

# Influence of intramammary infection of a single gland in dairy cows on the cow's milk quality

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Intramammary infection (IMI), comprises a group of costly diseases affecting dairy animals worldwide. Many dairy parlours are equipped with on-line computerised data acquisition systems designed to detect IMI. However, the data collected is related to the cow level, therefore the contribution of infected glands to the recorded parameters may be over estimated. The present study aimed at evaluating the influence of single gland IMI by different bacteria species on the cow's overall milk quality. A total of 130 cows were tested 239 times; 79 cows were tested once and the others were examined 2–8 times. All of the analysed data refer to the number of tests performed, taking into account the repeated testing of the same cows. Of the cows tested ~50% were free of infection in all 4 glands and the others were infected in one gland with different coagulase negative staphylococci (CNS), *Streptococcus dysgalactiae*, or were post infected with *Escherichia coli* (PIEc), i.e., free of bacterial infection at the time of sampling but 1–2 months after clinical infection by *E. coli*. Overall, infection with bacteria had significant effects on somatic cell count (SCC) and lactose concentration. Examining each bacterium reveals that the major influence on those parameters was the sharp decrease in lactose in the PIEc and curd firmness in PIEc and *Strep.* Individual gland milk production decreased ~20% in *Strep. dysgalactiae*- and ~50% in PIEc-infected glands with respect to glands with no bacterial findings. Significant differences were found in lactose, SCC, rennet clotting time and curd firmness in the milk of infected glands and among those, these parameters were significantly higher in *Strep. dysgalactiae* and PIEc than in CNS infected cows. The current results using quarter-milking reinforces the importance of accurate IMI detection in relation to economic and welfare factors, and moreover, emphasises the need for technical sensing and constant reporting to the farmer about changes in the milk quality of every animal.

**Keywords:** Mammary gland, cow milk quality, intramammary infection, automatic sensing.

Intramammary infection (IMI) in dairy cows still comprises one of the major costly diseases of dairy animals worldwide. In most of the herds IMI exists in its clinical form in ~1–2% of the animals at any given time, while subclinical mastitis caused by coagulase negative staphylococci (CNS) at the udder quarter level may vary between 6–55% (Djabri et al. 2002; Thorberg et al. 2009; De Vliegher et al. 2012). The direct costs of treating clinical mastitis and the decrease in milk quantity and quality due to both types of infection vary among countries and among the bacteria species. In general, it is higher in countries with higher animal value and where milk quality payment schemes prevail. A decision to treat a cow or to ignore the infection is not simple: antibiotic treatment of cows that are not at risk needs to

be justified with respect to cost of medicine and milk loss (Steenefeld et al. 2007; Barlow et al. 2009; van den Borne et al. 2010a, b) as well as with possible health hazard to consumers. Comprehending the economics of bacterial infection is essential. In the presence of 4 anatomically and physiologically separated milk glands, understanding the effect of infection in one gland on the total milk production of a cow could help in making treatment decisions like drying off the gland or ignoring it, particularly in animals which are not at risk.

At present, many dairy parlours are equipped with on-line computerised data acquisition systems. Among them, the software AfiFarm Herd Management includes the AfiLab™ milk analyser (afimilk, Afikim, Israel), which provides on-line data on gross milk composition (fat, protein, lactose and coagulation properties) and/or the AfiFree milk meter, which measures milk flow, milk quantity and milk conductivity (a common indication of

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mastitis). However, the data collected is related to the cow level and therefore the contribution of infected glands to the recorded parameters are over estimated in many cases. For instance, low milk production in the infected or post-infected glands with extremely high somatic cell count (SCC), which result in overall high SCC on the cow level, leads to withdrawal of that cow's milk from the bulk tank. In a recent study, it was found that a single infected gland (quarter) is sufficient to change and impair the whole cow's milk recorded parameters (Blum et al. 2014). Moreover, it was noted that the day-to-day variations in the level of milk constituents of healthy udder-quarters of a cow were similar and/or smaller than that of the infected ones (Forsbäck et al. 2010).

