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# ABSTRACT

Daily milk production, and fluctuations therein, can provide information on health and resilience of dairy cows. We studied variance and autocorrelation of deviations in daily milk yield in relation to the occurrence of clinical mastitis (no, early or later in lactation). Individual lactation curves were fitted to 305-d lactations of 414 dairy cows using quantile regression. Log-transformed variance (InVar) and autocorrelation of the quantile residuals of daily milk yield (predicted - observed) were evaluated for intervals until 30 and until 305 days in milk (**DIM**). Cows were classified as having no mastitis (*n* = 249), early mastitis that first occurred before 30 DIM (n = 29); or later mastitis (n = 136). Subsequently, linear models were used to assess effects of mastitis and parity class (primiparous or multiparous) on InVar and autocorrelations; and logistic regression analyses were performed to predict mastitis from InVar or autocorrelation and parity. From 10 to 30 DIM, InVar was greater for cows with early mastitis than for cows with no or late mastitis, and autocorrelation tended to be lower for cows with early mastitis than for cows with no mastitis. The InVar and autocorrelation from 10 to 30 DIM were not predictive of late mastitis. From 10 to 305 DIM, InVar was greater and autocorrelation was lower for both cows with early and late mastitis than for cows with no mastitis; and both were predictive of having mastitis in the 305-d lactation. Primiparous cows had lower InVar than multiparous cows. In cows without mastitis, autocorrelation values were positively correlated with InVar. Results confirm that increased InVar is associated with clinical mastitis.

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# Implications

We assessed whether variance and autocorrelation of deviations in daily milk yield were associated with clinical mastitis in dairy cows. Variance was increased and autocorrelation was decreased in case of mastitis. Results indicate that variance and autocorrelation are suitable for evaluation of udder health.

#### Introduction

Daily milk production, and fluctuations therein, can provide information on health and resilience of dairy cows. The occurrence of mastitis generally is associated with a drop in milk yield on the day of, or the days preceding, diagnosis and treatment of clinical mastitis (Gröhn et al., 2004). Aside from pathogens, milk production may fluctuate in response to disturbances such as estrus, heat stress or changes in nutrition (Kadzere et al., 2002). Fluctuations in milk yield are expected to be smaller for more resilient cows that are less affected by disturbances or recover more quickly (Berghof et al., 2019). Recently, the variance and autocorrelation of fluctuations in milk yield throughout complete lactations have been associated with breeding values for udder health, fertility, dry matter intake and longevity in heifers (Poppe et al., 2020).

To our knowledge, it is unclear whether variance and autocorrelation of deviations in daily milk yield indicate vulnerability for disturbances, or whether these fluctuations mainly result from clinical disease. If healthy, but more vulnerable cows (i.e. less resilient) would have larger fluctuations in daily milk yield throughout their lactation due to a larger impact of minor day-to-day disturbances (Berghof et al., 2019), deviations in the absence of disease may be informative of future disease risk.

The aim of our research is to relate variance and autocorrelation of deviations in daily milk yield in the first month and the 305-d

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lactation with clinical mastitis occurrence. A classification of expected vulnerability for mastitis in the first month based on these variables could be used for customised management. A full-lactation association between clinical mastitis and variance or autocorrelation would confirm the value of these variables for evaluation of udder health for breeding purposes.

## Materials and methods

The study was conducted using data from a previous experiment that evaluated effects of extended lactations in dairy cows (Niozas et al., 2019). The dataset contained individual daily milk records (n = 117 275) of 414 complete lactations of 414 unique cows until 305 days in milk (DIM), or dry-off or culling before 305 DIM. For daily milk yields, milk yield per milking was converted to hourly milk production in the hours since the previous milking and summed for the 24 hours per day. Also, the dataset included treatment records for clinical mastitis. Cows were diagnosed with and treated for clinical mastitis in case of increased milk conductivity (>70 standardised units, as measured by the milking system: Lely Astronaut A4, Maassluis, the Netherlands), in combination with macroscopic alterations in milk (flakes, watery, clots or bloody; Niozas et al., 2019). During mastitis treatment, when milk was discarded, no milk records were available. Cow lactations were classified as having no mastitis in the 305day lactation (No; n = 249); first mastitis occurrence within the first 30 DIM (**Early**; *n* = 29); or first mastitis occurrence later than 30 DIM (Late; n = 136). Cow lactations were classified as primiparous (n = 157) or multiparous (n = 257; supplement S1).

