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Restlessness behaviour, heart rate and heart-rate variability of dairy cows milked in two types of automatic milking systems and auto-tandem milking parlours

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Abstract

In order to assess the welfare of cows milked in automatic milking systems (AMS), restlessness behaviour during milking (stepping, foot-lifting), heart rate (HR) and heart-rate variability (HRV) were investigated in cows milked in two different AMS models (AMS-1, AMS-2) and in auto-tandem milking parlours (ATM) on four commercial farms in each case.

Stepping rates and proportions of milkings with foot-lifting were calculated based on video recordings. Non-invasive radio equipment was used for measurements of HR and the HRV variable mean-squared differences of successive beat-to-beat intervals (rMSSD). HR data were recorded during milking and resting, thus allowing an assessment of the milking process in relation to the baseline level of each individual cow. Data were evaluated using generalised mixed-effects models.

Restlessness behaviour was shown more often in AMS-2 than in AMS-1 and ATM. HR increased from resting to milking, and this difference was greatest in AMS-2. HR in AMS-1 and AMS-2 was higher during resting and milking than in ATM. rMSSD decreased from resting to milking, although to a lesser extent in AMS-1 due to lower values during resting. The lowest rMSSD values during milking were observed in AMS 2.

In conclusion, statistically detectable differences in restlessness behaviour, HR and HRV indicated a slightly increased stress level in dairy cows in automatic milking systems as opposed to auto-tandem milking parlours. Viewed in absolute terms, however, all differences between the milking systems were

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minor. The results thus indicate that being milked in an AMS rather than in an ATM did not seriously impair the welfare of dairy cows.

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1. Introduction

In contrast to conventional milking parlours, automatic milking systems (AMS) accomplish milking without the presence and supervision of a human. Situations that are stressful for the cows might arise during the milking process, with no chance of the instant intervention by the farmer as in conventional milking parlours. Stress during milking not only impairs the welfare of the cows, but also has a negative influence on milk ejection, resulting e.g. in an increase in residual milk which may in turn negatively influence health (Rushen et al., 2001).

Behaviour patterns and changes in heart rate (HR) and heart-rate variability (HRV) have previously been used to assess stress in dairy cows milked in AMS. Results were contradictory: some studies found higher levels of restlessness behaviour such as stepping, foot-lifting and kicking in AMS than in an auto-tandem milking parlour (ATM; Wenzel et al., 2003), while others found no such differences (Hopster et al., 2002), or even noted less restlessness (Hagen et al., 2004, 2005) than in a herringbone parlour. The same studies found higher (Wenzel et al., 2003), lower (Hopster et al., 2002) or similar HR levels (Hagen et al., 2004, 2005) during milking in AMS and ATM. In addition, Hagen et al. (2005) observed a lower HRV during lying bouts in cows milked in an AMS.

Whereas Hopster et al. (2002) concluded that there are no differences in animal welfare terms between cows milked in ATM and those milked in AMS, Wenzel et al. (2003) and Hagen et al. (2004, 2005) saw some signs of increased stress in the AMS system in their data. These studies were limited, insofar as they were carried out on a single experimental farm, and only one type of AMS (Lely Astronaut) was investigated. It is thus questionable whether the results of these studies are representative of the behavioural and physiological reactions of cows milked on working farms. Similarly, data collected on one type of AMS may not reflect the cow's reactions when faced with another type of AMS.

Several confounding variables could complicate a direct comparison of different cows in different milking systems. Previous experience with being milked could influence the cows' reactions. With increasing age (parity), cows might accumulate negative milking experiences. Greater physiological demands placed on the cows, such as higher milk yield or challenged immune status (reflected by e.g. somatic cell counts, SCC) could also reduce tolerance of stressful situations such as being milked (Moberg, 2000), and bring about changes in metabolism which could affect the heart-rate variables.

In this study, we compared restlessness as a behavioural indicator of a stress response to milking, and heart-rate variables as physiological measures of stress, in two different types of AMS and in auto-tandem milking parlours (ATM) on four working farms in each case, while controlling for the suggested confounding variables. If being milked in AMS was more stressful for the cows than being milked in the ATM, we would expect to observe more intense restlessness behaviour during milking, higher HR levels and lower HRV levels during milking, and possibly while resting, in the AMS than in the ATM.

2. Material and methods

2.1. Farms, milking systems and animals

We investigated 12 Swiss working farms with cubicle housing systems, equipped with either one of two AMS models or an ATM (four farms each). The milking systems were in use for at least 6 months prior to the study on all farms. The investigation was carried out in autumn/winter 2001/2002, spring 2002 and autumn/winter 2002/2003. Each farm was visited once, and all different milking systems were observed in each season.