An outline procedure has been suggested lately as a management tool to cope with subclinical mastitis during the lactation utilising on-line computerised data (Leitner et al. 2012a). The underlying idea was to exploit the on-line computerised data available in modern farms, particularly those related to milk yield and information related to SCC, in combination with the data yielded by the routine monthly testing of individual cows' milk in order to cope with each udder and gland.

The variation in bacteria species involved in mastitis and the different response by the host in the context of clinical symptoms, milk yield and quality calls for continuous attention to management. Separation of milk of infected glands from the bulk milk, treatment of infected glands and identification of animals at risk are essential in achieving mastitis control. Infection which results in inflammation, as exhibited by an increase in SCC, is considered today as the major factor associated with milk quality at the individual gland and the bulk milk tank level. However, regardless of SCC on the individual gland, different pathogens induce damaging modifications to the milk proteins during infection (Merin et al. 2008; Fleminger et al. 2011, 2013). Moreover, the variability in quality of the bulk milk minimises the overall SCC in the bulk milk tank to <300 000 cell/ml. The questions that arise from this data are: Disregarding the bulk milk SCC, which gland's milk should not be allowed to enter the bulk milk tank? What is the cut-off level for bulk milk SCC that indicates a decrease in milk quality that should be considered in a payment scheme?

The objective of the present study was to evaluate the influence of IMI in one gland by different bacteria species on the cow's milk quality, using on-line acquisition of data relating to milk yield and composition at a single gland level.

## Materials and methods

### Animals

A commercial dairy herd of ~350 Israeli Holstein cows producing >11 000 l during 305 d were the source for the study. The cows were milked thrice daily (4:00, 11:00 and 19:00) and were fed a typical Israeli total mixed

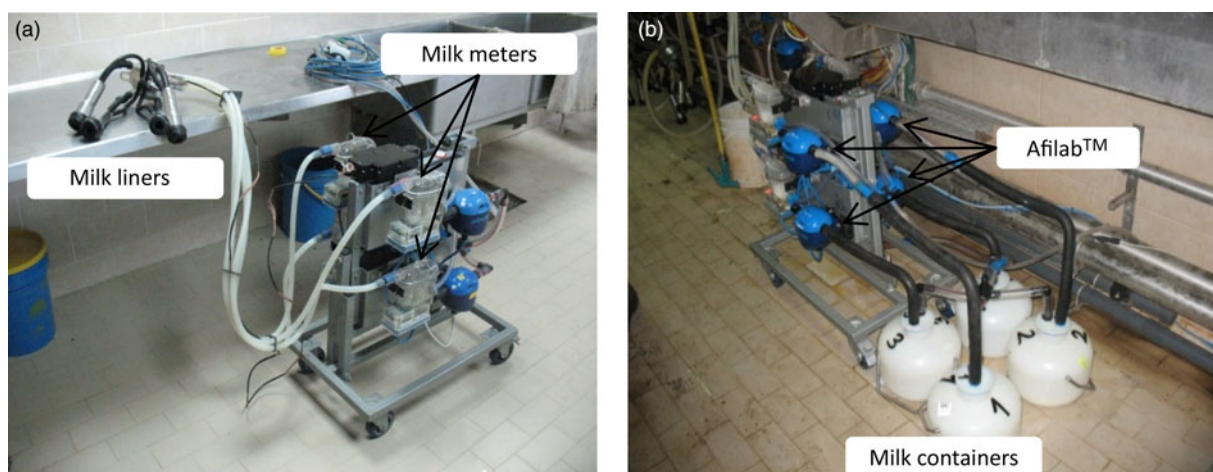
ration (TMR) containing 65% concentrate and 35% forage (17% protein). Food was offered *ad lib* in mangers located in the sheds. The milking parlour was a double-sided parallel equipped with afimilk on-line milking stations. Cow's data included: parity, days in milk (DIM), days in pregnancy (DIP), milk yield (l/d), fat, protein, lactose, SCC and history of health, which were all taken from the Israeli Herd Book records. Pregnancy was determined at day 45 post-insemination, therefore, average DIP and per cent of pregnant cows were calculated only from that day.

