Visual appearance and CMT score of foremilk of individual quarters in relation to cell count of cows milked automatically

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The objectives of the study were: to evaluate the interaction between visual appearance and California mastitis test (CMT) score of the foremilk in relation to the cell count of the milk; to evaluate the consequences of sorting milk according to these criteria; and to explore whether visual appearance and CMT score of foremilk depended on the time interval between milkings. Measuring somatic cell count (SCC) in composite milk only and discarding milk above certain thresholds will not ensure that milk from all cows with visually abnormal foremilk is withheld from delivery. Low thresholds of SCC will reduce the frequency of cows with abnormal milk but increase the discarding of milk from cows with visually normal foremilk. CMT score of foremilk differentiated better between cows with high and low SCC in composite milk than visual inspection of foremilk. CMT scores of foremilk decreased with increasing interval between milkings within cow, whereas the visual appearance was independent of the interval. We propose that visual appearance of the foremilk should be kept as a criterion for sorting milk at time of milking. For test purposes, the use of visual appearance of foremilk for differentiation between normal and abnormal milk has to be done on multiple milkings. Additionally, CMT scoring of foremilk improves correct classification of normal and abnormal quarters and especially when including data from the previous milking.

Keywords: Automatic milking, foremilk, somatic cell count, milking interval.

Consumers of milk and milk products are increasingly concerned about animal welfare and production methods. The introduction of automatic milking into dairy farming reduces the number of man-hours in the barn as several manual functions are replaced by this new technology, including the test for the normality of the milk at every milking. The current EU Hygiene Directive (89/362/EEC) requires that the milk be inspected before milking. Milk from cows with clinical mastitis must be withheld from delivery so that only healthy cows produce milk for consumption. Milk from mastitic cows may differ in colour and homogeneity and have high somatic cell counts (SCC). The gold standard of acceptable milk is based on visual inspection of foremilk. However, the control is based on the measurement of bulk milk SCC and consequently a monitoring and control system could be based on some form of visual inspection of the milk as well as a direct or indirect measurement of SCC. SCC is used as a measurement of inflammation and, as such, of abnormal milk. Milk

from healthy quarters is normally not affected by one quarter having clinical mastitis but this milk shall not be delivered for consumption according to the EU Hygiene Directive (89/362/EEC). Clearly, the fewer quarters producing abnormal milk, the more suitable the product becomes (Smith et al. 2001).

Cows without clots or blood in the foremilk normally yield milk for consumption. In relation to conventional milking, detection of clots in the foremilk depends on the skills of the milker and the practical conditions under which the foremilking is performed. Hillerton (2000) states that the sensitivity is 80% for detecting cows with clinical mastitis during foremilking, but the specificity is 100%. If clots are found in the milk at any time during milking, the quarter suffers from clinical mastitis. It becomes more difficult if the selection criterion is determined on the basis of SCC because SCC at the guarter or cow level may not always be the best determinant of the mastitis status of a quarter or cow. However, SCC is currently the only parameter that can be interpreted from quarter to bulk tank milk and is widely used for this purpose and as an indicator of the milk quality.

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Herd	AMU† n	Cows n	Cow milkings n	Normal appearance, %	CMT<4, %	Mean log SCC	Yield kg/milking	Interval h
1	4	149	667	81	70	5.16	9.0	10.3
2	2	129	571	87	67	5.26	11.3	11.0
3	2	121	517	89	76	5.07	13.1	11.1
4a	2	78	372	84	78	5.01	8·1	9.8
4b	3	114	678	92	82	4.92	7.2	8.3
Total		591	2835	87	74	5.09	9.6	10.1

Table 1. Numbers (*n*) of cows and basic statistics of milk yield and quality of the four herds used in the study

+ Automatic milking units

Classification of the inflammatory status of a quarter was earlier based on a threshold of 500 000 cells/ml, but recently Hillerton (1999) suggested that a threshold of 200 000 cells/ml would improve discrimination between infected and uninfected quarters. Based on the likelihood of infection and altered manufacturing properties, Smith et al. (2001) conclude that milk from quarters with SCC >200 000 cells/ml, with or without clinical signs, is abnormal milk.

