

# Major Considerations in Managing Subclinical Mastitis During Lactation in Modern Dairy Farms

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## ABSTRACT

The decision on whether to treat cows' subclinical udder infections or to ignore it is not straightforward as antibiotic treatment of animals that are not at risk should be justified with respect to the cost of treatment and milk loss. Data regarding 152 dairy cows was used to evaluate the economics of mastitis-control according to five categories: a) No intervention; b) Antibiotic treatment; c) Drying off quarter/s; d) Drying-off the whole udder and e) Culling. The data was analyzed according to parity, bacteria, time in lactation at infection recording, treatment, time elapsed between infection and treatment and somatic cell count at treatment. Cure of first lactation cows was significantly higher than that of cows at their 2<sup>nd</sup> and 3<sup>rd</sup> onward lactations and depended on the bacteria causing the infection. It was higher in cows infected with coagulase negative staphylococci than with various types of *Streptococci*, and lowest in cows previously infected with *Escherichia coli*. The effect of day of treatment after onset of the infection was significant. It was also demonstrated that use of casein hydrolysate (a drug in development that can dry-off the inflamed quarter with modest reduction in overall milk yield by avoiding the problem of withholding milk), eliminates the need to use antibiotics and the cost of treatment becomes highly economical. In conclusion, antibiotic treatment is unavoidably associated with milk waste; thus, when the alternative is no intervention it is the preferable option. In cases where the infected gland produces low quality milk with somatic cell count  $\sim 1,000 \times 10^3$  cells/mL milk, drying-off the gland by using a drug such as casein hydrolysate is the preferable option.

**Keywords:** Subclinical mastitis; Treatment; Casein hydrolysate; Herd management.

## INTRODUCTION

Mastitis is one of the most important factors that imposes economic burden on dairy farms worldwide. It is estimated that mastitis infections (clinical and subclinical) affects ~30% of dairy cattle and cost the EU dairy industry about €1.55 billion in 2005 (1, 2, 3) and up to \$2 billion in the USA (4). Cost of mastitis treatment drives modern dairy farmers to exert continuous efforts for its reduction, which is achieved by constant improvement of genetic selection, nutrition and herd management. However, despite the tremendous efforts to solve the problem of mastitis the number of new udder

infections as well as the increase of chronic cases of mastitis remains very high. Nevertheless, changes do occur in the bacteria involved in mastitis reduction in cases caused by *Streptococcus agalactiae* or *Staphylococcus aureus* accompanied by a steady or increased infection by other bacterial species, mainly coagulase negative staphylococci (CNS) (5, 6).

In general, antibiotics are used during lactation to cure clinical forms of infections when the animal is at risk and therefore intervention is required (7, 8). On the farm level, the time and type of intervention depends on the farm manager and the veterinarian. Frequently, antibiotics are administered

before identification of the cause of infection. The economic losses from clinical mastitis are related to direct cost of drug, milk withdrawal and increased culling and death (9, 10, 11). Moreover, intramammary infections (IMI) with bacteria such as *Escherichia coli* can lead to negative long lasting effects on the quantity and mainly the quality of the gland's milk after the bacteria are eradicated. Such an effect might persist for months and in some cases for the entire animal's life and has been termed "udders post infected by *E. coli*" (PIEc) (12, 13, 14).

Subclinical (SC) infections result in decreased milk yield, reduction in fertility, deterioration of milk quality and increased risk of culling (3, 15, 16), especially owing to its wide prevalence, which may reach about 20 to 40% of the udders in some herds (1, 17). Many of the cows with chronic SC infection are not diagnosed because there are no recognizable symptoms and the milk appears normal. As a consequence, in most countries the treatment of SC mastitis is performed during the dry-off period for eliminating prevailing infections and preventing new ones (7) by using broad spectrum antibiotics that cover mainly Gram positive bacteria (18, 19, 20).