In order to test the cow's mammary health status, teats were cleaned before milking, and disinfected with non-woven towelettes moistened with chlorhexidine, cetrimide and ethanol (MediWipes, Albaad, Massuot Yitzhak, Israel). The first few squirts of milk were discarded and a 5 ml sample was then taken in a sterile tube for bacteriological testing, performed according to accepted standards (Oliver et al. 2004). Positive quarters were those from which the same bacteria species was isolated in milk at least twice in different days. Animals infected with coagulase negative staphylococci (CNS) and *Streptococcus dysgalactiae* (*Strep.*) were regarded to be subclinically infected. *Escherichia coli*-infected glands were those assessed about 1–2 months after showing clinical infection with isolation of *E. coli*, but with no bacteria isolation at the time of the study. The latter cows were designated as post infected with *E. coli* (PIEc). Cows of which all 4 glands were found free of infection and its SCC was <100 000 cels/ml were considered as with no bacterial finding (NBF).

### Study layout

Overall, 130 cows were tested 239 times; 79 cows were tested once and the others were examined 2–8 times. The layout chosen enabled us to test the differences between cows as well as to compare the same cow at different times. Therefore, all the analysed data refer to the number of tests performed (239 tests), taking into account repeated testing of the same cows.

An AfiLab™ units connected to a corresponding milk meter was installed in each of 4 different stations of the milking parlour. All quarters were simultaneously, but separately, milked through the afimilk quarter milkers, thus allowing on-line milk analysis at the gland level of milk constituents as well as coagulation properties. The milk was collected for reference sampling and quarter milk yield was established for each tested cow. Fig. 1a, b illustrates the gland (quarter) milkers. Each day during the period of the study (5 weeks), 8–12 cows that entered the milking parlour during the noon milking and randomly entered the station where the individual gland milkers were installed were milked by the apparatus. After milking, first, individual milk samples were taken from each quarter-milking container, thus representing the gland's milk and then, according to the gland's milk volume, an additional sample representing the cow's whole udder milk for the session was taken, resulting in 5 samples for each cow.



**Fig. 1.** (a, b) The afimilk experimental quarter milker apparatus equipped with 4 milk meters, 4 Afilab™ units and 4 milk containers.

Milk samples were tested for SCC by Fossomatic 360 and for milk composition by Milkoscan 6000 (Foss Electric, Hillerød, Denmark), coagulation properties, i.e., curd firmness (CF) and rennet clotting time (RCT), by Optigraph (Ysebaert, Frepillon, France) and Afi parameters by the Afilab™ (afimilk, Afikim): Afi-fat, Afi-protein, Afi-lactose, Afi-RCT and Afi-CF.

The influence of the infected gland with the different bacteria on the cow's milk SCC and CF was calculated according to Eq. 1.

$y =$

$$\frac{MY_{\text{uninfected}} \times [\text{SCC or CF}] + MY_{\text{infected}} \times [\text{SCC or CF}]}{MY_{\text{cow}}} \quad (1)$$

where MY is the milk yield of the quarter and its infection status.

#### Statistical analysis

All statistical analyses were carried out with JMP software (SAS Institute, 2002). The analyses performed were both on the cow and on the quarter levels.

**Cow level analysis.** The 'bacteria type' model including the cow effect: The major unit was a cow and the minor unit was a day within a cow. The effects of bacteria: NBF, PIEc, CNS or *Strep.* and parity number (1st, 2nd, 3rd or higher than 4th) were used as fixed effects. The continuous variables DIM and DIP served as co-variance. The effects of bacteria, parity, DIM and DIP on the analysed parameters were determined by a four-way ANOVA in a 'split-plot' design. The analysed parameters were the cows' milk yield (l/d), fat (g/l), protein (g/l), lactose (g/l), log SCC, RCT (min), CF (V), Afi-fat (g/kg), Afi-protein (g/kg), Afi-lactose (g/kg), Afi-RCT (min) and Afi-CF (V). The ANOVA model on the cow level used bacteria species and added the cow effect.

The statistical model used was:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + e^1 + \gamma_k + \delta_l + e^2_{ijklm} \quad \text{Model (1)}$$

where:  $\mu$  = Mean of all data,  $\alpha_i$  = The difference between the bacteria  $i$  from the trial mean (fixed effect),  $\beta_j$  = The difference between the parity  $j$  from the trial mean (fixed effect),  $e^1$  = Variance between cows (random error),  $\gamma_k$  = The covariance of DIM,  $\delta_l$  = The co-variance of DIP and  $e^2_{ijklm}$  = Residual variance between measurements (random error). In this model, the bacteria and parity were tested against the variance between cows; and the DIM and DIP were tested against the residual.