Regulations for bulk tank milk SCC are not a matter of human safety but of suitability. The EU enforces a maximum bulk milk SCC of 400 000 cells/ml. This level is intended as an indirect control of the number of subclinically infected cows delivering milk for consumption. Eberhart et al. (1982) estimated that 13% of the cows were infected at this bulk milk SCC level. Rasmussen (2002) found that the bulk milk SCC was likely to be <400000 cells/ml if fewer than 5% of the cows had visually abnormal foremilk. However, the absence of cows with clinical signs did not ensure that bulk milk SCC was <200000 cells/ml. SCC is normally low in foremilk, drops slightly in the first part of milking, then increases during milking, and is highest in strippings at which time it gradually drops until the next milking (Smith & Schultze, 1967; Woolford et al. 1998; Hamann & Gyodi, 1999). Infected quarters may have higher SCC in foremilk than in strippings. Discrimination between infected and uninfected quarters based on SCC is normally best done on foremilk, and Smith & Schultze (1967) conclude that variation in time of sampling can introduce uncertainty in the interpretation of SCC. The interval between automatic milkings may vary from 6 to 18 h and may consequently influence the classification of cows and quarters based on SCC or California mastitis test (CMT) score. Large variations are normally seen in milking interval, milk yield, and milk composition of cows being milked automatically, and Friggens & Rasmussen (2001) found that SCC within cow decreased with increasing milking interval. The drop in SCC follows the drop seen in fat percentage and probably reflects dilution by newly produced milk.

The objectives of the study were: (1) to evaluate the interaction between visual appearance and CMT score of the foremilk; (2) to evaluate the effects of sorting milk based on visual inspection and CMT score of foremilk on the mean cell count of quarter and composite milk; (3) to

evaluate the effects of sorting milk based on SCC on the frequency of cows with abnormal foremilk; and (4) to explore whether visual appearance and CMT score of foremilk depend on the time interval between milkings.

Materials and Methods

Data collection

All guarters of 544 cows from four herds milked automatically were sampled at every milking within a 48-h period. Herds had from two to four automatic milking units (Table 1). Herd 4 was sampled twice, 47 cows appear in both periods. Bulk milk SCC of the four herds was between 250 000 and 350 000 cells/ml and at least 5% of the cows were expected to have clinical mastitis. Milk quality inspectors examined the foremilk visually in a strip cup with a black plate and then scored the milk by the CMT system on a 1-5 scale after stripping into a white CMT plate. Visual appearance of the foremilk was scored as 1: normal, 2: watery or containing small flakes, 3: clinical mastitis with clots, 4: straw yellow (colour different from other quarters), 6: containing blood, or 8: dry. After stripping into the CMT-plate, the plate was tipped so that about 2 ml of milk remained per quarter. An equal amount of reagent (Kruse, Denmark) was mixed with the milk and the CMT score was noted within one minute. Expected cell counts of the CMT scores were: (1) <150000, (2) 150000-300 000, (3) 300 000-800 000, (4) >800 000, and (5) $>3 \times 10^6$ cells/ml. The automatic milking systems (AMS) were put on hold (manual mode) for each individual cow milking until foremilk sampling had been completed and were then switched to automatic. Milk samples were taken from the mounted milk meters and composite milk of the four quarters was analysed for SCC with a Fossomatic 5000 (Foss Electric, Hillerød, Denmark). A total of 2835 cow milkings, 11043 visual inspections of foremilk and scores of CMT comprised the dataset. One automatic milking unit of herd 4 was equipped with sampling devices for quarter milk. Quarter milk yield was measured and samples analysed for SCC. This dataset included 1425 milk samples from 287 quarters of 77 cows. Time of milking, milk yield, and cow identity were transferred electronically from the AMS databases and merged with foremilk scores and SCC.