Lely Astronaut[®] (Lely Industries N.V., Maassluis, The Netherlands), hereinafter called AMS-1, is a one-box system with a service arm on which the teat-cleaning brushes and teat-cups are mounted, and which remains stationary beneath the cow during the whole of the milking process. The milking boxes of AMS-1 had metal floor surfaces. DeLaval Voluntary Milking System VMS[®] (DeLaval International AB, Tumba, Sweden), hereinafter called AMS-2, is another one-box system, but with a multipurpose arm that first fetches the teat-cleaning cup, then the teat-cups, from a mounting at the side of the milking stall, and finally remains beside the cow as a support for the milk tubes during the milking process. In AMS-2, the floor was covered by a rubber mat. ATM (three farms with a 2 × 2 and one farm with a 2 × 3 parlour) were chosen for comparison, because they are similar to the AMS in terms of the individual milking stalls and individual entry and exit to and from the milking parlour. In the AMS models, teat-cups were removed by quarter, whereas the complete milking cluster was removed all at once in the ATM. Cows on farms with AMS-1 could freely access and alternate between feeding and lying areas (free cow traffic), while on farms with AMS-2 they had to pass selection gates from the lying to the feeding area (guided cow traffic with selection gates). In the latter case, a cow exceeding a certain time limit since the previous milking could only access the feeding area by moving through the milking unit of the AMS.

Though feeding and grazing regimes differed among the farms, there were no systematic differences between the milking systems. On all farms there were at least as many lying cubicles as there were dairy cows. On 11 of the 12 farms, only breeds selected for high milk yield (Holstein, Red Holstein and Brown Swiss) were present. One farm with an ATM kept Simmental cows, representing a dual-purpose breed.

Median herd size was 45 lactating cows on the investigated farms (range 21–56). Twenty lactating cows were chosen as focal animals from each herd with the assistance of the farmer. Cows were selected that the farmer considered to be healthy and trouble-free during milking. The selection was made because we wished to evaluate well-functioning milking systems and avoid cows with either problematic anatomy or troubles in adjusting to an AMS. The 20 focal cows were selected from among the healthy, trouble-free specimens so as to represent a good cross-section of the herd in terms of age and lactation stage. On two farms with AMS-2 and ATM (H and J, Table 1), only 18 and 16 cows were considered healthy and trouble-free by the farmer and were thus included as focal animals. Even though considered as a good choice by the farmer, four of the focal cows on farm E with AMS-2 were never milked successfully during the time of observations, i.e. they had only failed and assisted milkings. On this farm observations were restricted to daytime because the light at the milking unit was switched off contrary to the agreement with the farmer. Another five cows lacked data that we wanted to include as explanatory variables. For three cows we did not have SCC measures (milk quantity too small for one cow on farm H with AMS-2; no measurements for two cows on farm L with ATM due to participation in a competition on the day of data collection and due to being milked separately because of medical treatment), for one cow on farm F with AMS-2 we lacked the number of days in milk and for one cow on farm J with ATM we had no measurement of milk quantity. Thus, nine of the initially selected cows were dropped from the analyses. Details on the remaining 225 cows are given in Table 1. Average milking frequency for these cows did not differ between the two types of AMS and was 2.5 ± 0.1 milkings/day over all (mean $\pm$ S.E. across farms), while cows were milked twice daily in the ATM. Milk yield (Table 1) did not differ among all three types of milking systems. In each herd, 10 of the focal cows were randomly chosen for HR and HRV measurements.

Table 1
Cow characteristics for which behaviour was observed, and sample sizes for the behavioural observations and measurements of heart rate (HR) and heart-rate variability (HRV) in the two different types of automatic milking systems (AMS1, AMS-2) and auto-tandem milking parlours (ATM) for each farm (A–L)

	Sample size for behavioural data and cow characteristics					Sample size HR/HRV		
	No. of cows $\sum \sum 225$	No. of milkings ^a	Parity ^b	Days in milk ^b	Milk yield (Ø day^{-1}) ^b	No. of cows $\sum \sum 73$	No. of milkings ^a	No. of resting bouts ^a
AMS-1	$\sum 80$					$\sum 21$		
A	20	5 (1–7)	3.8 ± 0.4	140 ± 22	17.5 ± 1.4	0	–	–
B	20	9 (5–13)	4.0 ± 0.5	174 ± 12	22.4 ± 1.8	6	3 (2–5)	3 (3–3)
C	20	9 (6–14)	2.7 ± 0.3	130 ± 16	30.7 ± 2.1	5	3 (2–6)	3 (3–3)
D	20	9.5 (3–14)	2.7 ± 0.3	149 ± 20	25.7 ± 2.4	10	5 (1–12)	3 (3–4)
AMS-2	$\sum 72$					$\sum 26$		
E	16	3 (2–4)	2.8 ± 0.5	187 ± 27	22.6 ± 2.4	4	2.5 (2–3)	3 (3–3)
F	19	7 (2–10)	2.4 ± 0.4	184 ± 18	23.0 ± 1.4	4	3.5 (2–5)	3 (3–3)
G	20	8 (4–11)	2.9 ± 0.4	173 ± 23	23.4 ± 1.6	10	3 (1–4)	3 (3–3)
H	17	8 (6–11)	2.2 ± 0.3	174 ± 20	15.6 ± 1.3	8	5.5 (3–9)	3 (3–3)
ATM	$\sum 73$					$\sum 26$		
I	20	6 (3–6)	3.3 ± 0.4	91 ± 15	24.4 ± 2.1	7	5 (3–6)	3 (3–3)
J	15	6 (5–6)	2.4 ± 0.4	180 ± 21	24.1 ± 1.0	5	3 (2–4)	3 (3–3)
K	20	6 (6–6)	2.6 ± 0.3	204 ± 21	21.5 ± 1.6	8	3 (2–4)	3 (3–3)
L	18	5 (3–6)	2.4 ± 0.5	137 ± 13	22.8 ± 1.7	6	2.5 (2–3)	3 (2–3)

^a Median (min–max).

