Milkability of Holstein cows significantly affected by the incidence of clinical mastitis for weeks after diagnosis

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Supplementary File

Materials and methods

Animals and management

Cows were housed in free stall housing with recycled manure solids as bedding (herd size around 480 heads, 412 m above sea level, annual rainfall 615 mm). Cows were milked twice a day in the herringbone milking parlour. Stimulation was done by milking first streaks from each teat, followed by udder cleaning, followed by machine stimulation. The threshold milk flow for the automatic detachment system was set to 0.5 kg/min. The pulsation was set to 60:40 ratio with 55 pulses per minute. Vacuum level was set to 42 kPa. Teat liners had three-sided concave design with 22.5 mm orifice diameter (Milkrite triangular Impulse IP10 AIR; Johnson Creek; Wisconsin; USA).

Ultimately, 127 cows participated in the experiment (first lactation = 50; second lactation = 32, third lactation = 26, fourth lactation = 10, fifth and more lactation = 9). A veterinarian diagnosed 27 cows with clinical mastitis within tested period (first lactation = 3; second lactation = 7, third lactation = 8, fourth lactation = 5, fifth and more lactation = 4). All the diagnosed cases were clinical type of mastitis, therefore with visible changes in milk and on the udder, increased SCC, and in need of immediate treatment.

Four cows were diagnosed with mastitis towards the end of monitored period (in 100DIM, 108DIM, 109DIM, and 118DIM), and therefore milkings after 120DIM were missing in the statistical evaluation for post-mastitis period. Clinical mastitis re-occurred for two animals within the observed period, and only the first incidence was counted for the statistical evaluation. Second incidence of mastitis did not interfere with the 6 week observational period for the first incidence, and was ignored for statistical evaluation.

Data collection

Bimodal milk flows were detected when two increments of milk flow were followed by clear drop in milk flow by more than 0.2 kg/min within 1 min after the start of milking (Džidić et al., 2004).

Data for every given week in the period surrounding mastitis incidence consisted of 14 individual milking records for each cow diagnosed with mastitis. Data for control group consisted of 240 individual milking records for each cow not diagnosed with mastitis.

The model equation

The model equation used for the evaluation was as follows:

$$Y_{ijkl} = \mu + TM_i + PAR_j + WEEK_k + b1*(DIM) + b2*(DATE) + e_{ijkl}$$

where:

Y_{ijkl} = dependent variable (milk yield; average milk flow; milking time; milk flow during 0-15 sec; milk flow during 15-30 sec; milk flow during 30-60 sec; milk flow during 60-120 sec; the occurrence of bimodal milk flows[1]);

μ = mean value of dependent variable;

TM_i = fixed effect of ith time of milking (i = morning, n = 13419; i = evening, n = 13469);

PAR_j = fixed effect of jth parity (j= 1, n = 11585; j= 2, n = 6805; j= 3, n = 5153; j= 4, n = 1736; j= 5 and more, n = 1609);

$WEEK_k$ = fixed effect of kth period around mastitis incidence (k= two weeks before mastitis diagnosis, n = 333; k= one week before mastitis diagnosis, n = 358; k= one week after mastitis diagnosis, n = 365; k= two weeks after mastitis diagnosis, n = 350; k= three weeks after mastitis diagnosis, n = 331; k= four weeks after mastitis diagnosis, n = 283; k= control group without mastitis diagnosis, n = 23999);

b1*(DIM) = linear regression on days in milk (DIM);

b2*(DATE) = linear regression on the date of milking;

e_{ijkl} = random error.

[1]Milkings with no milk flow during first 30 sec of milking were marked as "delayed milk flow" and were not counted for bimodal milk flow evaluation.

Few milking records were missing because of: cow not being at the milking parlour due colostrum production, milking was outside of the monitored period, or cow was not identified at the parlour and the record is missing.