	CMT 1	CMT 2	CMT 3	CMT 4	CMT 5	Total
	п	п	п	п	п	п
Normal	7211(68)	1853(17)	795(8)	446(4)	285(3)	10590(100)
Blood	5(14)	7(19)	4(11)	10(27)	11(30)	137(100)
Flakes	20(13)	20(13)	25(16)	38(24)	55(35)	158(100)
Clots	11(5)	10(4)	10(4)	26(11)	173(75)	230(100)
Yellowish	1(4)	4(14)	1(4)	7(25)	15(54)	28(100)
Total	7248(66)	1894(17)	835(8)	527(5)	539(5)	11 043(100)

Table 2. Relationship between visual inspection and CMT score of foremilk, number (*n*) of samples and, in brackets, the percentage of the row

Based on all milkings in the 48-h period, 2066 quarters could be classified as normal, as there was no abnormal visual appearance, 94 quarters appeared abnormal in more than 50% of the samples; the remaining quarters had at least one abnormal visual appearance.

Statistical analysis

To evaluate the effects of the varying interval between milkings, the following analyses of visual appearance and CMT scores of foremilk were made. To obtain an estimate of the deviation from the cow's normal milking interval, the average milking interval of a cow was calculated, and the observed Milking Interval Deviation (MID) was calculated for each milking.

The visual appearance and CMT scores were modelled in terms of indicators where the score Y_{ijt} , of cow i, quarter j observed at occasion t, was abnormal, which in the case of CMT was defined as score 4 or 5. The logit of the probability of observing an abnormal quarter level sample was modelled as

 $logit(p_{iit}) = F - B_i + Herd_i + MID_t(Herd_i) + MID_t^2 + MID_t^3$

where p_{ijt} =Prob(Y_{ijt} ='Abnormal'), F-B_j was the indicator of a front/back quarter, Herd_i was the indicator of the herd, MID_t was milking interval deviation, hence entering the model in terms of a third order polynomial. The models were elaborated by including: $Y_{ijt'}$ indicating whether the quarter was abnormal at the previous milking, N_{it'} indicator of the number of abnormal quarters at the previous milking. In the model for visual appearance, the indicator of whether the current CMT score was abnormal was also used.

Observations from the same quarter were assumed to be correlated with constant correlation, being modelled by means of GEE, using the repeated statement of PROC GENMOD (SAS Institute v8.12).

The worst score observed, $MaxY_{it} = max_j(Y_{ijt})$, was analysed analogously.

Results and Discussion

Visual appearance and CMT score of foremilk

The appearance of the milk was normal in 87% of cow milkings and correspondingly abnormal in 13% (Table 1)

with a variation between the four herds from 8 to 19% of the cow milkings. This frequency was well above the expected frequency of 5%. Foremilk appeared abnormal in 4·1% of the quarters. CMT scores <4 appeared in 67 to 82% of the cow milkings and correspondingly high CMT scores were found in 18 to 33% of the cow milkings. Average milk yield per milking ranged from about 7 to 13 kg and the mean time interval between milkings from about 8 to 11 h. Variations within herds and between cows were large with milk yields ranging from 1·6 to 21·0 kg per milking and intervals from 3·5 to 22·9 h (1 and 99% fractiles). Likewise, SCC ranged from 10 000 to 7×10^6 cells/ml.

Three percent of the quarters with visually normal foremilk had a CMT score of 5 (Table 2) whereas 53% with a CMT score of 5 appeared normal. Ten percent of the quarters with clots in the foremilk scored a CMT of 1 or 2. Foremilk samples containing blood were almost evenly distributed among CMT scores but within CMT score the frequency of blood increased from 0.07% of CMT scores 1 to 2% of CMT scores 5. Samples with blood were not included in the calculations of sensitivity and specificity. The sensitivity of the quarters with abnormal milk was 77% for a CMT score of 4-5 and the specificity was 93% (quarters with normal milk that had CMT score 1–3). The use of CMT score as the true state and for classifying normal and abnormal milk were quite different and resulted in a sensitivity of 30% and a specificity of 99% (quarters with low CMT scores on foremilk probably do not show visually abnormal milk). This is a very high specificity but it emphasizes that quarters without inflammation (low CMT score) are likely to have milk of normal appearance. Consequently, if the purpose of the test on foremilk is to identify quarters with clinical mastitis, the appearance of the milk has to be checked. If, however, the purpose is to avoid quarters with abnormal foremilk, CMT scoring does a reasonable job.

