Subclinical mastitis assessed by deviations in milk yield and electrical resistance

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Summary. Concurrent falls in milk production and electrical resistance of composite milk were examined in Israeli Holstein cows. The cows were milked three times a day by a system that recorded yield and the lowest electrical resistance in the composite milk from the four glands. The study included two groups: cows that experienced on day 0 a decline in resistance and milk production $\geqslant 20\%$ from the mean of the previous 9 d (62 cows, case group) and cows that experienced no such episodes over 9 d before and after a fixed day (118 cows, control group). Bacteriological status and somatic cell count (SCC) or California mastitis test scores were assessed on the fixed day in the control group, and on days 0, 1 and 2 in the case group. California mastitis test scores greater than 2 and SCC thresholds of 5×10^5 cells/ml were used to create two classes of leucocytosis. There was no statistically significant difference between the two groups in frequency distributions of pathogens and their types: in 30% of cows infection was not detected, 33% were infected by major pathogens (95% of which were *Staphylococcus aureus*), and 53·5% by minor pathogens (80% *Micrococcus* spp.). Cows in the case group had lower milk production during the 8 d following day 0. Mean electrical resistance was lower in infected cows and particularly in cows infected by *Staph. aureus*. High leucocytosis was associated with reduced electrical resistance in both groups, and was found in 93% of cows in the case group *v*. 25% in the control group. The results suggest that falls in electrical resistance of milk and in milk production were not linked to a specific pathogen, and were followed by 3–8 d of reduced milk production and electrical resistance. The study suggests that there are episodic aggravations in mammary health that do not evolve into clinical mastitis but may induce significant losses in milk yield and quality.

The electrical resistance of milk (ER), has been suggested as a means of detecting mastitis (Linzell *et al.* 1974; Gebre-Egziabher *et al.* 1979; Fernando *et al.* 1985). The use of ER data in milking systems may make it possible to detect mastitis in real time, and for this to be possible a consistent relationship with mastitis should be demonstrated.

ER varies between individual cows and is lower in infected than uninfected quarters of a cow (Gebre-Egziabher *et al.* 1979), but the difference between infected and uninfected quarters may vary at various phases of milking (Fernando *et al.* 1985). Correlations between ER and somatic cell count (SCC), an indicator of mammary infection, are not always consistent; these correlations may even be lower than those between ER and other indices of infection, such as Na, K, Cl or albumin

concentrations in milk (Fernando *et al.* 1982, 1985; Isaksson *et al.* 1987). This suggests that sporadic measurement of ER might be of little assistance in the management of mammary health.

Measuring ER in individual glands during routine milking may considerably increase costs while the alternative of measuring ER in composite milk reduces the potential accuracy of mastitis detection. This deficiency might be compensated for by storing the lowest ER recorded during milkings and by comparing, within individual cows, this ER with the mean of a previous period (Carmi, 1987). Individual quarter health has been found to be relatively stable over time (Linzell *et al.* 1974; Jensen & Knudsen, 1991), which lends further support to the use of within-cow changes as indicators of mammary health.

The efficiency of mastitis detection might be further increased by combining changes in both ER and milk yield as indicators of inflammation. The diagnostic value of changes in milk yield alone is not obvious. Falls in milk production that last for one or two milkings and are not associated with changes in ER do occur; they might be related to digestive, metabolic, heat stress and milking problems (Berman & Shoshani, 1995), or associated with oestrus (Arney *et al.* 1994). Such reductions are not significantly associated with rises in SCC (Nielen *et al.* 1995). However, using changes in both ER and milk production for the detection of mastitis might enhance the sensitivity of detection.

A computerized milking system widely used in Israel (Afimilk system; Zaham Ltd, Afikim 15148, Israel) uses combined changes in milk production and electrical resistance of composite milk to warn of possible mastitis episodes. This system indicates the presence of combined falls in ER and milk production, most of which do not develop into clinical mastitis. A preliminary study indicated that such falls may occur 0·3–0·6 times/cow per month and are associated with milk losses of 50–120 kg/episode (Berman & Shoshani, 1995).

This present study examined whether combined falls in yield and ER of composite milk might indicate subclinical inflammation in infected glands. The detection of such increases in inflammation may provide the opportunity for earlier treatment of cases that may develop into clinical mastitis.