Note: in this model, the REML Estimation of Variance Components method was used, matching SAS PROC MIXED (Creighton et al. 2005).

**Gland level analysis.** The individual glands' milk yield (%) according to the gland's location: front left (FL), front right (FR), rear left (RL) and rear right (RR) was corrected due to a significant effect between the milk yields (kg/d) of glands FL + FR vs. glands RL + RR in NBF cows. Since the FL and FR glands produce less milk than the RL and RR glands thus a correction of 25% to estimate the contribution of each gland to the udder's milk production was implied. Glands effects on milk yield (kg/d) were determined by a one-way ANOVA in a 'block' design. In this model, the cows were used as blocks. The analysed parameters were the gland's milk yield (%), fat (g/l), protein (g/l), lactose (g/l), log SCC, RCT, CF, Afi-fat (g/kg), Afi-protein (g/kg), Afi-lactose (g/kg), Afi-RCT (min) and Afi-CF (V).

The statistical model was:

$$Y_{ij} = \mu + \alpha_i + B_j + e_{ij} \quad \text{Model (2)}$$

where:  $\mu$  = Mean of all data,  $\alpha_i$  = The difference between gland  $i$  from the trial mean (fixed effect),  $B_j$  = The variance between cows (blocks, random effect),  $e_{ij}$  = Residual variance between measurements (random error). The ANOVA

**Table 1.** Data on cows that entered the study: Parity number, days in milk (DIM) and days in pregnancy† (DIP) on the cow level according to bacterial infection

	NBF‡	CNS§	<i>Strep.</i> ¶	PIEc††
Number of cows	137	73	13	16
Parity	2.3 ± 0.1	2.6 ± 0.2	3.9 ± 0.3	3.7 ± 0.4
DIM	161.6 ± 10.6	244.3 ± 15.7	301.1 ± 37.5	245.9 ± 51.1
Min.-Max.	4–517	5–571	58–495	39–703
DIP (% cows over 45 DIP)	119.2 ± 5.7 (31.4%)	88.0 ± 8.1 (42.9%)	49.0 ± 10.8 (23.1%)	71.5 ± 3.0 (10.0%)

Mean and SE

†Pregnancy was determined at day 45 post-insemination, therefore average DIP and % pregnant cows were calculated only from that day

‡No bacterial finding

§Coagulase negative staphylococci

¶Streptococci

††Post infection with *E. coli*

model was on the quarter level with the random effects of cow and date within a cow.

The effects of the bacteria type (NBF, PIEc, CNS or *Strep.* – fixed effect); cow (random effect) and date within a cow (random effect) on the analysed parameters were determined by a three-way ANOVA in a random design.

The statistical model was:

$$Y_{ijk} = \mu + \alpha_i + B_j + D_k[B_j] + e_{ijk} \quad \text{Model (3)}$$

where:  $\mu$  = Mean of all data,  $\alpha_i$  = The difference between the bacteria species  $i$  from the trial mean,  $B_j$  = Variance between cows,  $D_k[B_j]$  = Variance between dates within a cow,  $e_{ijk}$  = Residual variance between measurements (random error). In this model, the variance between cows was tested against the variance between dates within a cow. The bacteria and the variance between dates were tested against the residual.

Multiple comparisons between parities and bacteria species were done by Tukey HSD T-test. Correlation between Afi parameters and Milkoscan and Optigraph results were previously performed (Leitner et al. 2011, 2012b), hence, correlation was done between the parameters Afi-RCT, and Afi-CF and the parameters log SCC, Afi-fat, Afi-protein and Afi-lactose.

## Results

### Cow level analysis

Of the tests performed, 137 cows were free of infection in all 4 glands and the remaining cows were infected in one gland with CNS (70% with *S. chromogenes* and 15% each with *Staphylococcus haemolyticus* or *Staphylococcus Simulans*), *Strept. dysgalactiae* and PIEc (Table 1). On the average, cows infected with *Strep. dysgalactiae* and PIEc had more parities than NBF and CNS infected ones. In addition, NBF cows were on the average earlier in the current lactation than all the infected cows. Both DIP and pregnancy were lower in the cows infected with *Strep. dysgalactiae* or PIEc.