Visual appearance of foremilk and cell count in composite milk

Cows where all quarters had normal milk had low cell counts in the composite milk and only 3.6% of the samples contained $>10^6$ cells/ml (Table 3). Cows that had at least one quarter with clots in the foremilk had a geometric

Table 3. Log SCC values in composite milk and the percentage of samples $>200\,000$, $>400\,000$, and $>10^6$ cells/ml by visual appearance of foremilk of the worst quarter

CCC colle/m

		SCC, Cens/III					
Worst quarter	n	Log	>200 000	>400 000	>10 ⁶		
Normal	2450	4.99	23.8	11.8	3.6		
Blood	25	5.41	54.2	29.2	8.3		
Flakes	126	5.36	52.5	30.0	11.7		
Clots	220	6.01	82.3	73.0	53.7		

mean SCC of about 10^6 cells/ml and 53.7% were $>10^6$ cells/ml. Even when all quarters had visually normal foremilk, 11.8% of the composite milk samples exceeded 400 000 cells/ml, but not all samples (73%) were above this threshold if one or more quarters had clots in the foremilk. Cows with blood or flakes in the foremilk had intermediate cell counts in composite milk.

AMS are able to retain information about previous milkings and sorting of milk could be dependent on historical information. SCC was slightly lower if two consecutive milkings had visually normal foremilk compared with scoring at a single milking and it also resulted in fewer samples with SCC >10⁶ cells/ml (2·7 v. 3·6% for single milkings). Cows for which foremilk appeared abnormal at both the previous and present milking had high SCC whereas lower values were found when only one of the foremilkings appeared abnormal.

Hillerton (1999) suggested that bulk milk quality is acceptable if <10% of the cows have SCC between 200000 and 400000 cells/ml and there are none above this level. Generally, this is not achievable just by discarding milk from cows with visually abnormal foremilk even if consecutive milkings are taken into account. It is necessary to perform tests on foremilk more directly associated with SCC in composite milk (such as CMT scoring) if milk from cows with SCC >400000 cells/ml is undesirable.

CMT score of foremilk and cell count in composite milk

CMT differentiated better between cows with high and low SCC than visual inspection of foremilk. Only 4 (0·23 %) of the cow milkings with CMT scores <3 in the foremilk had SCC >10⁶ cells/ml in the composite milk (Table 4), and 29 (1·7%) were higher than 400 000 cells/ml. Cows with at least one quarter with a CMT score of 5 had approximately a mean SCC of 10⁶ cells/ml and 45·5% of the composite milk samples exceeded 10⁶ cells/ml. Cows with at least one quarter with a CMT score of 5 were most likely to have a SCC above 200 000 cells/ml and be mastitic according to the definition of Hillerton (1999). However, only 43% of the cows with a CMT score 5.

Milk from cows with consecutively CMT scores of 1 to 3 on foremilk only exceeded SCC >10⁶ cells/ml in 0.1% of cases. Cows that increased in CMT score from one

Table 4. Log SCC in composite milk and the percentage of samples $>200\,000$, $>400\,000$, and $>10^6$ cells/ml by CMT score of foremilk (highest quarter)

			SCC, cells/ml					
Highest quarter	п	Log	>200000	>400 000	>10 ⁶			
CMT 1	997	4.65	4.4	1.6	0.3			
CMT 2	700	4.95	11.4	2.0	0.1			
CMT 3	409	5.23	39.4	14.9	2.1			
CMT 4	311	5.47	65.2	34.1	7.5			
CMT 5	412	5.96	88·1	72.9	45.5			