MATERIALS AND METHODS

Management

The study was carried out on Israeli Holstein cows in three commercial herds, milked three times daily. The cows were kept in open sheds that provided 10 m² of shade per cow, and an adjacent open lot of similar area per cow. Feed was offered in mangers located in either shaded or unshaded parts of the sheds. The shaded area was bedded with wheat straw or coarse sawdust. Cows were kept in management groups of 60–100, grouped by calving date until final group size was reached and kept in a group until ∼ 100 d before the end of the lactation or until culling. Housing, bedding material used in resting area, feeding, management, milking routine and veterinary care (including mammary health care) were closely similar in the three herds, and were stable during the observation period. The three herds also had similar mean milk yields (8500–9000 kg per 305 d), calving to conception intervals (108–115 d) and mean SCC (260 000–300 000 cells/ml). The incidence of subclinical episodes was 0·3–0·4 cases/cow per year, and the incidence of clinical mastitis 0·4–0·6 cases/cow per year.

Milking system

In the three herds a computerized milking system (Afimilk) was used for milking the cows three times daily. In this system cows were identified automatically, and milk yield, ER, pedometer recordings and milking times were stored for every milking. Milk yield was measured volumetrically, by counting numbers of 200 ± 4 ml volumes passing through the milk meter. ER was measured to $\pm 1\%$ using a low frequency AC current between stainless steel electrodes (5 mm diam., extremities 43 mm apart) located in the milk meter. ER was measured in each milk volume that passed through the milk meter and the milking system stored the lowest ER value recorded during each milking. Fore milk from individual glands was examined and glands were palpated during washing and massage before milking. Suspect mastitis cases were treated by two or three additional daily milkings for 1–3 d.

Study design

Cows in which isolated subclinical episodes of spontaneous falls in both milk yield and ER were detected served as the case (SEP) group. Such changes in ER and milk yield may precede the development of clinical mastitis. Only isolated subclinical episodes were considered in this study. An episode was considered subclinical if there had been no changes in milk appearance and glands were not observed to be painful, hot or swollen. An episode was considered isolated if at least 10 d separated it from preceding or subsequent episodes, as such episodes usually consist of lower ER and yield lasting for 3–8 d. The occurrence of these episodes is unpredictable, and they occur in mammary glands infected by various pathogens. For the control group (C) to consist of cows infected by pathogens similar to those in the case group, it had to be created after completing the SEP group. It was not possible to produce such a control group in the herd of the SEP group, as most dairy herds in this country contain 250–350 lactating cows. In order to produce a C group with infection rate (number of cows with at least one quarter infected/total number of cows) and distribution of pathogens similar to that of the SEP group, the C group was formed by pooling two management groups, each of 80–100 cows and located in other herds.

The SEP group comprised 62 cows considered to have had a subclinical mastitis episode during a 120 d period. A subclinical mastitis episode was considered to have occurred by either of two criteria: if the ER for two consecutive milkings deviated $\geqslant 20\%$ below the mean of 9 preceding days for the particular milking time or, alternatively, the ER deviated for one milking and milk yield fell by $\geqslant 20\%$ below the means for the particular milking time over the 9 preceding days. Duplicate samples of quarter milk were taken aseptically for bacteriological examination on the day of detection of the episode and on the following 2 d. California mastitis test (CMT) determinations on individual quarters (on a scale of 1–4) were performed on the same schedule. Technical difficulties prevented the determination of SCC in this group. The CMT score is, however, correlated well with SCC (Schneider & Jasper, 1964).

The C group consisted of 118 lactating cows, and was formed as follows. Two management groups, one in each herd, were pooled to produce a frequency of mammary pathogens similar to that in the SEP group. Cows were then selected in which no subclinical mastitis episodes were detected during the 9 d preceding day 0. From this initial group we excluded cows in which such episodes occurred during the 9 d following day 0. This left 118 cows to form the C group. Duplicate quarter milk samples were collected aseptically after the morning milking on day 0 for

bacteriological assessment. SCC of composite milk samples collected in the three milkings on day 0 were determined to $\pm 10\%$ by a cell counter (Fossomatic model 215; Foss Electric, DK-3400 Hillerød, Denmark) at the official Dairy Herd Book Laboratory (Bitan Aharon, Israel). Milk yield and ER values were collected during the 9 d before day 0 and for 9 d thereafter.