^b Mean $\pm$ S.E.

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2.2. Behavioural observations

On each farm, data on behaviour during milking were recorded on three successive days. With an expected minimum milking frequency of twice a day, this would allow data to be collected for about six milkings per cow. One to four video cameras were installed at the milking box or in the milking parlours such that the udder and hind legs of cows being milked were visible.

Behavioural data consisted of the number of steps taken (shifting weight from one hind foot to the other, lifting the foot less than 10 cm), occurrence of foot lifts (one hind foot lifted more than 10 cm) and occurrence of kicks (fast movement, often directed at items such as the teat-cups or the stanchions of the milking box) during the time period from the first tactile contact between the cow and the milking machine or the milker, i.e. the beginning of udder cleaning, until the removal of the last teat-cup. Leg movements before and after this time span were ignored. At the same time, information on the length of the complete milking bout and the length of two milking phases was collected based on the video time signal. The preparation phase consisting of teat-cleaning and teat-cup attachment was distinguished from the milking phase proper. The number of steps taken was calculated as a rate per minute to allow comparisons between milkings and milking phases of different lengths. Foot-lifting and kicks were observed more rarely. The proportion of milkings with foot-lifting was calculated for each cow in order to compare the complete bout among the systems. For the comparison of the two milking phases, the proportion of cows on each farm that displayed foot-lifting or kicking behaviour in any of the given milking phases was calculated.

2.3. SCC measurements

On the farms with AMS, milk samples were collected with automatic milk-sampling units on at least 1 day, with the intention of collecting at least two samples per cow. Where conditions allowed, milk samples were taken on up to three consecutive days. In ATM, milk samples were generally collected on 1 day (but not on a behavioural-observation day) and the farmer's usual routine for taking milk samples during milk recordings was followed. Median SCC were calculated over all samples for each cow (SCC analysis by the Swiss Brown Cattle Breeders' Federation, 6300 Zug, Switzerland). Of the focal cows, 649 milk samples (median per cow: 3, range 1–7) were analysed for SCC.

2.4. Measurements of heart rate and heart-rate variability

Focal animals wore a custom-made belt with three electrodes for the recording of beat-to-beat intervals. The cows were fitted with the belts at least 12 h before measurements started.

The pulse signal as registered by the electrodes was processed by an amplifier and led to a voltage-controlled oscillator, generating a frequency-proportional signal in the telephony spectrum. This signal was continuously transmitted from the cows to a nearby recording station by low-power radio units, each cow transmitting on an individual frequency. With a phase-locked loop circuit, the signal was reprocessed into its original form of a voltage signal and digitised (Daq-Book 112, IOTech Inc., Cleveland, Ohio, USA) at a sampling rate of 1 kHz (as recommended by Hejzel and Roth, 2004). DC drifts originating in the hardware that could influence the analysis were corrected with a fourth-grade low-pass filter and a threshold frequency of 0.1 Hz. Graphic data acquisition software running on a Windows computer recorded the intervals between successive heartbeats (DasyLab 5.5; IOTech Inc., Cleveland, Ohio, USA). Since the amplitude of the signal depended on factors such as the cow's movements and the wetness of the electrodes, a threshold value was continuously calculated as the sliding median of the maximum amplitude over the last 60 heartbeats, and multiplied by 0.8. The time intervals between points where the threshold value was exceeded were stored to the closest ms and used as estimates for beat-to-beat intervals.

HR and HRV measurements during resting bouts were used as a baseline in the comparison with values during milking. These bouts were roughly identified by a time-sampling method looking for cows lying in a cubicle (sampling every 5 min during daylight hours, i.e. 8.00–18.00 h). The exact lying-down and standing-up times were clearly visible in the HR data, and data was not evaluated for the first 10 min

after a cow lay down. In the remaining lying period, 5-min intervals were identified in which there were errors of under 5% in the single beat-to-beat measurements (Polar Precision Performance 2.0; Polar Electro Oy, Kempele, Finland). These intervals were used for evaluation. With very few exceptions, the data on resting included for a given cow were taken from different resting bouts. Milking bouts were identified using the time of day included in the recordings of behaviour in the milking unit. Only the first 5 min following contact between the milker/robot arm and the udder were included for analysis of milking bouts exceeding 5 min in length and containing measurement errors of under 5%. Hence, only measurements from recordings of the same length (5 min) were compared, as is advised for the comparison of HRV measures (Task Force, 1996). The errors in the resting and milking bouts were then corrected using the algorithm provided by the software mentioned above (validated by Hopster and Blokhuis, 1994; Marchant-Forde et al., 2004). Based on these restrictions, 1–12 milking bouts and three to four resting bouts were evaluated per cow (Table 1).