The decision as whether to treat the cows or to ignore the infection is not simple: antibiotic treatment of cows that are not at risk as in the case of SC mastitis, needs to be justified with respect to the cost of medication and milk loss (21-23). Moreover, the global rise of antimicrobial resistance of bacteria, combined with the decreasing number of innovative antibacterial agents has led to the need of new thinking for using antibiotics (24-27). One alternative of coping with SC infection when the quality (such as somatic cell count - SCC) of the gland's milk does not meet the criteria for acceptance by dairy plants is to dry-off the gland, while continuing milking the other glands. However, conventional procedures to attain this goal by surgical or chemical means, imposes irreversible damage to the glands and are problematic from the animal welfare point of view. Recent studies showed that treating glands by infusion of casein hydrolysate (CNH) was effective in drying-off milk secretion in the treated glands (28-32). This procedure imitates the process induced during mammary gland involution in an accelerated mode. Infusion of CNH into the gland immediately improved milk quality by forcefully activating the glandular innate immune system and was also reflected by high cure of up to 90% of the glands and reversion of the gland to full activity in the subsequent lactation (28, 30, 33, 34).

A side effect of modernization is an increase in herd

size to hundreds and even thousands of cows (35, 36). One outcome of this trend is the development of a concept that cow herd management and health control should be focused on the herd level (37) rather than on the individual cow, as practiced in traditional dairy farming. On the same line of conceptual thinking is the prevailing use of blanket antibiotic treatment in many countries (i.e., treating all the cows in the herd) upon drying-off the cows at the end of lactation rather than selectively treating only infected glands.

Routine monthly milk recording, which includes SCC is a practical procedure in dairy farms in many countries and this information can serve as the basis for treatment decisions (21, 23). However, the elapsed time between two such tests results in delayed acquisition of the relevant information for a decision (29). Modern dairy farms are characterized by high levels of computerized data acquisition, which provide on-line information on cows' milk yield, milk composition and body weight, and input on cows' behavior such as step count, lying duration and rumination, all of which could help farmers to reach helpful decisions on how to encounter daily problems on the farm. Thus, paradoxically – or apparently so at first glance – the prevalence of on-line computerized data enables modern farmers to reach decisions on individual cows even among large herds (38). On-line computerized milking systems which provide measures of changes in milk yield and conductivity and more, can help in the identification of cows with udder infections soon after the occurrence of the infection (39, 40). Thus, in addition to the identification of visible clinical mastitis, the system can provide valuable information on new infections that are not visible to the farmer close to its occurrence, which could help in shortening the elapsed time until treatment.

In earlier studies efforts were made to exploit the on-line data to provide dairy farm managers with the most optimal decision on how to treat mastitis, where the major principles of treatments consideration were treatment effectiveness and success (29, 41). One important element in deriving optimal treatment strategy is the treatments' effect on economic output of the farm. Such information is difficult to obtain in conventional farms because of lack of alternatives for comparison.

In light of the above, the present study was carried out at the research dairy farm of the Agricultural Research Organization, which allowed testing conditions that were too complicated to be justified when studied in a commercial herd. The treatment options considered in this study were: no

intervention, antibiotic treatment, drying-off milk secretion from an infected gland, drying-off the whole udder or culling. This information was used to calculate the economic effects of treatments of SC mastitis during the lactation. Based on the information, we derived a set of simple equations, which enable choosing the most optimal treatments in terms of maximizing the economic benefit for a given dairy farm.

## MATERIALS AND METHODS

### Study protocol

All treatment protocols were approved by the Institutional Animal Care Committee of the Agricultural Research Organization, which is the legitimate body for such authorizations in Israel.

The study was carried out in a dairy herd of 220 lactating Israeli-Holstein cows, at the Agricultural Research Organization, the Volcani Center. The dairy parlor was equipped with an on-line computerized AfiFarm Herd Management data acquisition system that included the AfiLab milk analyzer (Afirmilk, Afikim, Israel), which provides on-line data on milk gross composition (fat, protein and lactose) and milk conductivity (<http://www.afimilk.com>). The cows were milked thrice daily and the average milk yield in this farm throughout 2008-2013 was ~11,500 L during 305 days of lactation. Food was offered *ad lib* in mangers located in the sheds. Routine monthly milk yield and SCC were recorded by the Israeli Cattle Breeders Association. During the study period the monthly average bulk tank SCC varied between  $160 \times 10^3$  and  $245 \times 10^3$  cells/mL.