Leucocytosis is a typical response to infection; in latent infections the SCC are in the lower 10^5 cells/ml range and SCC increases to the 10^6 cells/ml range in more severe inflammations (Dohoo et al. 1981; International Dairy Federation, 1981). The content of inflammation markers in milk rises at SCC $> 5 \times 10^5$ cells/ml (Honkanen-Buzalski & Sandholm, 1981). In order to distinguish between latent infections and more severe inflammation states the cows were segregated into 'low' and 'high' leucocytosis classes. The thresholds used for segregation were an SCC of $> 0.5 \times 10^6$ cells/ml in the C group and in the SEP group a CMT score of 2 or more in one or more quarters. Both SCC and CMT score relate to the DNA content of milk, though in different ways. CMT gives a visual score and thus provides a subjective estimate of the DNA content of milk, while in the SCC the number of particles containing DNA is counted automatically (Carroll & Schalm, 1962; Clarke et al. 1995). Leucocytes have a short life span in milk, which leads to a large variation in cell size, in the content of DNA per cell, and the viability of cells counted (Schalm et al. 1971). Hence, CMT scores are indicative of a range of cell numbers in milk, although under controlled conditions a good estimate of cell numbers may be attained (Schneider & Jasper, 1964; Daniel et al. 1966; Astermak et al. 1969). A CMT score of 2 represents cell numbers in the 1×10^6 range (Schneider & Jasper, 1964; Daniel et al. 1966; Astermak et al. 1969). Setting a stricter criterion for 'high' leucocytosis in the SEP group than in the C group reduced the probability of erroneously defining a high inflammation state in the SEP group. This was thought appropriate, considering the observational nature of this study.

Bacteriological procedures

Quarter samples were taken aseptically according to recommended procedures (International Dairy Federation, 1981). Duplicate fore milk samples (~ 10 ml) were drawn aseptically from each quarter for bacterial culture and were stored at -20 °C until processing. The interval between collection of milk samples and culturing varied between 14 and 21 d. The samples were kept at room temperature for ~ 2 h prior to plating. Bacteriological analysis was carried out by the Central Mastitis Laboratory at the Kimron Veterinary Institute (Bet Dagan, Israel) according to NMC standard procedures (National Mastitis Council, 1987). Portions (0.03 ml) from each sample of milk were spread over a blood agar plate (Bacto-Agar; Difco Laboratories, Detroit, MI 48232-7058, USA) containing washed sheep red blood cells (50 g/l), and on to MacConkey agar plates. The minimal detection limit was > 5 cfu/plate except for *Escherichia coli*, for which it was > 2 cfu/plate. A cow was defined as 'infected' if a pathogen was isolated in one of the duplicates in the C group or in one of the triplicates in the SEP group. This definition of infection reduced the probability of false negatives. Infecting organisms were classified into major or minor pathogens according to the International Dairy Federation (1981) classification.

Analysis of results

Frequencies of cows by classes was assessed by χ^2 analysis. The other analyses were carried out on daily means of milk yield and ER, computed from the three daily milkings. This comprised a period of 9 d in the C group and a period of 19 d (from

9 d before until 9 d after day of episode) in the SEP group. General linear model procedures of SAS (1990) and type III mean squares were used for evaluation of factors affecting milk ER. The model used was

$$Y_{ijkl} = \mu + G_i + T_j + G_i T_j + C_k(G_i T_j) + D_l + e_{ijkl},$$

where Y_{ijkl} is ER within group, pathogen, cow and day, G_i the group class effect, T_j the pathogen class effect, $C_k(G_i T_j)$ is the average effect of cow k nested within pathogen and group, error term for group and pathogen effects, D_l the day class effect, $G_i T_j$ the group × pathogen interaction and e_{ijkl} the residual error.

Pathogen and leucocytosis effects were not computed simultaneously in one model because the unbalanced distribution of degrees of freedom did not make possible the calculation of all least mean squares. Hence, a similar GLM model was used in which the leucocytosis term replaced the pathogen term in order to assess its effects on ER.

The relationship of day-to-day changes in ER to the day-to-day changes in milk yield was examined in both groups. In the SEP group the analysis comprised the results recorded > 2 d before the episode of combined falls in ER and milk yield.

The correlations between daily milk yield and ER were low in both groups ($R^2 \sim 0.05$, $P > 0.05$). The absence of a significant correlation might be due to differences between cows in both mean milk yield and mean ER. The mean ER of a cow might be affected by its mineral balance as well as by its acid–base balance, independent of changes in its milk yield. To correct for this, the following procedures were carried out. In the first step, the differences between cows in mean ER were minimized by computing the within-cow day-to-day changes in ER. These were obtained as the residual ER of a model that consisted of ER as dependent variable and cow as independent variable. These residual ER were used in a subsequent general linear model (model 2).

$$Y_{ijk} = \mu + G_i + C_j(G_i) + \beta_i R_k + e_{ijk},$$

where Y_{ijk} is the mean daily milk yield within cow and group, G_i the group effect, $C_j(G_i)$ the average effect of cow j nested within group class, R_k the residual ER effect, $\beta_i R_k$ the effects of residual ER in groups i and e_{ijk} the residual error.