One HRV variable was chosen for analysis: the square root of the mean-squared differences of successive beat-to-beat intervals (rMSSD). rMSSD takes into account short-term, high-frequency components of HRV (Task Force, 1996), strongly reflects vagal tone (Kleiger et al., 1992; confirmed in calves by Després et al., 2002), and is thus highly correlated to other heart-rate variability measures such as HFnorm, LF/HF, recurrence, determinism, entropy and maxline (see Hagen et al., 2005). For each milking and resting bout analysed, mean HR and one rMSSD value was calculated and stored for evaluation.

2.5. Statistical analyses

In our analyses, we used generalised linear mixed-effects models (Pinheiro and Bates, 2000; Pinheiro et al., 2005) in R 2.1.1 to 2.3.1 (R Development Core Team, 2005). These models allow for accommodating dependencies in the data (cows nested in farms nested in milking systems) in the random effects while searching for systematic differences between e.g. the milking systems (fixed effects).

For farms equipped with AMS, only those milkings were included in the analysis that were completed without technical problems (i.e. with successful attachment of all four teat-cups) and without human intervention (e.g. manual teat-cup attachment). Altogether, behavioural data of 1550 milkings of 225 cows (Table 1) were evaluated in all three systems.

Complete milking bouts with kicks as well as milking phases with foot-lifting or kicks were observed only very rarely in all milking systems. These occurrences were so rare that even a generalised model based on the binomial distribution led to numerical problems. No further attempt was made to evaluate these behaviour patterns statistically, although some descriptive statistics are presented in the results (Table 2).

Stepping rate over the complete milking bout was evaluated as a continuous-response variable using milking system (factor: AMS-1, AMS-2, ATM), parity (continuous), mean daily milk yield (continuous) and median SCC (continuous) as explanatory variables (fixed effects). Milkings with or without foot-lifting were evaluated as a response variable in a generalised mixed-effects model based on the binomial distribution including the same fixed effects. This model evaluates the dependence of the probability that foot-lifting occurred in a milking bout on the explanatory variables (function `glmmPQL`; Venables and Ripley, 2002). In the generalised models, it is not advisable to conduct likelihood-ratio tests (Bateson, Pinheiro and Ripley, personal communications), so the corresponding approximate *t*-statistics are reported. In the model with stepping rate per milking phase, the fixed effects were supplemented by a variable ‘phase’ (factor: preparation/cleaning versus milking) and an interaction of milking system and phase.

Due to the great inter-individual variability in the absolute levels of HRV, data for a given cow obtained during the visits to the AMS or the milking parlour were compared to the baseline data of the same cow (while resting). HR and rMSSD served as response variables in two different models. The milking system, the situation (factor: being milked versus resting) and the interaction of milking system and situation were included in the models as main explanatory variables. Parity, mean daily milk yield and median SCC (all as described above) were added as possible confounding effects. Heart-rate measurements were possible during 277 milking and 217 resting bouts for 73 of the 120 cows chosen (kept on 11 farms; Table 1).

Table 2

Behaviour during the preparation and milking phases in two different types of automatic milking systems (AMS-1, AMS-2) and in auto-tandem milking parlours (ATM)

Milking system	Stepping rate (min ⁻¹)		Cows with foot-lifting during milking (%)		Cows with kicks during milking (%)	
	Preparation	Milking	Preparation	Milking	Preparation	Milking
AMS-1	0.32 (0.22; 0.46)	0.54 (0.33; 0.87)	42 (29; 56)	66 (57; 75)	9 (3; 21)	11 (5; 24)
AMS-2	0.82 (0.56; 1.21)	0.81 (0.42; 1.57)	73 (46; 89)	79 (57; 91)	23 (10; 46)	25 (9; 53)
ATM	0.13 (0.02; 0.69)	0.70 (0.52; 0.96)	10 (5; 21)	36 (5; 85)	0 (0; 12)	3 (0; 98)

Mean and 95% confidence intervals calculated on the transformed scale (stepping rate: log; proportions: logit) and back-transformed to original scale.

Technical problems, e.g. damaged belts or dried-out electrodes, were the main factors leading to loss of data. On farm A, the first farm visited for this study, HR and HRV data were only saved to file at long intervals. A power outage caused a large loss of data, with the result that no evaluation was possible for this farm.

To account for the repeated milkings of the cows and the potential dependency among cows on each farm, the milking system, farm and individual cow were included as hierarchically nested random effects in all models. Using a stepwise backward procedure, variables with non-significant influences on the response variables were eliminated from the models. Stepping rate was log-transformed in the model to meet statistical assumptions on the distribution of the residuals (normality and homoscedasticity). These assumptions were checked using graphical displays of the residuals in all models.

Mean values, standard errors (S.E.) and confidence intervals (CI) in Tables 1 and 2 were based on farm means, which were in turn based on mean values of the individual cows. These summary statistics are thus corrected for differences in number of cows observed per farm.

3. Results

Stepping during the entire milking process was observed in more than 95% of all milkings (AMS-1 95%, AMS-2 98%, ATM 96%). On average, the stepping rate was higher in AMS-2 than in ATM and decreased slightly more for AMS-1 ($F_{2,9} = 7.03$, $p = 0.014$, Fig. 1a). In addition, the stepping rate increased with higher parity ($F_{1,211} = 4.85$, $p = 0.029$) and higher SCC ($F_{1,211} = 3.90$, $p = 0.049$). Foot-lifting was observed in 33% of all milkings (AMS-1 31%, AMS-2 47%, ATM 18%). The probability of foot-lifting was higher in AMS-2 than in ATM ($t_9 = 4.01$, $p = 0.003$) and tended to be higher in AMS-1 than in ATM ($t_9 = 2.08$, $p = 0.068$, Fig. 1b). One or more kicks were observed in only 9% of all milkings. In AMS-1, kicking was seen on average in 4% of all milkings, whereas in AMS-2 and ATM, higher percentages were recorded (13% and 10%, respectively).