To analyse fluctuations in milk yield, differences between daily yields and predicted yields based on an individual lactation curve were assessed. Individual lactation curves were fitted in R version 3.6.1 (R core team, 2019) using a 4th-order polynomial quantile regression with a 0.7 quantile (Supplement S2), using the quantreg package (Koenker, 2018), as described by Poppe et al. (2020). Out of four curve-fitting methods evaluated, quantile regression resulted in the strongest genetic correlations with health traits (Poppe et al., 2020). Quantile residuals were defined as daily deviations of realised milk yields from this lactation curve. From these quantile residuals, two traits were assessed: the log-transformed variance (InVar) and the lag-1 autocorrelation, i.e. the correlation between quantile residuals of subsequent days over the assessed time period (Poppe et al., 2020). The InVar gives an indication of the deviation of milk production from the predicted lactation curve. The InVar and autocorrelation were assessed over two different time periods, i.e. 10-30 DIM and 10-305 DIM, where 1-9 DIM was excluded because of poor model fit and following the approach of Poppe et al. (2020). These time periods were chosen to assess whether InVar and autocorrelation in the first month of lactation were informative of current or later occurrence of mastitis; and to confirm the value of these variables for evaluation of udder health.

To assess whether and how mastitis class (no, early or late), parity class (primi- or multiparous), and their interaction affected InVar and autocorrelation values from 10 to 30 and from 10 to 305 DIM, linear models with Tukey-adjusted posthoc analyses in SAS version 9.4 (SAS Institute Inc., 2011) were performed. Pearson correlations were evaluated between InVar and autocorrelation from 10 to 305 DIM for the six mastitis  $\times$  parity classes. Multiple logistic regression models were used to assess whether InVar and autocorrelation from 10 to 30 DIM and parity class were predictive of staying healthy (event = 0) or having late mastitis (event = 1); and to assess whether InVar and autocorrelation from 10 to 305 DIM and parity class were retrospectively informative of staying healthy (event = 0) or having (early or late) mastitis (event = 1). Model specifications and diagnostics of statistical analyses can be found in Supplement S3.

## Results

In early lactation, from 10 to 30 DIM, lnVar was greater for cows with early mastitis than for cows with no or late mastitis (Table 1). Based on the whole lactation, from 10 to 305 DIM, lnVar was greater for cows with early and late mastitis than for cows with no mastitis. Primiparous cows had lower lnVar than multiparous cows from 10 to 30 DIM and from 10 to 305 DIM.

From 10 to 30 DIM, autocorrelation values tended to be lower for cows with early mastitis than for cows with no mastitis (Table 1). From 10 to 305 DIM, autocorrelation values were smaller for cows with early or late mastitis than for cows with no mastitis. The null-hypothesis statistical test (NHST) on the association of parity with autocorrelation was inconclusive. Pearson correlations per mastitis × parity class indicated positive correlations between InVar and autocorrelation values for primiparous (r = 0.39, P < 0.01) and multiparous cows (r = 0.55, P < 0.01) in cows without mastitis (Fig. 1). For multiparous cows without mastitis, however, the high InVar values for lowest autocorrelation values suggest that the relation between the two variables may not be linear (Fig. 1D). In cows with late or early mastitis, NHSTs on the correlation between InVar and autocorrelation values were inconclusive.

The logistic regression to predict late mastitis vs. staying healthy using parity and lnVar and autocorrelation from 10 to 30 DIM had a reasonable model fit, yet poor discrimination, with an AUC of 0.69 (Supplement S3). Multiparous cows were more likely to develop late

Table 1

Log-transformed variance (InVar) and autocorrelation of daily deviations in milk yield from individual lactation curves for cows with no clinical mastitis, mastitis within the first 30 days in milk (DIM; early) and mastitis later in lactation (late), and for cows of different parity classes. Values represent LSMEANS with 95% confidence intervals between brackets.