Changes in ER and milk yield relative to day of subclinical episode and to infection state are the subject of a separate detailed study.

<div align="center">RESULTS</div>

Mean daily milk yield was similar in C and SEP cows, 31.3 ± 0.6 and 28.8 ± 1.0 kg/d respectively. Mean numbers of daily consecutive observations per cow (means $\pm$ SE) were 6.6 ± 0.1 and 18.8 ± 0.4 in the C and SEP groups respectively. The discrepancy in the C group between the 9 d study period and the 6.6 daily observation records/cow was due to some data acquisition and storage problems. In both groups, the within-cow variation in ER and in milk yield between days and that between milkings within day was small, constituting 5 % of the total variance. The relative stability of ER in a given cow was essential for making feasible the use of ER for the detection of mastitis episodes. In contrast, the variance in ER and daily milk yield between cows was high and constituted a major component of total variance ($P < 0.05$).

In both groups *Staph. aureus* was the predominant major pathogen, constituting 92 and 98 % of infections caused by major pathogens in the C and SEP groups respectively. Similarly, in both groups *Micrococcus* spp. infections constituted

Table 1. *Frequency of infections (%) in cows with subclinical episodes of mastitis and in control cows*

	Infection category		
Group†	Undetected	Major pathogen‡	Minor pathogen§
Control	34	12	54
Subclinical	26	21	53

† Differences between groups in distribution of cows between categories of infection were not significant.

‡ *Staphylococcus aureus* constituted 92 and 98% of major pathogen infections in the control and subclinical groups respectively.

§ *Micrococcus* spp. infections constituted 80 and 81% of minor pathogen infections in the control and subclinical groups respectively.

Table 2. *Electrical resistance in composite milk and milk yields during the 8 d before and after decline in electrical resistance and milk yield*

(Values are least square means ± SE)

Period	Electrical resistance, mΩ	Milk yield, kg/d
Before†	42·4 ± 0·3	29·7 ± 0·4
After	40·6 ± 0·4	27·4 ± 0·5

† Values between rows were significantly different: $P < 0.01$.

> 80% of infections caused by minor pathogens (Table 1). Other infections (12% of cases in the C group and 11% in the SEP group) were caused by various organisms.

The impact of combined falls in ER and milk yield on mammary function was examined in the SEP group by comparing ER and milk yield before and after such falls had occurred (Table 2). The period before the occurrence of combined reductions was represented by the results recorded > 2 d before the falls occurred, to avoid effects of changes that might have preceded the falls in ER and milk yield. The results indicate that falls in ER and milk yield were followed by significant reductions in both mean ER and mean milk yield that extended into the following 8 d with no observable symptoms of clinical mastitis.

Group differences in ER

The grand mean of ER for the observation period was significantly lower in the SEP group than in the C group (39·8 v. 45·2 mΩ respectively, $P < 0.01$). This difference might stem from a herd difference, unrelated to mammary health, as the mean ER of the herd that included the SEP animals ($n = 376$, excepting the SEP group) during the study period was 40·9 ± 0·08 mΩ and a similar value was found in the same season of the following year. On the other hand, differences in mean ER between the two herds from which the C animals originated were quite small, < 2%. Differences between herds in ER and periodic changes in mean ER of lactating cows, probably unrelated to mammary health, were also observed in two other herds (A. Berman, E. Shoshani & B. Hanochi, unpublished results). This reduces the probability that the lower ER of SEP cows was related to their mammary health.

Pathogen effects on ER

Pathogen effects on ER were significant (Table 3). Contrast procedures indicated that the three infection categories (major or minor pathogens or no infection detected) were associated with ER values significantly different from one another

Table 3. *Effect of class of bacterial infection on electrical resistance of composite milk in control cows and in cows in which there had been a subclinical mastitis episode*†

(Values for electrical resistance are mΩ, least square means $\pm$ SE)

	Pathogens detected		
Group	Major‡	Minor‡	None
Control			
n	23	63	32
Electrical resistance	45·6 ± 0·9	48·0 ± 0·6	50·6 ± 0·8
Subclinical			
n	6	38	18
Electrical resistance	39·6 ± 1·4	42·9 ± 0·8	44·2 ± 1·1

† Differences between groups and pathogens were significant: $P < 0.01$. However, the effect of pathogen did not differ for the two groups.