The significance ( $P$  value) of the ANOVA results for the fixed effects of bacteria (NBF, PIEc, CNS or *Strep.*) and

parity (1st, 2nd, 3rd or 4th+) when including the cow effect and for the covariance effects of DIP and DIM on the analysed parameters are summarised in Table 2. Overall, no significant differences were found among milk composition measured with the Milkoscan 6000, AfiLab™ and milk coagulation properties measured with Optigraph and AfiLab™. Parity had a significant effect on five parameters, however, the differences could not be explained and appeared as random. Bacterial infection had significant effects on log SCC and lactose concentration but differences in coagulation properties were not significant due to large variations, particularly in PIEc and *Strep.* infected glands. Examining each of the bacteria species in Table 3 revealed that the major influence on those parameters was the significant sharp decrease in lactose level in the PIEc and the lower CF value ( $P < 0.05$ ) in PIEc and *Strep.* cows.

The continuous variables of DIM and DIP (as determined at day 45 post insemination) had a significant co-variance effect on most parameters (Table 2). Overall, increases in both parameters had a significant negative effect on milk yield and a positive effect on milk constituents and had no effect on SCC. The correlations between the parameters Afi-RCT and Afi-CF and the parameters log SCC, Afi-fat, Afi-protein and Afi-lactose are presented in Table 4. Higher log SCC increased RCT and decreased CF while higher fat or protein concentrations shortened RCT and increased CF.

### Gland level analysis

In order to calculate and correct for the influence of the bacterial infection on the gland's milk production, gland location: FL, FR, RL and RR were tested only in cows whose four quarters were free of infection. The gland's locations had a significant effect ( $n = 515$ ;  $P < 0.0001$ ;  $R^2 = 0.810$ ) on milk yield. The FL and FR glands produce less milk than the RL and RR glands thus a correction of 25% to estimate the contribution of each gland to the udder's milk production was implied (Table 5).

Overall, 920 glands were examined because several cows had only 3 functioning glands. Of all the glands, 745 were free of infection, 134 were infected with CNS,

**Table 2.** The ANOVA significance levels and coefficient of determination ( $R^2$ ) of the analysed parameters and % variance between cows from overall variance (% var.) of 239 tests performed

Parameter	ANOVA results		$P$ values			
	$R^2$	% var.				
			Bacteria	Parity	DIP†	DIM‡
milk yield (l/d)	0.782	59.6	NS	NS	0.004	<0.001
fat (g/l)	0.563	58.8	NS	NS	0.005	NS
protein (g/l)	0.876	84.7	NS	NS	<0.001	<0.001
lactose (g/l)	0.727	64.3	<0.001	0.01	0.05	<0.001
log SCC	0.732	53.0	<0.001	NS	NS	NS
RCT (min)	0.654	54.8	0.1	NS	NS	<0.001
CF 60 (V)	0.792	80.5	0.1	0.02	<0.001	NS
Afi-fat (g/kg)	0.609	58.3	NS	NS	<0.001	0.021
Afi-protein (g/kg)	0.797	75.5	NS	NS	<0.001	<0.001
Afi-lactose (g/kg)	0.682	53.2	0.03	0.03	NS	<0.001
Afi-RCT (min)	0.730	56.8	0.1	0.02	NS	<0.001
Afi-CF (V)	0.696	67.4	0.1	0.01	0.008	NS

†Days in pregnancy

‡Days in milk

**Table 3.** Lactose, log SCC, rennet clotting time (RCT) and curd firmness (CF) on the cow level according to bacterial infection (Mean  $\pm$  SE)