Clinical udder infections were treated with antibiotics according to the herd veterinarian's decision, where most of the cases were treated before the causative agent was diagnosed. Reports on daily cow's performance and behavior (conductivity, milk yield and animal behavior) were automatically recorded by the computerized AfiFarm system and the monthly routine milk recording (Israel Cattle Breeding Association) served as the basis for identification of cows suspected to be SC infected. Milk from each quarter was aseptically sampled from cows with SCC  $>200 \times 10^3$  cells/mL and the milk samples were analyzed for bacteriology, California mastitis test and SCC (42). If a bacterium was isolated accompanied with SCC  $>200 \times 10^3$  cells/mL, an antimicrobial susceptibility test was performed in accordance with NCCLS guidelines (43) with commercially available disks – Dispens-O-Disc

(Susceptibility Test System, Difco) or BBL Sensi-Disc Antimicrobial Susceptibility Test Discs (Becton Dickinson, MD, USA), according to the manufacturer's instructions. The plates were incubated at 30°C for methicillin sensitivity (5 mg/disk), and at 37°C for sensitivity for other antibiotics: Penicillin G (10 units/disk), Erythromycin (15 mg/disk), Cephalothin (30 mg/disk), Neomycin (30 mg/disk), trimethoprim-sulfamethoxazol (1.25-23.75 mg/disk). The results were interpreted in terms of susceptibility or resistance according to the manufacturers' recommendations.

The above information was used to assign cows to treatments by applying the treatment decision scheme for achieving optimal treatment of Leitner *et al.* (29). Cows were designated into 5 groups: 1) no intervention, 2) antibiotic treatment, 3) drying-off milk secretion from an infected gland, 4) drying-off the whole udder and 5) culling, which was done upon decision of the dairy herd manager and the clinical veterinarian and the availability of cows to be treated (cows involved in experiments).

### Treatment of subclinical infection

In the case where the choice was antibiotic treatment, the following procedures were applied: The cows were infused with a tube of Nafpenzal MC (Intervet, Boxmeer, The Netherlands), composed of 180 mg Penicillin G (300,000 IU), 100 mg Dihydrostreptomycin, 100 mg Nafcillin) or Nafpenzal DC (300 mg Procaine benzylpenicillin (300,000 IU), 100 mg Dihydrostreptomycin, 100 mg Nafcillin). The antibiotic was administered daily for 3 days to all quarters, both infected and uninfected, together with intramuscularly injection of 50 mL Benzylpenicillin procaine (30 PEN) and GENTAJECT (ABIC Biological Laboratories, Teva Ltd., Israel). COBACTAN™ (Intervet, International B.V. Boxmeer, The Netherlands) was used only in cases of *Strep. Uberis* infection. Treatments were carried out after the mid-day milking and the treated cows were not milked at the evening milking. The milk was discarded for 7-10 days in accordance with the instructions of Delvotest (DSM Food Specialties, Delft, The Netherlands) and was then tested daily for antibiotic residues until complete disappearance of bacterial growth inhibition. Cure was defined as the disappearance of the bacteria and decline of SCC to  $<150 \times 10^3$  cells/mL. Milk was sampled from the treated quarters and was tested monthly during the first 100 days following treatment.

Cows treated with CNH prepared under Good

Laboratory Practice conditions received one or two infusions of 10 mL of ~7 mg/mL peptide concentrate into the infected quarter at midday milking and were not milked in the evening (28).