‡ For pathogens, see Table 1.

Table 4. *Effect of leucocytosis class on electrical resistance of composite milk in control cows and in cows in which there had been a subclinical mastitis episode*†

(Values for electrical resistance are mΩ, least square means $\pm$ SE)

	Leucocytosis class‡	
Group	Low	High
Control		
n	89	29
Electrical resistance	48·7 ± 0·5	46·7 ± 0·9
Subclinical		
n	4	58
Electrical resistance	47·7 ± 2·2	42·3 ± 0·6

† Differences between groups and classes were significant: $P < 0.01$. However, the effect of leucocyte class did not differ for the two groups.

‡ Low if SCC $< 5 \times 10^5$ or California mastitis test < 2, high if SCC $\geqslant 5 \times 10^5$ or California mastitis test $\geqslant 2$ in one or more quarters.

($P < 0.02$). The ER was lower when major pathogen infections were present than with major pathogen infections (37·6 *v*. 40·1 mΩ respectively, $P < 0.02$). Mean ER was also lower with minor pathogen infections than when pathogens were not detected (42·3 *v*. 44·2 mΩ respectively, $P < 0.01$). The absence of a statistically significant group × pathogen interaction indicated that the effect of pathogens on ER did not differ for C and SEP groups. This suggests that the effect of pathogen class on ER may well be independent of that of a decline in milk yield and ER. As the major pathogen diagnosed was predominantly *Staph. aureus*, and the predominant minor pathogens were *Micrococcus* spp., these effects might be specific for these two organisms.

Leucocytosis effects

In order to assess the effect of leucocytosis on ER, the results were divided (Table 4) into two classes of leucocytosis, 'high' and 'low', as previously detailed. The incidence of 'high' leucocytosis cases was higher in the SEP than the C group (94 *v*. 25 % of total cases respectively, $P < 0.01$). Mean ER was lower in the 'high' than in the 'low' leucocytosis class (46·2 ± 1·7 *v*. 41·9 ± 1·9 mΩ respectively, $P < 0.01$). There was no significant group × leucocytosis interaction. The absence of such an interaction suggests that the effect of leucocytosis on ER might be similar in the two groups.

Table 5. *Effects of infection status and leucocytosis class on electrical resistance of milk*†

(Values for electrical resistance are mΩ, least square means ± SE)

Infection	Leucocytosis class‡	
	Low	High
Not detected		
n	27	23
Electrical resistance	50·4 ± 0·9	46·1 ± 1·1
Detected		
n	66	64
Electrical resistance	48·0 ± 0·6	43·1 ± 0·6

† Differences between leucocytosis classes and infection states were significant: $P < 0.01$. There was no significant interaction between the two classifications.

‡ Low if SCC $< 5 \times 10^5$ or California mastitis test < 2, high if SCC $\geqslant 5 \times 10^5$ or California mastitis test $\geqslant 2$ in one or more quarters.

The distribution of infection status classes between leucocytosis classes did not permit calculation of least squares means for these subgroups. Therefore the effect of infection on ER was examined by comparing all cases in which infection was detected with those in which it was not detected (Table 5). The effects of both infection and leucocytosis class were significant ($P < 0.01$), and no significant interaction between the two terms was evident. These suggest that the effects of infection class and of leucocytosis class on ER may be independent of each other.

Relationships of ER and milk yield

Episodes of combined falls in ER and milk yield may be preceded by a period in which the mammary host–pathogen relationships are unstable, and this could be reflected in concurrent, correlated fluctuations in ER and milk yield. In the SEP group the relationships between ER and milk yield were examined in the period from 2 to 9 d before the advent of combined declines in ER and milk yield.

The correlations between daily milk yield and daily mean ER were low in both C and SEP groups ($r = 0.22$, $P > 0.05$). However, when the within-cow between-days variance in ER was examined, higher correlations were found ($r = 0.93$, $P < 0.01$) that differed between groups (model 2, group × residual ER interaction, $P < 0.01$). In the C group, the coefficient estimating the effect of changes in residual ER on milk yield was of little biological significance ($b = -0.03$ kg/mΩ). In the SEP group the coefficient was 11-fold that in the C group ($b = 0.34$ kg/mΩ). This indicates that in the SEP group, in the period preceding the subclinical episode, day-to-day decreases in ER were associated with concurrent decreases in milk yield. These concurrent fluctuations occurred from 9 to 2 d before the detection of the subclinical episode. The concurrent fluctuations were not linked to any marked rise in variation in ER and milk yield, as the day-to-day variation in ER and in milk within cow was only $\sim 15\%$ greater in the SEP group than in the C group.