		Mastitis class			Parity class		<i>P</i> -values <sup>1</sup>		
		No	Late	Early	Primiparous	Multiparous	Mast.	Par.	Mast. $\times$ Par.
N cows		249	136	29	157	257			
InVar	10-30 DIM	2.0 <sup>a</sup>	2.2 <sup>a</sup>	3.0 <sup>b</sup>	2.2 <sup>a</sup>	2.6 <sup>b</sup>	< 0.01	0.01	0.7
		(1.9-2.1)	(2.0 - 2.4)	(2.7 - 3.4)	(2.0 - 2.4)	(2.4 - 2.8)			
	10-305 DIM	2.3 <sup>a</sup>	2.7 <sup>b</sup>	2.6 <sup>b</sup>	2.2 <sup>a</sup>	2.8 <sup>b</sup>	< 0.01	< 0.01	1
		(2.2 - 2.4)	(2.6 - 2.9)	(2.4 - 2.8)	(2.1 - 2.4)	(2.7 - 2.9)			
Autocorrelation	10-30 DIM	0.57*	0.58	0.48*	0.56	0.53	0.09	0.3	0.9
		(0.55 - 0.60)	(0.53-0.62)	(0.40 - 0.56)	(0.51-0.61)	(0.49-0.57)			
	10-305 DIM	0.73 <sup>a</sup>	0.58 <sup>b</sup>	0.63 <sup>b</sup>	0.65	0.64	< 0.01	0.6	0.5
		(0.72-0.75)	(0.55-0.61)	(0.58 - 0.68)	(0.62-0.68)	(0.62-0.67)			

a,b In each row, values in the same class that do not share the same superscript are statistically significantly different (*P* < 0.05); values in the same class that both have a \* tend to differ (*P* < 0.1).

<sup>1</sup> Mast. = mastitis class; Par. = parity class.



**Fig. 1.** Scatter plots of autocorrelation and lnVar of deviations in daily milk yield from 10 to 305 days in milk (DIM) for primiparous cows with no mastitis (*n* = 120, A), early mastitis (*n* = 14, B) and late mastitis (*n* = 23, C), and multiparous cows with no mastitis (*n* = 129, D), early mastitis (*n* = 15, E) and late mastitis (*n* = 113, F).

mastitis vs. staying healthy than primiparous cows (odds ratio = 4.1, 95% Cl<sub>95</sub> = 2.4–6.9, P < 0.001), but NHSTs on the associations with InVar from 10 to 30 DIM (P = 0.1) or autocorrelation from 10 to 30 DIM (P = 0.4) were inconclusive. The logistic regression to posthoc predict having mastitis vs. staying healthy using parity and InVar and autocorrelation from 10 to 305 DIM had a good model fit, and a good discrimination, with an AUC of 0.87 (Supplement S3). The odds of having (early or late) mastitis vs. staying healthy increased for every 0.1 increase in InVar from 10 to 305 DIM (odds ratio = 1.2, Cl<sub>95</sub> = 1.2 to 1.3, P < 0.001), and reduced for every 0.1 increase in autocorrelation from 10 to 305 DIM (odds ratio = 0.34, 95% Cl<sub>95</sub> = 0.27–0.44, P < 0.001), and the NHST on the association with parity class was inconclusive (P = 0.7).

# Discussion

This study investigated whether variance and autocorrelation of deviations in milk yield could provide information regarding occurrence of clinical mastitis. Cows with mastitis in early lactation had a higher InVar and tended to have a lower autocorrelation from 10 to 30 DIM than cows that had no mastitis. Late mastitis (i.e. first occurrence after 30 DIM) could not be predicted based on lnVar or autocorrelation from 10 to 30 DIM. In contrast, both cows with early and late mastitis had a higher lnVar and lower autocorrelation from 10 to 305 DIM than cows that had no mastitis, and increased lnVar and reduced autocorrelation values were related with an increased likelihood of mastitis. These results seem to be in the same direction as the reported genetic correlation (of -0.32) between full lactation lnVar and breeding values for udder health, but opposite to the reported genetic correlation (-0.21) between full lactation autocorrelation and breeding values for udder health (Poppe et al., 2020).