	$n$	Lactose (g/l)	log SCC	RCT (min)	CF (V)
NBF†	137	48.8 $\pm$ 0.2 <sup>a</sup>	4.69 $\pm$ 0.04 <sup>c</sup>	24.0 $\pm$ 0.9	11.53 $\pm$ 0.4 <sup>a</sup>
PIEc‡	16	45.5 $\pm$ 0.1 <sup>b</sup>	5.75 $\pm$ 0.11 <sup>a</sup>	31.9 $\pm$ 3.1	8.27 $\pm$ 1.1 <sup>b</sup>
CNS§	73	48.4 $\pm$ 0.3 <sup>a</sup>	5.27 $\pm$ 0.07 <sup>b</sup>	27.3 $\pm$ 1.4	11.56 $\pm$ 0.8 <sup>a</sup>
<i>Strep.</i> ¶	13	48.0 $\pm$ 0.1 <sup>ab</sup>	5.72 $\pm$ 0.13 <sup>ab</sup>	30.6 $\pm$ 4.2	7.83 $\pm$ 1.3 <sup>b</sup>
$P$ value		<0.001	<0.001	0.1	0.1

†No bacterial finding

‡Post infected with *E. coli*

§Coagulase negative staphylococci

¶*Streptococci*<sup>a,b</sup>Results within column with no common superscript differ significantly ( $P < 0.05$ )**Table 4.** Correlations of Afi-RCT (rennet clotting time) and Afi-CF (curd firmness) with milk composition measured by AfiMilk and somatic cell count (SCC) by Fossomatic 360 of the whole udder milk ( $P < 0.001$ ).

Variables	$r$
Afi-RCT	
log SCC	0.498
Afi-fat	0.263
Afi-protein	0.248
Afi-CF	
Afi-lactose	-0.884
log SCC	-0.241
Afi-fat	0.328
Afi-protein	0.519
Afi-lactose	0.511

19 with *Strep. dysgalactiae* and 22 were PIEc. The significance ( $P$  values) of the ANOVA results for the fixed effect of bacteria (NBF, PIEc, CNS or *Strep.*) and the random effect of the cow on the analysed parameters are summarised in Table 6.

Gland's milk production decreased by ~20% in *Strep. dysgalactiae*- and by ~50% in PIEc-infected glands. The significant difference found among the bacteria in total fat and protein was probably due to a random effect. Lactose was significantly lower and log SCC significantly higher in the milk of infected glands than in NBF, and among the infected glands, both parameters were significantly higher in *Strep. dysgalactiae* and PIEc than in CNS. RCT measured by the Optigraph and Afi-RCT significantly increased and CF and Afi-CF decreased in the milk of infected glands, and among the infected glands, both parameters were greater and significant in *Strep. dysgalactiae* and PIEc than in CNS. The correlations of the gland's level were similar to that calculated for the cow level (Table 7).

In order to calculate the values in Eq. 1, all actual milk yields were standardised to 34.8 l/d (the real MY yield of a NBF cow) including the 25% correction for each quarter. Multiplying the MY of each gland by either its actual SCC or CF recorded value (assuming a linear relation for CF, as found at the laboratory – not presented), resulted in the

**Table 5.** Gland location in the quarter, front left (FL), front right (FR), rear left (RL) and rear right (RR) and the correction process for milk yield ( $P < 0.0001$ )

Quarter	<i>n</i>	Mean (kg/d)	Sum	%	Correction (25%)
FL	133	2.56 <sup>b</sup>	5.19 (43.3%)	21.65	+3.35
FR	133	2.63 <sup>b</sup>		21.65	+3.35
RL	133	3.43 <sup>a</sup>	6.80 (56.7%)	28.35	-3.35
RR	133	3.37 <sup>a</sup>		28.35	-3.35

<sup>a,b</sup>Results within column with no common superscript differ significantly ( $P < 0.05$ )

**Table 6.** The ANOVA significance levels and coefficient of determination ( $R^2$ ) of the analysed parameters, % variance between cows and dates within a cow from overall variance (% var), and the bacteria types LSM means