### Statistical analysis

A multivariate model was designed with a logistic model statement using the GLIMMIX procedure of SAS (44). The results of this procedure served as the dependent variable. Cure per treatment was calculated as the number of cases cured divided by the number of total treatments. The entire data set (n = 152 treatments) was analyzed, with the general form:

$$\text{Cure} = \text{Exp}_{\cdot j=1,2} + \text{Parity}_{k=1,2,3} + \text{Bacteria}_{l=1,2,3} + \text{day\_inf}_{n=1,2,3} (\text{I}) + \text{day\_t} (\text{T})_{0=1,2,3} + \text{Period} (\text{I-T})_{p=1,2,3} + \text{LSCCi}_{q=1,2,3} + \text{Parity}_{k=1,2,3} \times \text{LSCCi}_{q=1,2,3} + \text{error}$$

where:  $\text{Exp}_{\cdot j=1,2}$  = two periods of the study,  $\text{Parity}_{k=1,2,3}$  = 1<sup>st</sup>, 2<sup>nd</sup>, or 3 and more lactations,  $\text{Bacteria}_{l=1,2,3}$  = causing agents Streptococcus, PIEc or CNS,  $\text{day\_inf}_{n=1,2,3} (\text{I})$  = estimated day of infection according to high SCC > 200×10<sup>3</sup> cells/mL (divided into three sub-groups: parturition, 1-50, 51-100 d, >100 d),  $\text{day} (\text{t}) (\text{T})_{0=1,2,3}$  = days in milk (DIM) at time of treatment (divided in to three sub-groups: <50, 51-100, >100),  $\text{Period} (\text{I-T})_{p=1,2,3}$  = difference between estimated day of infection and treatment (divided into three sub-groups: <50, 51-100, >100),  $\text{LSCCi}_{q=1,2,3}$  = Log SCC level at day of infection (divided in to three sub-groups: <300, 301-1,000, >1,000×10<sup>3</sup> cells/mL. Data are presented as mean percentage of success.

### Cost of handling SC mastitis during lactation according to treatment choice

The treatment cost of a SC infected cow may be carried out by summing several variables, as follows: 1) Cost of treatment of the cow; 2) The cow value; and 3) The expected recovery of the cow. Calculation of the cost of treatment (CT) per cow includes:

$$\text{CT} = a + b + c \quad (1)$$

where: CT - cost of treatment; a - cost of medication; b - cost of veterinary service; c - cost of the milk discarded because of antibiotic residues in milk (kg/d × d × milk price).

The cow value (CV) (\$US) was integrated in the equation according to the following categories:

$$\text{CV} = [\alpha (1, 2, 3) \times \beta (1, 2, 3) \times \gamma (1, 2, 3)] + \delta (1, 2) \quad (2)$$

where:  $\alpha (1, 2, 3)$  = An estimation of the relative value of the cows according to parity. A value of 1 was assigned for the

first parity, 0.8 for the 2<sup>nd</sup> parity and 0.5 for ≥3<sup>rd</sup>.  $\beta (1, 2, 3)$  = estimation of the relative value of the cows according to stage of lactation. Duration of lactation was fixed to 305 days and was used to calculate the time from treatment to the end of the lactation. Accordingly, a value of 1 was assigned to cows treated during the first 100 days in lactation, 0.8 for those treated between 100 and 200 days DIM and 0.5 for those treated >200 DIM.  $\gamma (1, 2, 3)$  = the net income from milk was calculated as the average daily milk yield during 10 days before treatment multiplied by 0.1.  $\delta (1, 2)$  = the net income from a pregnant cow. If a cows was pregnant (>45 days in DIM) it contributed \$100 to the value of the cow (CV).

Expected recovery of the cow (ER):

$$\text{ER} = \text{CT} \times \{100/[\varepsilon (1, 2, 3) + \chi (1, 2) + \mu (1, 2)/3]\} \quad (3)$$

where:  $\varepsilon (1, 2, 3)$  – Cure according to type of bacteria, calculated as 60% cure for CNS, 50% for *Streptococci* and 20% for PIEc;  $\chi (1, 2)$  – is the cure during periods (I-T), with 50% cure for treatment within 50 days from infection, 30% for 50-100 d and 20% for >100 days;  $\mu (1, 2)$  is the cure according to SCCi, 50% cure for <300×10<sup>3</sup> and 30% for >300×10<sup>3</sup> cells/mL.