Table 5. Log SCC in quarter milk and the percentage of quarters $>200\,000$, $>400\,000$, and $>10^6$ cells/ml by visual appearance of foremilk

		SCC, cells/ml						
Appearance of foremilk	n	Log	>200 000	>400 000	>10 ⁶			
Normal	1362	4.8	15	9	4			
Watery and flakes	22	6.1	86	77	59			
Clots	40	6.8	93	93	88			

milking to the next had a higher SCC (log 5·47) than those remaining on a low CMT score (log 4·79) but not as high as when the last milking had high CMT scores as well (log 5·83). The sensitivity was 83% of two consecutive high CMT scorings to identify cows with SCC >200000 cells/ ml. The specificity was 91% of two consecutive low CMT scorings to identify cows with SCC <200000 cells/ml.

Visual appearance and CMT score of foremilk and cell count on quarter milk

Four percent of the quarters with milk appearing normal had SCC $> 10^6$ cells per ml, which was mainly in newly calved cows. Out of the guarters with clots in the foremilk, 88% had SCC $>10^6$ cells per ml in the milk produced specifically from that quarter (Table 5). According to Smith et al. (2001), discarding the milk from quarters with SCC >200 000 cells/ml would dump the milk of 204 quarters (15% of 1362 quarters, Table 5) with visually normal foremilk and this number should be compared with 40 being visually abnormal. Such a low SCC threshold on the quarter basis will ensure that most abnormal milk is withheld from delivery but will also cause large amounts of milk to be dumped. The CMT score of foremilk correlated well with the cell count of milk from the quarter. About 0.7% only of the quarters with CMT 1 or 2 on foremilk had SCC $> 10^6$ cells/ml and 91% out of the guarters with CMT score 5 on foremilk had guarter SCC above this value. All guarters with CMT score 5 had SCC >200 000 cells/ml. However, only 11% of the quarters with a SCC >200 000 cells/ml had a CMT score 5 in foremilk and this SCC threshold is not able to distinguish clearly between quarters with high or low CMT score of the foremilk. In the

Table 6. Log SCC in composite milk and the percentage of samples $>200\,000$, $>400\,000$, and $>10^6$ cells/ml by deviation in milking interval from cow average and visual appearance of the worst quarter

Visual appearance of foremilk, worst quarter

			Normal				Abnormal			
	SCC, cells/ml						SCC, cells/ml			
Deviation, h	п	Log	>200 000	>400 000	>10 ⁶	п	Log	>200 000	>400 000	>10 ⁶
<-3	221	5.22	38.0	22.0	8.3	52	5.89	84.8	65.2	37.0
-31	595	5.02	24.7	13.5	3.5	87	5.86	75.0	61.3	42.5
-1-1	943	4.95	22.2	10.1	3.2	119	5.67	66.1	53·0	33.0
1–3	427	4.94	20.2	10.5	3.4	70	5.72	66.7	48.5	36.4
3<	272	4.94	21.3	7.9	1.9	43	5.62	60.0	47.5	30.0

very early stage of lactation, calculation of sensitivity and specificity is very dependent on SCC threshold of foremilk for identifying infected quarters since the SCC drops rapidly during these first days (Sargeant et al. 2001). Clearly, single thresholds of SCC in quarter or composite milk will not be sufficient to identify cows and quarters with abnormal milk.

Influence of hours since last milking on visual appearance and CMT score of foremilk and SCC in composite milk

The distribution of visually normal foremilk samples showed no clear relationship to the milking interval or the milk yield (data not shown) when data were not adjusted for variations within cow. Likewise, with no adjustment for cow, the frequency of CMT scores 1-3 seemed to be independent of milking interval but the frequency increased with increasing milk yield (data not shown). Looking at variations within cow (milking interval deviation), the geometric mean SCC dropped from a level of about 200 000 cells/ml to about 100 000 cells/ml when the milking interval deviation increased from being 3 h shorter than average to being average or longer. For cows with visually normal foremilk, 8.3% of the cows with a milking interval deviation at least 3 h shorter than the average had $SCC > 10^6$ cells/ml (Table 6). This percentage dropped to 1.9% when the milking interval deviation was at least 3 h longer for the cow. Cows with at least one quarter with abnormal foremilk did not show the same tendency where about one third of the cows had a SCC $>10^6$ cells/ml irrespective of the milking interval deviation.