An interaction effect between phase of the milking process and milking system was found for stepping rates ($F_{2,1542} = 24.55$, $p < 0.001$, Table 2). While only a slight increase in stepping rates was found from preparation phase to the actual milking phase, a pronounced increase was observed in ATM. In AMS-2, generally higher values for stepping were found in both phases, with little difference between the preparation and milking phase. A higher proportion of cows lifted a foot during the milking phase than during the preparation phase, with the smallest difference in AMS-2 (Table 2). In both phases, the proportion increased from ATM to AMS-1 and AMS-2. Milkings with kicks were only rarely observed. These occurred mostly during the milking phase and in AMS-2 (Table 2).

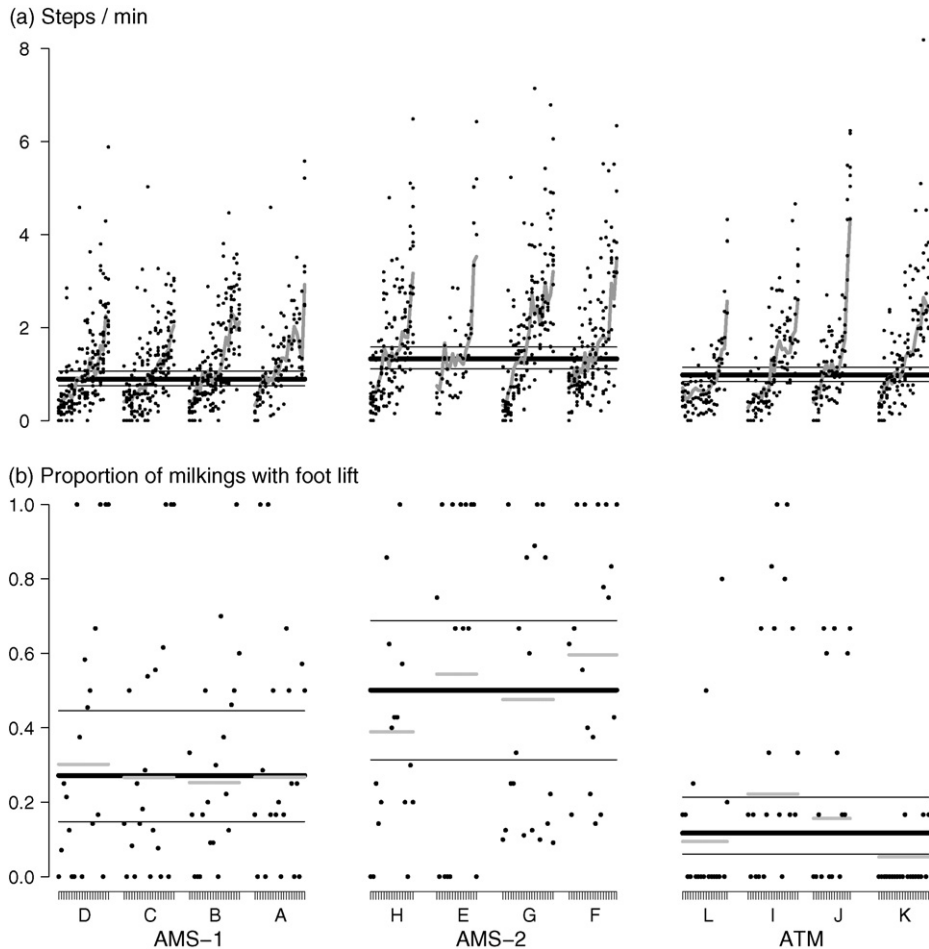


Fig. 1. Stepping rates during single milkings (a) and proportions of milkings with foot-lifting (b) of individual cows (tick marks) on 12 farms (A–L) with either one of two types of automatic milking systems (AMS-1, AMS-2) or auto-tandem milking parlours (ATM). Farms and cows on farms are sorted according to their ascending estimated random effects in the stepping-rate model. Dark grey curve in (a), estimates for individual cows; light grey horizontal lines in (b), estimates for each farm; black horizontal lines in (a) and (b), model estimate (thick line) and model 95% confidence interval (thin lines) for the three milking systems.

In all three milking systems, higher HR values were found during milking than during resting, with lower HR during both resting and milking on farms with ATM. While AMS-1 and AMS-2 showed comparable values during resting, the increase of the values during milking was greater in AMS-2 (interaction of milking system and resting versus milking: $F_{2,418} = 6.39$, $p = 0.002$, Fig. 2a). In all systems, rMSSD values decreased from resting to milking. Cows in AMS-1 had lower values in rMSSD during resting, and cows in AMS-2 had lower values during milking (interaction of milking system and situation $F_{2,418} = 9.61$, $p < 0.001$, Fig. 2b) than those in ATM. Values in rMSSD were lower in cows with a higher daily milk yield ($F_{1,61} = 6.92$, $p = 0.011$).