DISCUSSION

This was an observational study, whose main goal was to examine episodes in which milk yield and electrical resistance of milk fell concurrently. There was no *a priori* reason to presume that such falls are related to infection, as only a minority of such cases developed into clinical mastitis, and metabolic changes may also

provide causes. The episodes involved a marked reduction in milk yield, $> 20\%$ of total milk yield. If the episode is restricted to one quarter, as was found in a significant number of cases (A. Berman, B. Hanochi, and E. Shoshani, unpublished results), it implies a drastic temporary impairment of milk synthesis, with no outwardly observable symptoms. The episodes examined were not accompanied by symptoms of clinical mastitis, and were followed by lower milk production for periods of up to 8 d. These episodes are likely to be associated with infection, as infection was detected in 74% of SEP cows, which might have been close to the true infection rate, bearing in mind the sampling procedure and the infecting organisms (Erskine & Eberhart, 1988; Sears *et al.* 1990; Daley *et al.* 1991). Infection frequency was similar in C and SEP cows, 74 and 64% respectively. The frequency of *Staph. aureus* infection was higher in SEP than in the C cows (21 *v.* 12%), although this difference was not significant. The frequency of high leucocytosis was markedly higher in SEP than in C cows (93 *v.* 25% respectively, $P < 0\cdot01$). It is rather unlikely that such a large difference in the frequency of leucocytosis is attributable to the differences in infection found in this study. Subsequent studies in the other two herds indicated that episodes such as were observed in the SEP cows of one herd also prevailed in other herds and can occur against a background of various infections (A. Berman, E. Shoshani and B. Hanochi, unpublished results). It is thus probable that such episodes reflect a change in host–pathogen relationships. As the present study was observational in nature, such a presumption may seem speculative, unless sufficient experimental evidence can be provided. Some evidence may be found in the continuous and fluctuating effects of infection on milk yield.

Infection impairs milk production, as indicated by the negative relationship between monthly test day SCC values and milk production (Tyler *et al.* 1989; Bartlett *et al.* 1990). Leucocytosis alone, in the absence of infection, may damage mammary secretory cells both *in vitro* (Capuco *et al.* 1986) and *in vivo* (Akers & Thompson, 1987). *Staph. aureus* damages mammary parenchyma, which probably impairs its secretory capacity (Sordillo *et al.* 1989). Antibiotic treatment of *Staph. aureus* infections decreases SCC and increases milk yield by 14% (Mwakipesile *et al.* 1983). This suggests that chronic infection may have continuous, progressive deleterious effects on milk production which may represent both direct and indirect effects of infection. However, such effects may not explain the transient falls in milk yield and ER shown in this study. Such episodes occur when infection persists and may cause additional losses in milk yield (Bramley, 1991).

Variation across days in bacteria shed in milk, and in shed cells and their bactericidal capacity, is evident from studies of experimentally induced *Staph. aureus* infection (Sears *et al.* 1990; Daley *et al.* 1991). In these studies, an inverse relationship prevailed between numbers of bacteria shed in milk and SCC, with peak bactericidal efficiency occurring near peak SCC. However, the relationship between SCC and overall phagocytic capacity of neutrophils in milk is probably not linear, as milk may contain a factor that reduces the phagocytic capacity of leucocytes (Niemialtowski *et al.* 1988), and the phagocytic capacity of neutrophils is reduced by ingestion of milk particles (Paape & Guidry, 1977). Day-to-day fluctuations in milk yield were significantly correlated with concurrent fluctuations in ER in the SEP cows during the period of 9 to 2 d preceding the episodes. This relationship was significantly less strong in C cows, and similar to the poor relationship found between milk yield deviations and high leucocytosis periods in unselected cows (Nielen *et al.* 1995). The high leucocytosis in SEP cows occurred in cases in which milk yield deviations were associated with ER deviations. It is notable in this respect that in

uninfected glands ER tended to change in parallel from day to day, while in infected glands ER displayed significant departures from any association (Linzell *et al.* 1974). All these factors, taken collectively, suggest that transient changes in the host–pathogen relationship are probable.