The frequency of the cow milkings with abnormal foremilk and a SCC<200000 cells/ml increased from about 5 to 7% from the very short to the very long milking interval deviations. For cow milkings with SCC >200000 cells/ml, about 30% had abnormal foremilk irrespective of the interval. Calculation of sensitivity and specificity depended on the milking interval deviation when using SCC of 200000 cells/ml as the threshold for finding cows with normal or abnormal foremilk. Sensitivity decreased from

85% to 60% and specificity increased from 62% (100-38) to 79% (100-21) going from a milking interval deviation of at least 3 h shorter to at least 3 h longer than average for the cow. There was no clear influence of the milking interval deviation and SCC threshold of 400 000 cells/ml on the frequency of cow milkings with abnormal foremilk (although the percentage of SCC >400000 cells/ml depended on interval and appearance of the milk; Table 6). Out of 210 cow milkings with SCC $>10^6$ cells/ml, the frequency of cows with abnormal foremilk increased from 50% to 71% going from very short to long milking interval deviations. Consequently, cows with a longer milking interval deviation and SCC $> 10^6$ cells/ml are more likely to have abnormal foremilk. About 9% (222) of the cow milkings with SCC $<10^6$ cells/ml had at least one quarter with visually abnormal foremilk and accounted for considerably more milkings than cows with abnormal foremilk having SCC $>10^6$ cells/ml (125). In conclusion, SCC cannot be used accurately to discard milk from cows with visually abnormal foremilk and the outcome depends on the time interval between milkings.

The frequency of high cell counts decreased with increasing milking interval deviation of cows with a CMT score <4 of all quarters. For the cow milkings with at least one quarter with a CMT score of 4 or 5, the percentage of SCC >400 000 cells/ml dropped from 60% to 50% with increasing milking interval deviation, but the frequency of cell counts $>10^6$ cells/ml remained about 30%. As for visual appearance, the calculation of sensitivity and specificity depended on the milking interval deviation when using a SCC of 200 000 cells/ml as the threshold for finding cows with a low or high CMT score (CMT >3) of at least one quarter. Sensitivity decreased from 84% to 66% and specificity increased from 78% to 94% when going from a milking interval deviation at least 3 h shorter to at least 3 h longer than average for the cow. These sensitivities and specificities were generally higher than for detection of abnormal foremilk, which is not surprising since CMT scoring and SCC are related measurements.



Milking interval deviation (h)

2 3

5 6

Probability models of the relationship between milking interval deviation, visual appearance and CMT scores

The probability of observing a quarter with abnormal foremilk depended on the visual appearance at the previous milking and the present visual appearance of the other quarters but not on milking interval deviation (Fig. 1). The probability of observing abnormal foremilk of a quarter was 2-3% if the foremilk appeared normal at the previous milking. This probability increased to 18-24% if the foremilk from the guarter appeared abnormal at the previous milking (P < 0.001). Knowledge of the appearance of the foremilk from other quarters added information to the probabilities. The probability increased to 40-52% if both the quarter in question and additional quarters had abnormal foremilk at the previous milking but dropped to 15-21% if other quarters appeared normal (P< 0.01). The probability of observing a milking with at least one quarter with abnormal foremilk at cow level depended on the status of the cow at the previous milking but was independent of the milking interval deviation. Use of CMT scores at the actual milking added to the differentiation of the probability of observing a quarter with abnormal foremilk. The highest probability (60-67%) was seen if the previous milking had abnormal foremilk and the present milking a CMT score >3 (Fig. 2). Having abnormal foremilk at the previous milking but a low CMT score lowered the probability to 15-21% (P<0.001). The probability of observing abnormal foremilk was about 1% of quarters with a CMT score <4 at the present milking. This probability dropped to 0.8% if the quarter had normal foremilk at the previous milking, which may reflect the incidence rate of clinical mastitis in these herds. The probabilities of observing abnormal foremilk were independent of milking interval deviations. The slightly lower probability of observing abnormal foremilk of cows with high CMT scores and short milking interval