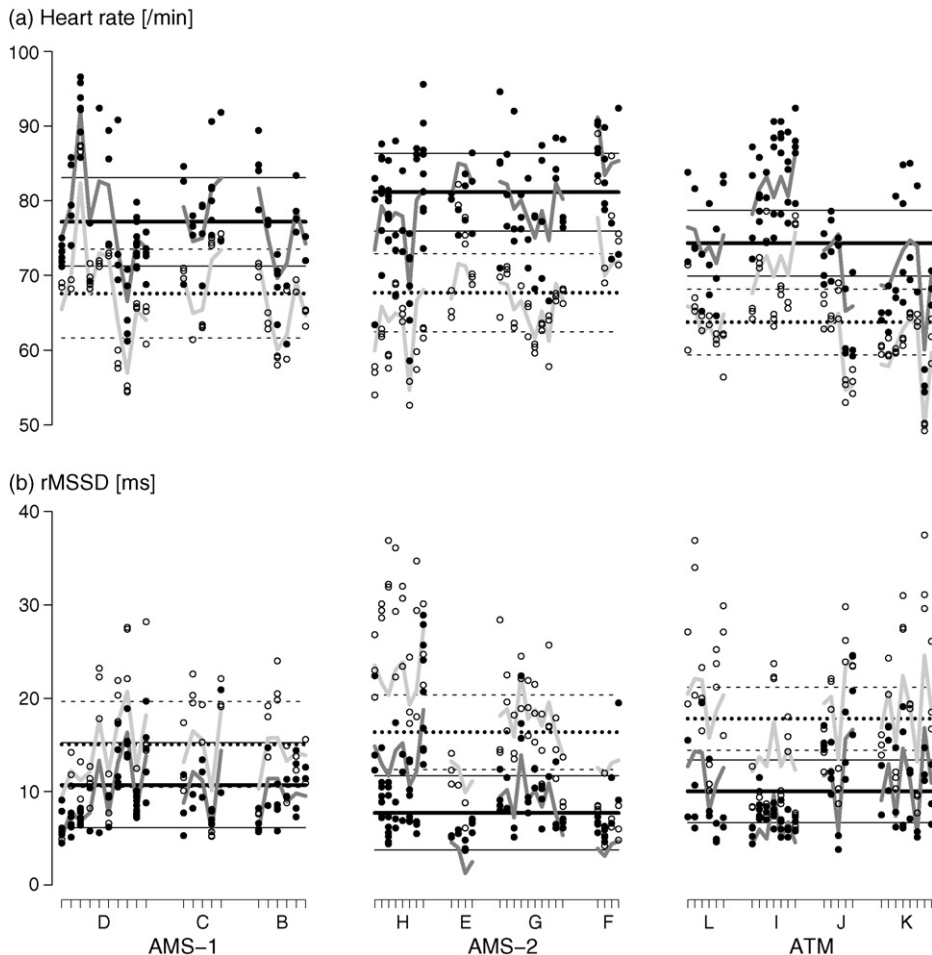


Fig. 2. Heart rate (a) and square root of the mean-squared differences of successive beat-to-beat intervals (rMSSD) (b) during single milking (●) and resting (○) bouts of individual cows (tick marks) on 11 farms (B–L) with either one of two types of automatic milking systems (AMS-1, AMS-2) or auto-tandem milking parlours (ATM). Sorting of farms and cows as in Fig. 1. Grey curves: estimates for individual cows during milking (dark grey) and at rest (light grey); black horizontal lines: model estimate (thick line) and model 95% confidence interval (thin line) for the three milking systems during milking (solid lines) and resting (dashed lines).

4. Discussion

Cows in AMS-2 (DeLaval) exhibited more restlessness than in ATM, showing a higher overall stepping rate, a higher stepping rate in both phases of the milking, a greater likelihood of foot-lifting, and a tendency towards some kicking. In addition, they had a higher HR during both resting and milking bouts, and lower rMSSD values during milking. These results suggest that cows on farms with an AMS-2 were subjected to an increased level of stress. There were also several – though fewer – indications in our study of higher stress in AMS-1 (Lely) than in ATM: a slightly higher likelihood of foot-lifting, higher HR during resting and milking, and lower rMSSD values during resting.

The comparison of our results with those of previous studies is complicated by the fact that the latter were restricted to one of the products (AMS-1, Lely). Contrary to the manufacturer's recommendations, however, partially forced cow traffic (Hagen et al., 2004, 2005) or forced cow traffic (Hopster et al., 2002) was used in three of the studies. (Free cow traffic was only used by Wenzel et al., 2003.) Behavioural reactions during milking are most probably a direct consequence of the milking system, so our results for AMS-1 should be comparable with those of the previous studies. The reactions recorded by us seemed to lie between the changes that were observed by Wenzel et al. (2003) and an absence of differences (Hopster et al., 2002). Hagen et al. (2004) investigated the behaviour of cows in both an AMS-1 and a herringbone parlour, and reported even stronger reactions in the latter milking system. The grouping, and in some cases, the combining of the different restlessness behaviours (stepping, foot-lifting, kicking) was, however, not the same in all these studies, making precise quantitative comparisons difficult. Nevertheless, as seen in the current data, the differences between milking systems were very similar for the different behavioural variables. Thus, even if the absolute number of incidents of restlessness behaviour may not be fully comparable among the studies, the relative changes between the different types of milking systems should not be strongly influenced by the grouping of behavioural patterns.