By subtracting equations 2 & 3 we obtain the treatment feasibility (TF):

$$\text{TF} = \text{CV} - \text{ER} \quad (4)$$

The value TF could be compared with the alternative options: no intervention, drying-off milk secretion of an infected gland, or culling.

According to the literature, cows with chronic subclinical mastitis exhibit mild inflammation (SCC <400×10<sup>3</sup> cells/mL milk) produce ~5% less milk with low influence on the bulk milk tank SCC (BMTSCC). Thus, no intervention can be calculated as 5% of the annual milk yield × milk price, i.e., for a cow producing 10,000 kg/305 days means a loss of 500 kg milk.

## RESULTS

No significant differences between the parameters analyzed in the 2 periods of the experiment (2007–2009 and 2012–2014) were found; therefore, the report covers the average of all data grouped together. First lactation cows had a significant higher cure ( $P = 0.003$ ) than 2<sup>nd</sup> lactation cows, while 3<sup>rd</sup> and higher lactation cows were in between (66.22%, 38.46% and 48.72%, respectively; Table 1). The main bacteria involved in the cases of SC mastitis were: various strains of CNS, mainly *Staphylococcus chromogams* (58.9%), *Streptococci*,

mainly *Strep. Dysgalactiae* (52.5%) and PIEc (30%). Cows identified as PIEc were treated 30-40 days after the infection, some of them with no isolation of the bacteria at that time. The effect of infection timing within lactation on cure was significant ( $P<0.05$ ): When treatment took place up to 50 days postpartum the cure was ~70%, while when treatment took place between 51-100 days, cure declined to 60% and to ~45% if the cows were >100 days into lactation. The effect of the time elapsed between estimated day of infection and treatment (I-T) was significant ( $P<0.05$ ): When treatment took place up to 50 days from infection, cure was ~65%. When treatment took place between 51-100 days from infection, cure declined to 51%, and was ~41% if the cows were treated >100 days after infection. The effect of SCC at time of treatment was significant ( $P<0.05$ ). When cows were treated while having up to  $300 \times 10^3$  cells/mL, cure was ~68% and with SCC  $>300 \times 10^3$  cells/mL cure was ~44% cure (Table 2).

The process of drying-off a single gland with CNH was applied to 27 cows, some of which were treated earlier with

antibiotics with treatment failure. The bacteria involved in the infected glands were mainly *Strep. uberis* and PIEc. At the time of treatment, cows had one infected gland with SCC  $>10^6$  cells/mL for at least 3 months or a “destroyed” gland with yellowish liquid secretion (not milk). The treated glands were not milked post treatment and involution occurred within 1-10 days after visual disappearance of pressure, swelling and pain. Drying off the infected glands and discarding its milk were reflected in an overall reduction of SCC to  $<200 \times 10^3$  cells/mL. During 30 days post-treatment, milk yield of the remaining three uninfected quarters decreased on the average by ~8%. High yielding cows, which were close to parturition, had the highest decrease in milk yield whereas in cows producing ~30 kg/d, almost no change in milk yield was recorded later in lactation. The cows treated with CNH had proceeded into their next lactation, all delivered healthy calves and they returned to ~85% functionality (~100% if the initially destroyed glands discounted) with no bacterial isolation in the subsequent lactation.

**Table 1.** Percent cure according to the major variables tested.

Variable	Level	N* <sup>1</sup>	Cure (%)	Significance <sup>2</sup>
Parity	1	74	66.22	A
	2	39	38.46	B
	3+	39	48.72	AB
Bacteria	PIEc	20	30.00	B
	CNS	73	58.90	A
	<i>Streptococci</i>	59	52.54	A
Day inf (I) (d) <sup>3</sup>	1	80	53.75	A
	2-100	36	61.11	A
	>100	36	50.00	A
Day_t (T)(d) <sup>4</sup>	0-50	27	70.37	A
	51-100	42	59.52	AB
	>100	83	46.99	B
Period (I-T) (d) <sup>5</sup>	0-50	66	65.15	A
	51-100	41	51.22	AB
	>100	44	40.91	B
LSCC <sup>6</sup>	0-300	28	67.86	A
	301-1,000	58	44.83	B
	>1,000	65	46.92	B

<sup>1</sup> N\* - number of cows

<sup>2</sup> A different letter within a variable denotes significant difference at  $P<0.05$  or lower.