Fig. 2. Probability of observing foremilk with abnormal visual appearance depending on deviation from cow average milking interval, previous visual appearance, and additional information on the present CMT score: (\blacklozenge), CMT >3; (\blacksquare), CMT <4; (\blacktriangle), CMT >3 and abnormal previous; (×), CMT <4 and abnormal previous.



Fig. 3. Probability of observing foremilk (worst quarter) with abnormal visual appearance depending on deviation from cow average milking interval and additional information on the actual CMT score (worst quarter) and previous visual appearance: (\blacklozenge), CMT >3 (worst quarter) and at least one abnormal previous; (\blacksquare), CMT <4 (worst quarter) and all normal previous; (\blacktriangle), CMT >3 (worst quarter).

deviations (Fig. 2) is due to overestimation of high CMT scores.

The probability of observing a cow with abnormal foremilk varied from 3% to 7% if the visual appearance of all quarters was normal at the previous milking and the CMT score of the worst quarter was <4 (Fig. 3). If one or more quarters had a CMT score >3, then the probability increased to 31-50% (*P*<0.001) and further to 48-72% (*P*<0.001) if one or more quarters had abnormal foremilk at the previous milking. The increase in probability of observing abnormal foremilk for long milking interval deviations is due to an underestimation of the true CMT score of the quarters, but the probability was independent of hours between milkings (*P*>0.20).

The probability of observing high CMT scores tended (P<0.1) to depend on the milking interval deviation and

60.0%

50.0%

40.0%

30.0%

20.0%

10.0%

0.0%

-5 -4 -3 -2 -1 0

Probability of abnormal visual appearance



Fig. 4. Probability of observing foremilk with a CMT score >3 depending on deviation from cow average milking interval and possible additional information on CMT score at the previous milking: (\blacklozenge), CMT <4 previous; (\blacksquare), CMT <3 previous; (\blacktriangle), no additional information; (*), all quarters CMT >3 previous.

especially if the last CMT score was high as well. The probability of observing a high CMT score of quarters having a high CMT score at the previous milking dropped from 53% to 10% going from short to long milking interval deviations (Fig. 4). Knowing that another quarter also had a high CMT score did not change the probability. The probability of observing a cow with a high CMT score decreased (P < 0.05) with increasing milking interval deviations. The probability of finding a high CMT score was highest if the CMT score was high at the previous milking as well (P < 0.001). The dependence of CMT score milking interval deviations makes it more complicated to use this tool to predict the probability of finding cows and quarters with abnormal milk. Likewise, low cut-off values of CMT scores are needed for identifying infected quarters and high cut-off values are needed for declaring a guarter free of infections in the first days after calving (Sargeant et al. 2001).

Sensitivity and specificity

The sensitivity of detecting a cow with abnormal foremilk from the highest CMT score depended on milking interval deviations (Fig. 5). The sensitivity dropped from 71% to 43% (average 58%) going from an interval at least 3 h shorter to at least 3 h longer than average. Parallel to this decrease, specificity increased from 78% to 93% (average 88%). Multiple scorings of visual appearance will help in classifying a cow as having normal or abnormal foremilk. The sensitivity of using CMT scores as a tool to detect cows with abnormal foremilk in multiple milkings was considerably higher than for one milking only (Fig. 5) and ranged from 76% to 93% (average 84%). So, if a cow really has clinical mastitis, its CMT score will be raised and will predict better. The sensitivity seemed to decrease with increasing milking interval deviations reflecting a dilution of the cells in the foremilk with the longer interval



Fig. 5. Sensitivity (SE1 and SE2) and specificity (SP) at quarter level for single and multiple milkings. True state: Free: No abnormal visual appearance, Infected: At least one abnormal visual appearance (SE1); More than 50% abnormal visual appearance (SE2). Test state: Free: CMT<4, Infected: CMT>3. (\blacklozenge), SE1; (\blacksquare), SE2; (\blacktriangle), SP2.