In both AMS-types investigated in the present study, the cows had higher HR during resting as well as during milking than in ATM. It would therefore seem that milking and resting bouts were less relaxed on farms with an AMS. This corresponds to the results of Wenzel et al. (2003), who observed higher HR during milking in an AMS than in an ATM, while contradicting Hopster et al. (2002), who consistently measured lower HR in an AMS. Hagen et al. (2005) found no differences in HR between a herringbone parlour and an AMS-1. The increase in stress both during resting and milking bouts could be associated with the more asynchronous activity of the cows on farms with an AMS, who are freer to choose their activities than cows on farms with an ATM, and are not synchronised by regular milking twice a day. During milking, the presence of a milker in ATM may also exert a relaxing influence (Rushen et al., 2001). As found in other studies (e.g. Lupoli et al., 2001), the milking process seems to be stressful in itself. In the present study, this was indicated by an increase in stepping rate, a greater likelihood of foot-lifting and kicking during milking than during the preparation phase in both AMS and ATM, and in clearly elevated HR and decreased rMSSD values during milking as compared to resting.

It seems that milking in AMS-1 was less stressful than in AMS-2. Possibly, the robot arm that rested at the udder throughout the milking process in AMS-1 was less disturbing to the cows than the moving robot arm in AMS-2. Given that the lowest values in rMSSD during resting were observed in AMS-1, the stressful influences on farms with an AMS-1 might not have been a direct consequence of the milking process itself, but rather a more indirect consequence of the housing system. This parallels the fact that – on the same farms as visited in the present study – cows milked in AMS-1 exhibited a disrupted daily periodicity in milk cortisol compared to the periodicity found in cows milked in AMS-2, a difference that might be attributed to different cow traffic strategies in the two AMS (Gygax et al., 2006). Why free cow traffic (AMS-1) should lead to less-relaxing resting bouts than guided cow traffic with selection gates (AMS-2) currently remains unknown, however.

We found that cows in higher parities and with higher levels of SCC were seemingly more prone to being unsettled by the milking situation judged by their higher stepping rates. The lower values in rMSSD with higher daily milk yield could have resulted from a higher basal energy requirement owing to the milk yield and the corresponding higher number of visits to the milking unit.

Restlessness behaviour, HR and HRV have all been previously used as indicators of stress in dairy cattle. We detected consistent patterns in these variables in the comparison among the milking systems. All variables provided information on the cows' direct reactions towards the milking process. HR and rMSSD additionally provided a measure at rest which potentially reflected the consequences of the housing/milking system as a whole and thus allowed to differentiate between the systems in more detail. The results on behaviour and HR/HRV corresponded well with other observations, in that teat-cup attachment success was lower and the cleaning/teat-cup attachment phase longest in AMS-2 (unpublished data) which may have caused stress during milking. On the other hand, milk cortisol data corroborated the view that the housing system may be a source of stress in AMS-1 (Gygax et al., 2006). The indicators used in this study all seemed to be meaningful, but it may be advisable for future studies to concentrate on variables that incorporate aspects of the housing system in general such as HR and rMSSD.

In our study, we wanted to investigate well-functioning AMS and thus restricted our analyses to the cows well adapted to the system and their successful milkings. Thus, our conclusions are restricted to such animals and situations, and the level of stress may be worse for cows that are not well adapted to being milked automatically and during failed milkings. Though many of the differences observed in the present study between the two types of AMS and ATM were still statistically detectable, their relevance for the animals included in the sample can be viewed as relatively minor. The largest absolute increases in mean stepping rate were of the order of 0.5 steps/min (Fig. 1a). Since leg movements of this type seem to have been used as a behavioural indicator of stress in cows in the context of milking only, there are no comparative data for other stressful situations. The largest absolute difference between the milking systems in terms of foot-lifting during milking bouts was 40% (Fig. 1b). Milking bouts, however, were considered to be 'with foot-lifting' if a single instance of foot-lifting occurred during milking. In about 75% of the milkings with foot-lifting, the frequency of foot-lifting was lower than 0.5 min^{-1} , resulting on average in 3.5 instances of foot-lifting for a typical milking bout lasting around 7 min (90% of the milkings with foot-lifting had frequencies $<1/\text{min}$). Kicking was so rare that a statistical evaluation of the milkings with or without kicking was not possible. Given their relatively minor nature (stepping and low-frequency foot-lifting), behavioural reactions during milking are unlikely to indicate a relevant impairment of the welfare of dairy cows. Mean heart rate differed in our study by up to 5 beats/min between the milking systems, and by about 10 beats/min between resting and milking bouts (Fig. 2a). Similarly, Hopster et al. (2002) found differences of about 10 beats/min comparing pre-milking and milking values in AMS and ATM with about the same increase from cows being milked in the AMS to those being milked in the ATM. In addition, Cook and Jacobson (1996) found that bulls trained to be loaded onto a trailer had lower heart rates by about 10 beats/min at loading than untrained bulls, though Waiblinger et al. (2004) found an increase of only about 5 beats/min between handled and non-handled cows during sham-insemination. We therefore found differences in heart rate corresponding to changes normal for milking, and reflecting differences based on subtle training effects. It would appear that these differences need not be considered extreme. As HRV measurements have only recently been used for the assessment of stress in cattle (Hagen et al., 2005), there is little comparative data to help determine the importance of the changes observed.