<sup>3</sup> Day\_inf (I) = day of estimate infection according to high SCC

<sup>4</sup> Day\_t (T) = days in milk at time of treatment

<sup>5</sup> Period (I-T) = difference between estimated day of infection and treatment

<sup>6</sup> LSCC<sub>i</sub> = log SCC level at day of infection

**Table 2.** Calculation of the economic cost of treating subclinical mastitis during lactation for examples reflecting common situations in modern dairy farms, according to the equations presented in the Material and Methods section.

	Example					
	1	2	3	4	5	6
Lactation	1	1	2	2	3	3
DIM	40	120	40	120	40	40
Milk yield (kg/d)	32	30	55	47	55	55
I-T (d) <sup>1</sup>	40	70	40	70	40	40
Bacteria	<i>Strep.</i>	CNS	<i>Strep.</i>	CNS	<i>Strep.</i>	PIEc
SCC ( $\times 10^3$ )	1,000	270	1,000	270	270	1,000
Pregnancy	No	Yes	No	Yes	No	No
CV (\$US) <sup>2</sup>	2,732	2,818	2,795	2,842	2,732	2,709
ER (\$US) <sup>3</sup>	594	462	894	733	917	968
TF (\$US) <sup>4</sup>	2,138	2,357	1,902	2,109	1,815	1,741
No intervention (\$US)	-	2,536	-	2,260	2,427	-
TF - No		-179		-451	-612	
Drying-off gland <sup>5</sup>	2,881		2,344			2,257
TF - dry-off	-143		-442			-517

<sup>1</sup> I-T = (I) estimated day of infection - (T) time of treatment

<sup>2</sup> CV (cow value) - An estimation of the relative value of the cows according to parity, stage of lactation, the net income from milk, the net income from the cow being pregnant

<sup>3</sup> ER (expected recovery) - CT (cost of treatment; medication, veterinary service, milk discarded) · Cure according to type of bacteria, periods (I-T), SCC

<sup>4</sup> TF (treatment feasibility) - TF = CV - ER

<sup>5</sup> Dry off gland with casein hydrolyzate (CNH)

The above-described information served for inserting numerical values into equations 1 to 4, which were used for calculating of the economic cost of treating SC mastitis during lactation (Table 2). According to the output from these equations, the main factor which affected treatment feasibility appeared to be the milk wasted during the antibiotic treatment, which in-turn depended on the time required for milk withdrawal owing to safety considerations.

## DISCUSSION

Accurate evaluation of the economic impacts of mastitis and cost of mastitis treatment has critical effects on the profitability of dairy farms (11, 45, 46). In the present study, simple equations based on the monthly routine milk recordings and on-line outputs available in modern dairy farms were derived, which could help farmers to attain an optimal solution on how to cope with cases of SC mastitis in their farms.

Milk quality is the key for high quality dairy products. The industry controls milk quality by imposing regulations for milk acceptance at the dairy plant level, such as bacterial count and BMTSCC. As a result, milk from clinically infected udders should not be milked into the bulk milk tank. On the other hand, milk of SC infected udders could be added into the bulk milk tank as long as the bacteria that caused the infection are not hazardous to humans through milk consumption and the quality of the milk is acceptable according to the regulations.