It is well established that milk ER is reduced in the presence of intramammary infection (Greatrix *et al.* 1968; Linzell *et al.* 1974; Gebre-Egziabher *et al.* 1979; Fernando *et al.* 1982, 1985; Jensen & Knudsen, 1991). This present study also suggested that ER might be lower in predominantly *Staph. aureus* infections than in minor pathogen infections, mostly *Micrococcus* spp. in this case. The interaction between infection, SCC and ER may be more complex, as although both *Staph. aureus* and *Streptococcus uberis* subclinical infections are associated with higher SCC, a reduced ER is evident only in *Staph. aureus* infections (Hillerton & Walton, 1991).

The advent of computer-assisted milking systems might offer additional tools for understanding such episodic subclinical mastitis events. In the particular ER measuring system used in this study, the ER value stored was the lowest measured during milking, as was the case in the study of Nielen *et al.* (1995). As the lowest ER of infected quarters is not consistently recorded in a particular milk fraction (Fernando *et al.* 1985), recording the lowest ER measured during milking could be an advantage.

In some agricultural systems SCC are determined monthly, bacterial cultures of quarters milk are carried out yearly and additional bacteriological examination may also be carried out. In such situations ER may be less important for the detection of chronic infection. The milking system used in this study evaluates mammary health from within-cow deviations of ER and milk yield from means of a previous period, and may assist in the detection of such subclinical mastitis episodes in real time. It may make earlier detection and treatment of clinical cases possible. The frequency of episodic deterioration might also serve for assessing the management of mammary health in the herd. The course of events following such episodes and the ways to treat them are not clear, and are the subject of further studies.

REFERENCES

Akers, R. M. & Thompson, W. 1987 Effect of induced leucocyte migration on mammary cell morphology and milk component biosynthesis. *Journal of Dairy Science* **70** 1685–1695

Arney, D. R., Kitwood, S. E. & Phillips, C. J. C. 1994 The increase in activity during oestrus in dairy cows. *Applied Animal Behaviour Science* **40** 211–218

Astermak, S., Funke, H. & Engan-Skei, I. 1969 The relationship between the California Mastitis Test, Whiteside test, Brabant Mastitis Reaction, catalase test, and direct cell counting of milk. *Acta Veterinaria Scandinavica* **10** 146–167

Bartlett, P. C., Miller, G. Y., Anderson, C. R. & Kirk, J. H. 1990 Milk production and somatic cell count in Michigan dairy herds. *Journal of Dairy Science* **73** 2794–2800

Berman, A. & Shoshani, E. 1995 Factors affecting deviations of milk production rate and electrical conductivity. *Proceedings, 3rd International Dairy Federation Seminar on Mastitis*, Tel Aviv 142–144

Bramley, A. J. 1991 Mastitis: physiology or pathology? In *New Insights into the Pathogenesis of Mastitis*, pp. 3–10 (Eds C. Burnevich, G. Vandeputte-Van Messom and A. W. Hill). *Vlaams Diergeneeskundig Tijdschrift*

Capuco, A. V., Paape, M. J. & Nickerson, S. C. 1986 In vitro study of polymorphonuclear leukocyte damage to mammary tissues of lactating cows. *American Journal of Veterinary Research* **47** 663–668

Carmi, S. 1987 Automatic computerized herd management: heat detection, mastitis and health monitoring. *Proceedings, 3rd Symposium on Automation in Dairying*, Wageningen, p. 18

Carroll, E. J. & Schalm, O. W. 1962 Effect of deoxyribonuclease on the California test for mastitis. *Journal of Dairy Science* **45** 1094–1097

Clarke, T., Evans, M. E., Hepworth, G., Moate, P. J. & Stewart, J. A. 1995 Mordant factors that affect the fluorescence and counting of somatic cells by instruments. *Journal of Dairy Research* **62** 373–394

Daley, M. J., Oldham, E. R., Williams, T. J. & Coyle, P. A. 1991 Quantitative and qualitative properties of host polymorphonuclear cells during experimentally induced *Staphylococcus aureus* mastitis in cows. *American Journal of Veterinary Research* **52** 474–479

Daniel, R. C. W., Smith, G. C. & Barnum, D. A. 1966 The relationship of California Mastitis Test (C.M.T.) scores with leucocyte counts on bucket milk samples. *Canadian Veterinary Journal* **7** (4) 80–83

Dohoo, I. R., Meek, A. H., Martin, S. W. & Barnum, D. A. 1981 Use of total and differential somatic cell counts from composite milk samples to detect mastitis in individual cows. *Canadian Journal of Comparative Medicine* **45** 8–14