Model equation explained variability from 0.6% for the occurrence of bimodal milk flows to 31.6% for milk yield and was statistically significant for all monitored parameters (Table S1, P<0.001). Individual effects in model equation (udder health status, parity, DIM, time and date of milking) were also statistically significant to monitored parameters, with the exception for the occurrence of bimodal milk flows (Table S1)

Comments on mastitis incidence rate in our study

Clinical mastitis was diagnosed to 21.3% of tested cows during the first 120 DIM, which would represent 5.3 cases per 100 cow-months at risk. Incidence rate rapidly increased with increasing parity, when almost half of the cows on fourth and higher lactation were diagnosed with clinical mastitis, while for the first lactation cows incidence rate was only 6%. Mean incidence rate of clinical mastitis on dairy farms ranges from 20 to 30 cases per 100 cow-years at risk (Naqvi et al., 2018). In the studies focusing on European Holstein population, Barkema et al. (1999) observed 26.3 cases per 100 cow-years at risk for Holstein population in Netherlands, while

Hagnestam et al. (2007) observed 26.1 to 34.7 clinical mastitis cases per 100 cow-years at risk for Swedish Holstein. Recalculating our 5.3 cases per 100 cow-months at risk into 63.6 cases per 100 cow-years at risk would be misleading, because our study was focused on the period in which majority of clinical mastitis cases occur (Barkema et al., 1998). Therefore, we would say that mastitis incidence in our study was similar to mean incidence rate on Holstein dairy farms.

A reason for higher MY in the month after the occurrence of mastitis might be partly due to the traditional intensive genetic selection for milk production traits and the genetic antagonism between milk production and mastitis resistance (Martin et al., 2018). The interpretation of the antagonism between mastitis resistance and milk production is not straightforward. Pleiotropic genes could be involved, but also biological competition for energy and nutrients between functions (Rogers, 2002). There is increasing economic justification to include the traits for mastitis resistance in the breeding objective of the breeds despite their antagonism to production traits. On the one hand, ongoing research aims to increase accuracy of such a selection by better modelling for SCC and clinical mastitis, combining these traits together and with predictor traits such as udder type (Rupp & Boichard, 2003).

Extended machine-on time may increase the incidence or severity of teat-end callosity (Neijenhuis et al., 2000), which would further damage uninfected quarters on susceptible cows. Frequent and long periods of overmilking (over 120 seconds) can develop udder problems such as hyperkeratosis and oedema. The long-term consequences of damaged teat-end are later reflected in increased somatic cell counts and deteriorated udder health (Edwards et al., 2013). Prolonged time of reduced milk flow is not only ineffective, but also increases the risk of damage to the teat tissue. Teats overmilked for 5 minutes during 16 milkings showed less injuries than teats overmilked for 20 minutes during 4 milkings (Pařilová et al., 2011; Neijenhuis et al., 2000; Gleeson et al., 2003). The changes in the udder may be irreversible if cows are exposed to improper milking for a long period, and these cows are at much higher risk of mastitis or culling (Pařilová et al., 2011).

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Table S1. Significance of the model equation for monitored parameters.

Monitored parameters	MODEL	$WEEK_{CM}$	Parity	DIM	Time of milking	Date of milking
	r^2	F-test	F-test	F-test	F-test	F-test
MY	0.316*	36.48*	2689.32*	25.63*	332.76*	148.84*
AMF	0.15*	52.25*	879.39*	596.79*	20.55*	251.61*
MT	0.056*	9.33*	172.56*	451.46*	56.64*	645.96*
BimMF	0.006*	1.1^{NS}	29.66*	0.03^{NS}	17.85*	0.92*
MF0-15	0.106*	23.57*	592.7*	379.87*	5.09***	304.95*
MF15-30	0.188*	26.07*	1145.23*	736.35*	37.98*	501.23*
MF30-60	0.188*	26.34*	1117.53*	757.74*	4.99***	622.98*
MF60-120	0.185*	32.94*	1183.67*	589.33*	7.67**	379.17*

Significance level P<0.001 - ***;** P<0.01 - ****;** P< 0.05 - *****;** no significance – NS. $WEEK_{CM}$ - period around mastitis incidence; DIM – days in milk; MY - milk yield per milking; AMF - average milk flow; MT - milking time; BimMF – occurrence of bimodal milk flows; MF0-15 - partial milk flow from 0 to 15 sec of milking in kg/min; MF15-30 - partial milk flow from 15 to 30 sec of milking in kg/min; MF30-60 - partial milk flow from 30 to 60 sec of milking in kg/min; MF60-120 - partial milk flow from 60 to 120 sec of milking in kg/min; r^2 - coefficient of determination.