In cows with mastitis, autocorrelation values were lower and NHSTs on the correlation between lnVar and autocorrelation were inconclusive. Scheffer et al. (2018) hypothesised that high resilience implies a limited impact of disturbances on biological functioning (including e.g. milk production), which results in low variance and autocorrelation of residuals because values fluctuate closely around a target value. In case of low resilience, disturbances result in larger and prolonged deviations, increasing variance and autocorrelation of residuals (Scheffer et al., 2018). Cows with (early or late) clinical mastitis could be considered less resilient than cows with no clinical mastitis, autocorrelation from 10 to 305 DIM was reduced. An explanation might be that, depending

on a cow's recovery capacity, subsequent fluctuations could be similar because of a low recovery (large autocorrelation) or very different because of a high recovery (low autocorrelation; Berghof et al., 2019). However, the dataset did not include milk records on the days cows were treated for mastitis. The absence of milk records during the treatment of mastitis, where milk yield is expected to have dropped and gradually recover (Gröhn et al., 2004), could have removed the large and similar deviations in milk yield that would increase autocorrelation among deviations in milk yield (Scheffer et al., 2018).

Cows without mastitis were not diagnosed and treated for clinical mastitis, and were generally healthy due to the study design that excluded cows with severe dystocia and puerperal diseases (Niozas et al., 2019). Still, cows without mastitis could contract other diseases such as ketosis or claw disorders (of which data were not available), and likely experienced disturbances unrelated to diseases. In this group of cows. larger values for InVar were positively correlated with larger autocorrelation values, in line with the hypothesis that these two parameters increase together in case of lower resilience (Scheffer et al., 2018; Poppe et al., 2020). However, aside from this correlation, scatter plots also showed some cows with no mastitis that had high InVar and low autocorrelation values, which might point to differences in recovery after disturbances (Berghof et al., 2019). Moreover, irrespective of mastitis class, multiparous cows had greater InVar than primiparous cows. In part, these greater fluctuations in milk yield were related to higher milk yield level in multiparous cows. Possibly, these larger deviations reflected that multiparous cows contracted more other diseases than primiparous cows (Berge and Vertenten, 2014) that were not documented or accounted for in this study. Further studies of disturbances and disease records are needed to gain insight in the versatile relation between variance and autocorrelation values and its relation to resilience.

Interestingly, InVar of cows with early or late mastitis was still increased compared with cows with no mastitis, despite the absence of records in the current dataset during the treatment of mastitis, when a lower milk yield would be expected (Gröhn et al., 2004). This implies that the reduction in milk yield before diagnosis of mastitis, or the recovery of milk production after treatment for mastitis or both were sufficient to increase InVar values. Whether data on milk production are recorded during treatment of mastitis differs between farms. Automatic milking systems can record and divert milk that is discarded; but farmers may choose to milk cows with clinical mastitis with a separate milking system for practical reasons, or to prevent the spread of mastitis (Barkema et al., 1999). The current results indicate that InVar is sufficiently robust to be used for evaluation of udder health from daily milk records, even when records are missing during treatment.

In conclusion, the occurrence of clinical mastitis was reflected by increased InVar and reduced autocorrelation of deviations in milk yield. Autocorrelation was positively correlated with InVar in cows without mastitis, but NHSTs on correlations with InVar in cows with mastitis were inconclusive, possibly due to a versatile relation with recovery or due to the absence of milk records during the treatment of mastitis. Independent from mastitis class, InVar was higher for multiparous than primiparous cows.

# Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100363.

#### **Ethics approval**

Not applicable, because only non-invasive procedures were conducted in this study.

# Data and model availability statement

The dataset is available as one of the supplements to this article.

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# **Declaration of interest**

None.

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None.

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