Erskine, R. J. & Eberhart, R. J. 1988 Comparison of duplicate and single quarter milk samples for the identification of intramammary infections. *Journal of Dairy Science* **71** 854–856

Fernando, R. S., Rindsig, R. B. & Spahr, S. L. 1982 Electrical conductivity of milk for detection of mastitis. *Journal of Dairy Science* **65** 659–664

Fernando, R. S., Spahr, S. L. & Jaster, E. H. 1985 Comparison of electrical conductivity of milk with other indirect methods for detection of subclinical mastitis. *Journal of Dairy Science* **68** 449–456

Gebre-Egziabher, A., Wood, H. C., Robar, J. D. & Blankenagel, G. 1979 Evaluation of automatic mastitis detection equipment. *Journal of Dairy Science* **62** 1108–1114

Greatrix, G. R., Quayle, J. C. & Coombe, R. A. 1968 The detection of abnormal milk by electrical means. *Journal of Dairy Research* **35** 213–221

Hillerton, J. E. & Walton, A. W. 1991 Identification of sub-clinical mastitis with a hand-held electrical conductivity meter. *Veterinary Record* **128** 513–515

Honkanen-Buzalski, T. & Sandholm, M. 1981 Trypsin inhibitors in mastitic milk and colostrum: correlation between trypsin inhibitor capacity, bovine serum albumin and somatic cell contents. *Journal of Dairy Research* **48** 213–223

International Dairy Federation 1981 *Laboratory Methods for Use in Mastitis Work*. Brussels: IDF (*International Dairy Federation Document* no. 132)

Isaksson, A., Philips, A.-C., Göransson, E. & Björkenfeldt, H. 1987 The electrical conductivity of bovine milk in mastitis diagnosis. *Acta Veterinaria Scandinavica* **28** 455–457

Jensen, N. E. & Knudsen, K. 1991 Interquarter comparison of markers of subclinical mastitis: somatic cell count, electrical conductivity, *N*-acetyl-β-glucosaminidase and antitrypsin. *Journal of Dairy Research* **58** 389–399

Linzell, J. L., Peaker, M. & Rowell, J. G. 1974 Electrical conductivity of foremilk for detecting subclinical mastitis in cows. *Journal of Agricultural Science* **83** 309–325

Mwakipesile, S. M., Holmes, C. W. & Moore, Y. F. 1983 Antibiotic therapy for subclinical mastitis in early lactation; effects on infection, somatic cell counts and milk production. *New Zealand Veterinary Journal* **31** 192–195

National Mastitis Council 1987 *Laboratory and Field Handbook on Bovine Mastitis*. Fort Atkinson, WI: W. D. Hoard & Sons

Nielen, M., Schukken, Y. H., Brand, A., Deluyker, H. A. & Maatje, K. 1995 Detection of subclinical mastitis from on-line milking parlor data. *Journal of Dairy Science* **78** 1039–1049

Niemialtowski, M., Nonnecke, B. J. & Targowski, S. P. 1988 Phagocytic activity of milk leukocytes during chronic staphylococcal mastitis. *Journal of Dairy Science* **71** 780–787

Paape, M. J. & Guidry, A. J. 1977 Effect of fat and casein on intracellular killing of *Staphylococcus aureus* by milk leukocytes. *Proceedings of the Society for Experimental Biology and Medicine* **155** 588–593

SAS 1990 *User's Guide. Statistics*, v. 6, 4th edn, vol. 2. Cary, NC: SAS Institute

Schalm, O. W., Carroll, E. J. & Jain, N. C. 1971 *Bovine Mastitis*. Philadelphia, PA: Lea and Febiger

Schneider, R. & Jasper, D. E. 1964 Standardization of the California Mastitis Test. *American Journal of Veterinary Research* **25** 1635–1641

Sears, P. M., Smith, B. S., English, P. S., Herer, P. S. & Gonzalez, R. N. 1990 Shedding pattern of Staphylococcus aureus from bovine intramammary infections. *Journal of Dairy Science* **73** 2785–2789

Sordillo, L. M., Nickerson, S. C. & Akers, R. M. 1989 Pathology of *Staphylococcus aureus* mastitis during lactogenesis: relationships with bovine mammary structure and function. *Journal of Dairy Science* **72** 228–240

Tyler, J. W., Thurmond, M. C. & Lasslo, L. 1989 Relationship between test-day measures of somatic cell count and milk production in California dairy cows. *Canadian Journal of Veterinary Research* **53** 182–187