCC threshold for mastitis diagnosis is still under debate (Bradley and Green, 2005; Dohoo et al., 1981, 1984; Fauteux et al., 2014; Petzer et al., 2017a; Sumon et al., 2020a). It was reported that the 200,000 cells/mL threshold has a sensitivity between 73 % and 89 %, and a specificity between 75 % and 86 % (Dohoo and Leslie, 1991; McDermott et al., 1982), but it predicts poorly in primiparous cows, where 15.8 % sensitivity and 84.4 % specificity were recently reported (Lipkens et al., 2019). This could represent a limitation of this study since more than one-third of the cows were primiparous which can result in an underestimation of mastitis cases. Regarding the distinction between an incident and a recurrent case, cows with SCC persistently over the threshold should be excluded from the incidence rate calculation. However, in chronic infections, the SCC fluctuates over time, and there is no consensus yet about the time that must elapse before a new case can be defined (Bradley et al., 2007; Petzer et al., 2017b; Sumon et al., 2020b). The choice of an eight-week timespan instead of a shorter one can partially explain the underestimation of the incidence rate of mastitis in this study.

To provide the state of the art of mastitis in the Piedmont region, both prevalence proportion and incidence rate were estimated since they measure different aspects of udder health. High incidence rates could be the result of transient infections as well as recurrent infections, while high prevalence proportions are likely caused by chronic infections. The simultaneous evaluation of prevalence, incidence rate, and recovery rate suggested that prevalent cases are the results of incident cases which failed to recover. Across the study period, the decreasing trend of both prevalent and incident mastitis cases could be explained by the rewarding systems for milk quality and increased awareness thanks to DHI programs (Barkema et al., 1998; Østerås and Sølverød, 2009). The burden of cases and the recovery rate followed a seasonal pattern typical of temperate areas, where during summer, the high temperatures stress the immune system and promote bacterial growth (Vitali et al., 2020).

On average, 30 % of cows recovered from mastitis. However, as mentioned above, some of them might have experienced a temporary decrease in SCC and not a complete recovery. This limitation is hardly avoidable using only TD data, and a more detailed analysis should comprehend a bacteriologic examination. The chance of recovering decreases as the cow grows older since the probability of developing a chronic infection increases while decreasing the possibility of being treated. As cows grow old, their market value decreases, leading to a lower cost-efficacy ratio of the therapy (De Vries and Marcondes, 2020). Also, although mastitis incidence was higher in the first lactation stage, the chance of recovering decreased as lactation progressed since a cow at the end of the lactation had more probability of developing a chronic infection. Eventually, our results suggested that milking three times a day reduced the risk of mastitis and increased the chance of recovery, in contrast to what was previously reported (Allen et al., 1986). However, the number of farms where cows were milked three times a day was considerably lower than those where they were milked twice.

Our findings confirmed that milk production and lactose concentration could serve as prognostic indicators of mastitis recovery. In vitro studies suggested that the lactose concentration has an association with the number of living bacteria, being the primary energy source for many mastitis pathogens (Stürmlin et al., 2021). On the other hand, an association has been reported between high-yielding cows, which are more prone to mastitis, and lactose concentration and between the latter and subclinical mastitis, thus suggesting that a more complex relationship exists (Antanaitis et al., 2021). Also, lactose concentration depends on many factors, e.g., energy balance, inflammation and infection occurrence, and amount of water activity (Antanaitis et al., 2021).

Italian red roan cows had a lower prevalence and incidence of mastitis as well as a higher chance of recovery than Holstein Friesian. On the contrary, Oropa red roan cows had all three mastitis indicators worse than Holstein Friesian. Some rustic breeds are linked to defined geographical areas, like in some valleys of Piedmont, with particular breeding traditions. This may bias estimates related to these breeds, as breeding methods and environmental conditions were not randomly distributed, although the large sample size and inclusion of the herd-effect as random effects in the estimation process should have mitigated this bias.

Cow-level risk factors explained most of the risk of mastitis (Hogeveen et al., 2010; Steeneveld et al., 2008), but herd-level risk factors exerted an effect which was responsible for part of the variability we observed within herds for all three mastitis indicators. However, herd-level variables need a thorough study since they are not available from TD records.

Ketosis

The beta-hydroxybutyric acid concentration in blood ($[\text{BHBA}]_{\text{blood}}$) is the best diagnostic tool for hyperketonaemia (Duffield et al., 2009; McArt et al., 2015; Ospina et al., 2010), while different thresholds for its concentration in milk ($[\text{BHBA}_{\text{milk}}]$) have been studied, yet no universal consensus has been achieved (de Roos et al., 2007; Denis-Robichaud et al., 2014; Ježek et al., 2017; Santschi et al., 2016; Tatone et al., 2017). Regarding ketosis, the major limitation of this study is that [$\mathrm{BHBA}_{\mathrm{milk}}$] was not consistently provided by all AIA laboratories at the time of the study, whence we were forced to use the fat-to-protein ratio as an indicator of ketosis. It has worse accuracy and precision, although the cut-off of 1.42 is reported to predict $[\mathrm{BHBA}]_{\mathrm{blood}} \geq 1.25$ mmol/mL with a 92 % sensitivity and 65 % specificity (Jenkins et al., 2015). Other thresholds have been suggested for the same or different blood concentrations, and the choice of F:P and $[\mathrm{BHBA}]_{\mathrm{blood}}$ cut-offs could result in over- or underestimation of ketosis. Also, the choice of a time frame of 60 days after calving during which ketosis can occur is questionable. It was selected based on the literature, to match the definition of fresh cow, even though the first TD after calving usually occurred earlier.