Parameter	ANOVA results			Bacteria type				
	$R^2$	% Var		<i>P</i> value Bacteria type	NBF† ( <i>n</i> = 745)	PIEc‡ ( <i>n</i> = 22)	CNS§ ( <i>n</i> = 134)	<i>Strep.</i> ¶ ( <i>n</i> = 19)
		Cows	Dates					
Milk (kg/gland/milking)	0.649	52.66	11.3	<0.001	2.90 <sup>a</sup>	1.69 <sup>c</sup>	2.46 <sup>a</sup>	2.27 <sup>b</sup>
Milk yield (%)	0.149	5.73	—	<0.001	25.7 <sup>a</sup>	12.9 <sup>c</sup>	24.5 <sup>a</sup>	18.4 <sup>b</sup>
Fat (g/l)	0.823	53.4	30.6	0.030	41.5 <sup>a</sup>	40.0 <sup>ab</sup>	41.4 <sup>ab</sup>	38.5 <sup>b</sup>
Protein (g/l)	0.960	83.5	12.9	<0.001	35.3 <sup>b</sup>	35.5 <sup>bc</sup>	34.9 <sup>c</sup>	36.6 <sup>a</sup>
Lactose (g/l)	0.641	56.2	2.5	<0.001	6.48 <sup>a</sup>	3.41 <sup>c</sup>	2.47 <sup>b</sup>	5.42 <sup>c</sup>
log SCC	0.697	44.6	8.3	<0.001	4.71 <sup>c</sup>	6.09 <sup>a</sup>	5.43 <sup>b</sup>	6.10 <sup>a</sup>
RCT <sup>5</sup> (min)	0.740	54.8	15.8	<0.001	23.1 <sup>c</sup>	38.7 <sup>a</sup>	27.5 <sup>b</sup>	35.0 <sup>a</sup>
CF <sup>6</sup> (V)	0.851	75.4	11.0	<0.001	12.63 <sup>a</sup>	3.60 <sup>d</sup>	9.90 <sup>b</sup>	7.18 <sup>c</sup>
Afi-fat (g/kg)	0.806	52.8	29.2	<0.001	41.4 <sup>a</sup>	40.3 <sup>ab</sup>	41.0 <sup>a</sup>	37.2 <sup>b</sup>
Afi-protein (g/kg)	0.894	69.0	21.3	<0.001	35.4 <sup>ab</sup>	34.6 <sup>bc</sup>	34.6 <sup>c</sup>	36.1 <sup>a</sup>
Afi-lactose (g/kg)	0.614	47.9	5.72	<0.001	48.5 <sup>a</sup>	43.8 <sup>c</sup>	47.7 <sup>b</sup>	43.1 <sup>c</sup>
Afi-RCT†† (min)	0.750	62.4	9.5	<0.001	24.1 <sup>d</sup>	36.6 <sup>a</sup>	27.2 <sup>c</sup>	30.8 <sup>b</sup>
Afi-CF‡‡ (V)	0.714	42.9	28.5	<0.001	11.87 <sup>a</sup>	5.232 <sup>c</sup>	10.10 <sup>b</sup>	6.73 <sup>c</sup>

†No bacterial finding

‡Post infection with *E. coli*

§Coagulase negative staphylococci

¶*Streptococci*

††Rennet clotting time

‡‡Curd firmness

<sup>a,b</sup>Results within column with no common superscript differ significantly ( $P < 0.05$ )

**Table 7.** Correlations of Afi-RCT (rennet clotting time) and Afi-CF (curd firmness) with milk compositions measured by Afilab™ and somatic cell count (SCC) by Fossomatic 360 on the gland (quarter) level ( $P < 0.001$ )

Variables	<i>r</i>
Afi-RCT	
log SCC	0.582
Afi-fat	0.174
Afi-protein	0.164
Afi-lactose	-0.836
log SCC	-0.307
Afi-CF	
Afi-fat	0.351
Afi-protein	0.485
Afi-lactose	0.643

estimated level of these parameters in the whole milk of the cow (Table 8). Infection in one gland with *Strep.* and PIEc had a major influence on both parameters in the whole cow's milk – increasing SCC and decreasing CF regardless of the lower MY. However, only a minor influence on SCC and CF was noted if the cows were infected with CNS.

## Discussion

The present study aimed to evaluate the influence of IMI by different bacteria species in one gland on the cow's milk quality. Overall it was found that at the cow level, infection with *Strep.* and PIEc had a negative effect on milk coagulation properties, unlike infection with CNS.