In summary, we conclude that there is little evidence that the welfare of dairy cows is seriously impaired by being milked in an AMS. This assessment is supported by the fact that the variability between different individuals and assigned to the statistical error were of the same order of magnitude as the observed fixed effects (as seen in the random effects and Figs. 1 and 2). Thus,

the prediction of a specific cow's reaction based on milking system alone would be highly imprecise (cf. confidence intervals in Figs. 1 and 2). Though we statistically detected differences in behaviour, as well as HR and HRV indicating a slightly increased stress level in dairy cows milked in automatic milking systems as opposed to auto-tandem milking parlours, we believe that the absolute size of these differences is so minor that no serious impairment of the welfare of dairy cows milked by automatic milking systems can be postulated.

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References

- Cook, C.J., Jacobson, L.H., 1996. Heart rate as a measure of adaptation to stress in cattle. *Aust. Vet.* 74, 471–472.
- Després, G., Veissier, I., Boissy, A., 2002. Effect of autonomic blockers on heart period variability in calves: evaluation of the sympatho-vagal balance. *Physiol. Res.* 51, 347–353.
- Gygax, L., Neuffer, I., Kaufmann, C., Hauser, R., Wechsler, B., 2006. Milk cortisol concentration in automatic milking systems compared with auto-tandem milking parlors. *J. Dairy Sci.* 89, 3447–3454.
- Hagen, K., Langbein, J., Schmied, C., Lexer, D., Waiblinger, S., 2005. Heart rate variability in dairy cows—influences of breed and milking system. *Physiol. Behav.* 85, 195–204.
- Hagen, K., Lexer, D., Palme, R., Troxler, J., Waiblinger, S., 2004. Milking of Brown Swiss and Austrian Simmental cows in a herringbone parlour or an automatic milking unit. *Appl. Anim. Behav. Sci.* 88, 209–225.
- Hejtel, L., Roth, E., 2004. What is the adequate sampling interval of the ECG signal for heart rate variability analysis in the time domain? *Physiol. Meas.* 25, 1405–1411.
- Hopster, H., Blokhuis, H.J., 1994. Validation of a heart rate monitor for measuring a stress response in dairy cows. *Can. J. Anim. Sci.* 74, 465–474.
- Hopster, H., Bruckmaier, R.M., van der Werf, J.T.N., Korte, S.M., Macuhova, J., Korte-Bouws, G., van Reenen, C.G., 2002. Stress responses during milking; comparing conventional and automatic milking in primiparous dairy cows. *J. Dairy Sci.* 85, 3206–3216.
- Kleiger, R.E., Stein, P.K., Bosner, M.S., Rottman, J.N., 1992. Time domain measurements of heart rate variability. *Ambulatory Electrocardiogr.* 10, 487–498.
- Lupoli, B., Johansson, B., Uvnäs-Moberg, K., Svennersten-Sjaunja, K., 2001. Effect of suckling on release of oxytocin, prolactin, cortisol, gastrin, cholecystokinin, somatostatin and insulin in dairy cows and their calves. *J. Dairy Res.* 68, 175–187.
- Marchant-Forde, R.M., Marlin, D.J., Marchant-Forde, J.N., 2004. Validation of a cardiac monitor for measuring heart rate variability in adult female pigs: accuracy, artefacts and editing. *Physiol. Behav.* 80, 449–458.
- Moberg, G.P., 2000. Biological response to stress: implications for animal welfare. In: Moberg, G.P., Mench, J.A. (Eds.), *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare*. CABI Publishing, Wallingford, pp. 1–21.
- Pinheiro, J.C., Bates, D.M., 2000. *Mixed-Effects Models in S and S-Plus*. Springer, New York.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2005. *NLME: Linear and Nonlinear Mixed Effects Models*. R Package Version 3.1-60.
- R Development Core Team, 2005. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: <http://www.r-project.org/>.
- Rushen, J., Munksgaard, L., Marnet, P.G., de Passillé, A.M., 2001. Human contact and the effect of acute stress on cows at milking. *Appl. Anim. Behav. Sci.* 73, 1–14.

- Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Eur. Heart J.* 17, 354–381.
- Venables, W.N., Ripley, B.D., 2002. *Modern Applied Statistics with S*, 4th ed. Springer, New York.
- Waiblinger, S., Menke, C., Korff, J., Bucher, A., 2004. Previous handling and gentle interactions affect behaviour and heart rate of dairy cows during a veterinary procedure. *Appl. Anim. Behav. Sci.* 85, 31–42.
- Wenzel, C., Schönreiter-Fischer, S., Unshelm, J., 2003. Studies on step-kick behavior and stress of cows during milking in an automatic milking system. *Livest. Prod. Sci.* 83, 237–246.