The 23 % incidence proportion of ketosis is consistent with previous reports (McArt et al., 2012; Tatone et al., 2017), but lower than what was estimated using [$\mathrm{BHBA}_{\mathrm{blood}}$] (Berge and Vertenten, 2014; Vanholder et al., 2015). Breeds were affected depending on their productivity since the risk increased with milk yield. The risk of ketosis remained high as late as sixty days postpartum which could be an overestimation due to the chosen threshold or the effect of undiagnosed hyperketonaemia in the earlier phase of the lactation. Ketosis incidence increased until the fourth lactation but decreased afterwards, probably because of the milk yield reduction in older cows. Like it was previously described (Tatone et al., 2017), ketosis peaked during winter and spring, possibly due to the peak of milk production and the poorer feed quality, as worse quality forages are available in the cold season. Our findings confirmed previously reported risk factors for ketosis, e.g., milk yield, stage of lactation, and parity (Tatone et al., 2017). The high covariance we observed within herds denoted that the risk of ketosis depended on herd-level as much as on cow-level risk factors.

Inter-calving interval

In dairy herds in Piedmont, the observed mean inter-calving interval was longer than 420 days and exceeded 500 days in more than 10 % of the cows. This average was inflated by extreme values which are mainly observed in rustic breeds. The difference among breeds likely reflected the herd management more than breeds diversity, as well as the age at first calving which proved to be a good predictor for long ICI and is strictly related to the breeder's choice. The ICI decreased significantly from 2015 to 2020, suggesting that DHI programs are increasing awareness of cows' reproductive health and efficiency, too. This trend is consistent with the general improvement observed for other health indicators like mastitis. Except for the first lactation, the ICI increased with parity, probably because reproductive problems arose progressively during the lifespan of the cow. The enlargement of ICI has many risk factors that occur during the milking and the dry period (Carvalho et al., 2019; McDougall, 2006), but the main limitation of ICI is that it is observed only once per lactation leaving all those risk factors occult. Also, it can be measured only in parturient cows, thus some issues of survival bias arise (Fetrow et al., 2007; Olori et al., 2002). Our findings confirmed that diseases like ketosis and mastitis heavily affected the reproductive performance of the cow (Carvalho et al., 2019; McArt et al., 2012), resulting in an increment in the duration of the subsequent ICI. Also, our results corroborated the hypothesis that productive and reproductive performances are associated (De Vries and Marcondes, 2020), since we observed that the ICI decreased as milk yield increased. On the other hand, we can partially exclude that longer ICI was due to the breeder’s choice to postpone the breeding of high-yielding cows. Regarding the reproductive performance estimation, the main limitation of this study is the choice of the inter-calving interval as an indicator, since other reproductive indicators are better suited for this purpose, e.g., the calving-conception interval or the pregnancy rate. However, those are not available from TD data thus requiring an additional effort to obtain, while the ICI was easily obtained and widely used.

Fresh-cow removal

The peak of milk yield occurs in the first two months after calving when the expenses for cow maintenance during pregnancy are paid back by the production. Therefore, a cow is most valuable during the first lactation phase, and severe problems should occur to make the breeder decide to remove a fresh-cow from the herd (Carvalho et al., 2019). However, our results were based on the previous assumption and could overestimate the occurrence of such severe problems, since from TD data it was not possible to distinguish health issues from voluntary sales.

We observed a seasonal pattern in the fresh-cow removal, which peaked during the summer months. It suggested that heat stress could aggravate the situation and jeopardise the ability of the cow to recover (Polsky and von Keyserlingk, 2017; Vitali et al., 2020). Ketosis and mastitis affected the removal of a fresh cow from the herd. Probably they influenced the farmer's decision to sell or slaughter the cow. Also, the risk of being removed in the first 60 days increased with parity, possibly because older cows are more prone to health problems and less valuable for the breeder (De Vries and Marcondes, 2020). High-producing cows had a lower risk of being reformed, although it is difficult to assess the direction of this association since healthier cows tend to live longer and produce more (De Vries and Marcondes, 2020). Eventually, cows with ICI longer than 440 days had more chance of being removed within 60 days from calving. This could depend on the recurrence of reproductive issues as well as the occurrence of additional problems. Nonetheless, it could be a planned decision of the breeder who, due to problems in getting the cow pregnant, decides in advance to sell the cow when it’s more valuable. The threshold for ICI was chosen based on the mean duration of ICI and assuming that 440-day ICI is an undesirable duration.

Conclusion

At the end of June 2021, in Piedmont, there were 298,023 cows in 2,125 dairy herds (ISTAT, 2021). In this regard, the estimates provided by this study are deemed generalisable to the dairy cattle population since most of the cows in Piedmont and about 9 % of all Italian dairy cows were evaluated for five years. The estimates of four HIs in Piedmont showed that there has been a constant improvement in dairy cows' health and performance during the last years, and they could be useful for the benchmarking of dairy herds in the next future.

The results of this study confirmed the importance of previously described cow-level risk factors but also suggested that they are not enough to explain the whole risk of mastitis, ketosis, long ICI, and fresh cow removal alone. Therefore, a detailed analysis of the herd-level risk factors is needed.

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