 Table 7. Number of cows detected as having abnormal milk

 depending on the sensitivity and specificity of the system

	Sensitivity/specificity						
Abnormal milk True status	90/99	80/99	90/90	80/80			
10/100 cows 1/100 cows	$\begin{array}{c} 10 \ (9{+}1) \\ 2 \ (1{+}1) \end{array}$	9 $(8+1)$ 2 $(1+1)$	18 (9+9) 11 (1+10)	26 (8+18) 21 (1+20)			

and higher milk yield. On average, CMT score and SCC are highest when milking interval deviation is short and finding a low CMT score or SCC a few hours after the last milking means that the cow or quarter in question is probably not infected. This information can be used to improve specificity. Concerning the long milking intervals where CMT score and SCC on average is low, high numbers will indicate a high probability that the cow or quarter is infected. Such information can improve the sensitivity. The finding of high CMT scores or SCC values with short milking interval deviations has to be used more carefully but aids to the diagnostic value if there are big differences within cow.

The sensitivity of detecting quarters with multiple milkings with abnormal foremilk using CMT score >3 ranged from 86% to 95% (average 88%) with no clear relation to milking interval deviation. The specificity of appointing quarters with normal milk using CMT score <4 increased steadily from 90% to 97% (average 95%) by increasing the milking interval deviation from <-3 to >+3 h. The confidence intervals for specificities were reasonably short reflecting a much higher proportion of normal than abnormal quarters.

A correct sorting of milk does not only make heavy demands on the detection system, but requires that the true status of normal or abnormal is precise. A sensitivity of 90% means that out of 10 cows with abnormal milk, 9 are identified. A combination with a specificity of 99% means that one out of 90 normal cows are identified as false positive, and in total 10 out of the 100 cows will be identified (Table 7). For a herd with one cow with abnormal milk out of 100 cows, a sensitivity of 80% or 90% and a specificity of 99% will identify two cows. Any reduction in the specificity will have a major effect on number of false positives, which becomes a factor 20 to the one having truly abnormal milk. Consequently, the sensitivity is the most important factor in a herd with a high prevalence of clinical mastitis and specificity is the most important factor in herds with a low prevalence. Although related to the visual appearance of foremilk, SCC and CMT score do not detect abnormal foremilk with a high enough precision, and it seems that the detection system should be more closely related to the property of clots in the milk. However, classification of milk from cows and quarters into a true state of either normal and abnormal can be supported by including CMT scores in the diagnosis since they may not show clinical signs at every milking. If the purpose of the classification is to test alarm systems of AMS, then the use of both visual appearance and CMT score of foremilk will differentiate better than using only one of them.

In conclusion, measurement of SCC in composite milk only and discarding milk above certain thresholds will not ensure that the milk from all cows with visually abnormal foremilk is withheld from delivery. Low thresholds of SCC will reduce the frequency of cows with abnormal milk but increase the discarding of milk from cows with visually normal foremilk. CMT scores of foremilk differentiated better between cows with high and low SCC in composite milk than visual inspection of foremilk. CMT scores of foremilk decreased with increasing milking interval deviation whereas the visual appearance was independent of the interval. We propose that visual appearance of the foremilk should be kept as a criterion for sorting milk at time of milking. The appearance of foremilk is the gold standard for sorting of milk. But evaluation and testing of the ability of AMS to detect and sort milk must be done with respect to a clear classification into normal and abnormal milk. For test purposes, the use of visual appearance of foremilk for differentiation between normal and abnormal milk has to be done on multiple milkings.

Additionally, CMT scoring of foremilk improves correct classification of normal and abnormal quarters and especially when including data from the previous milking.

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