One of the agents most frequently found in ruminant mastitis worldwide is *E. Coli*, which usually is associated

**Table 8.** The influence of the infected quarter with the different bacteria (NBF, no bacterial finding; PIEc, post infected with *E. coli*; CNS, coagulase negative staphylococci; Strep., Streptococci) on the cow's milk somatic cell count (SCC) and Afi-CF (curd firmness)

Cow	Gland Milk†		Cow's milk‡ (34.8 l/d)	Cow's SCC§ (/ml)	Cow's Afi-CF (V)
	+	–			
Bacteria					
NBF		8.7	(8.7 × 4)	51 286	11.53
PIEc	5.07	9.91	5.07 + (9.91 × 3)	227 226	8.27
CNS	7.20	9.20	7.20 + (9.20 × 3)	96 361	11.56
Strep.	6.81	9.33	6.81 + (9.33 × 3)	287 608	7.83

†Quarter milk yield/d was calculated as the average milk (kg quarter/milking – Table 6) × 3 milkings

‡Cow milk yield/d was calculated as yield of the infected quarter + the compensated milk yield of the 3 uninfected quarters with the assumption that all the cows produced 34.8 litres/d

§Cow SCC was calculated as the contribution of each infected and uninfected quarter milk to the cow milk yield

with clinical and acute mastitis. However, the economic impacts of *E. coli* infection persist far beyond its clinical appearance. Recently it was shown that mammary gland functionality remains depressed after *E. coli* mastitis resulting in decreased milk yield, increased SCC and decreased quality of the milk for producing dairy products, especially cheese (Blum et al. 2014; Silanikove et al. 2014). In the current study, all the cows referred to as PIEc, were at the time of sampling about 1–2 months after the infection. However, the recovered gland's average milk yield was ~50% below the cow's normal gland production, 12.9% instead of 25% of total udder yield. Nevertheless, no significant difference was found for the cow's daily milk yield, probably due to compensation of the other glands. These results support those of Blum et al. (2014), which showed that even though the bacteria infected only one gland and was cleared spontaneously or after medical treatment, the quantity and mainly the quality of the gland's milk decreased, and even if the other gland compensated for the milk yield loss, the overall influence on the milk quality needs to be profoundly considered. The animal's response to *Strep. dysgalactiae* and other *Strep.* species that are involved in mastitis are different from those of *E. coli*. The inflammation response in most cases is permanent but does not develop into clinical mastitis, despite the continuous presence of the bacteria for months, including increase of SCC (Leitner et al. 2013). Moreover, the changes in milk composition and decrease in the measures of coagulation properties of milk from such glands are significant (Merin et al. 2008; Silanikove et al. 2014). In the current study, infection with *Strep. dysgalactiae* decreased the gland's milk production by ~20% with no significant influence on the cow's milk yield. Nevertheless, a theoretical influence of the presence of a bacterium in a gland on the cow's SCC and CF revealed that the low quantity of milk of the infected gland increased the cow's SCC > 4 fold and decreased CF by ~20%, similar to that of the PIEc (Table 8). In contrast, infections with one of the different CNS species only moderately increased the cow's SCC and had no influence on the cow's milk coagulation properties, although on the glandular level these parameters were significantly different from the uninfected glands.

## Conclusions

The current results using quarter-milking reinforces the importance of accurate IML detection in relation to economic and welfare factors, and moreover, emphasises the need for technical sensing and constant reporting to the farmer about changes in the milk quality of every animal. Today, the criteria used for grading milk in the bulk milk tank for payment and for quality are made on the cow level, which according to the present study is insufficient, and it is suggested that in the future it should be attributed to the quarter level. This is due to the fact that CNS-infected glands have a relatively minor effect on the level of SCC and milk quality, while glands infected with other bacteria species, such as *Strep. dysgalactiae* (and probably other *Strep.* species) or glands post infected with *E. coli*, produce low amount of milk and this milk dramatically influences the whole cow's milk quality and therefore the milk in the bulk tank. These findings call for decisions regarding the question of how to treat the animal – (treat the udder or the quarter) based on a set of arguments that include: loss of milk due to the medical treatment, milk price and the value of the animal. By taking these lines of action into consideration one could draw new criteria regarding the value of milk for industrial processing according to its suitability for producing various dairy products, as well as keeping in mind the consumers safety, by preventing milk unsuitable for human consumption from entering the bulk milk tank.

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