On the farm level, in most instances SC mastitis cases are chronic and can persist through the entire lactation period until the dry-off period between lactations, which might result in lowered milk yield (47) and with negative effects on reproduction (15). Thus, the decisions for non-intervention, to treat infected udders with antibiotics or to dry-off infected quarters in SC-infected cows have economic implications. In the case of no treatment, the economic loss which relates to SC cases consists of a decrease in milk yield of ~5% through a lactation (11,000 - 550 = 10,450 kg/milk, 305 days), increase in the number of inseminations (~20%) and increase in extra open days (~15%) (15). Additionally, SC IMI could result in lower milk prices due to increased SCC, especially in countries with penalties for high SCC. Antibiotic treatments have a direct cost, which comprise mainly of wasted milk during the antibiotic clearing from the glands and the cost of the veterinarian visit, laboratory diagnostics and medication. The success of the antibiotic treatment depends on bacterial

species, parity and time elapsed from infection, as well as availability of appropriate medications. Accordingly, our data suggest that antibiotic treatment should be used only for treating high-value cows, such as first lactation (replacement of first year cows with heifers is particularly costly because the cows do not cover their rearing expenses). Of note, treatment of the 2<sup>nd</sup> lactation cows had the lowest success (~14% cure). It is suggested that these cows are the most sensitive to infections as they are not yet fully mature, but already reached increased milk production over the first lactation of an ~15-20% compared with cows at first lactation.

The timing of detecting SC infection is complicated and many of the cows are recognized as infected only at the routine monthly milk test. The importance of identifying cows with SC infection as close as possible after the bacteria entered the gland is a clear output of this study and it is consistent with previous reports (21, 22, 23). On-line computerized data, particularly those related to milk yield, SCC and conductivity, is available in many modern farms. Our data showed that such information can help in identifying cows close to the onset of the infection (unpublished data).

The results also suggest that if the medication is not highly successful in eradicating the bacteria, then the overall cost of treatment might be even higher, which is consistent with the conclusions of other researchers (1, 48). In 51 large dairy herds in WI, USA, where clinical mastitic cows were treated, ~35% of the cultures were negative for bacterial presence and in 17% antibiotic was administered without evidence of cure (7). In Finland, ~20-50% spontaneous cure of SC mastitis was found, which might actually be "false cures" (23, 49). Based on the above described results and present results, it can be concluded that diagnosing the bacteria that causes infection and its sensitivity to available medications is an essential step in fighting mastitis. Ignoring this step can lead to failing in curing the cow and in addition, it leads to extra expenses due to unneeded withholding of milk.

Drying-off a gland in cases where it produces low quality milk with SCC  $\sim 1,000 \times 10^3$  cells/mL is very important because it affects the overall cow milk yield and reproductive performance and because it may subject the milk to poorer categorization and pricing by dairy plants that collect the farm milk. However, the alternative is treatment with antibiotics and according to the present results, the cost in such case is even higher, as presented in the example in Table 2.

It seems that only new drugs such as CNH, where one

or more glands of a cow can be treated during milking and with minimal milk loss during the treatment (no withholding time) could be the solution to this problem. This aspect was demonstrated in an experiment on the farm level. When CNH was used to dry-off udders, many glands returned to full functionality with high cure of existing infections and even with increased milk production in the following lactation (28). The physiological basis for those effects and for the immediate improvement of milk quality are related to forceful activation of the glandular innate immune system, an effect that was reflected in high cure rates of up to 90% (14, 31) with reversion of glands to full activity in the subsequent lactation (28, 30, 33, 34). Moreover, in many cases, reduction in milk yield in the existing lactation is minor, most likely due to compensation of milk production by the other glands, consistent with previous findings (50, 51).

## CONCLUSIONS

An effort was made to provide farmers with a tool for making decisions in handling subclinical mastitis in a large dairy herd, based on data available from the herd management system and the economic value of the cow and with the associated expenses of the treatments. Our data clearly demonstrated that the cost of discarding milk during antibiotic treatment, the most conventional and frequently suggested procedure by veterinarians, is more costly than no intervention. The study also highlighted the importance of developing “green” alternative medications for treating mastitis, which will eliminate the need to discard milk following the treatment.

Overall, the combination of the equations reported here with the scheme reported before and verified once again here, allows a farm manager in a given farm to reach the best treatment decisions for treating cases of SC mastitis in terms of cure (or improved milk quality) and cost of treatment. The methodology is simple and flexible and can be adapted to various situations by modifying the constants of the equations to the relevant situation.

## REFERENCES

- Hillerton, J.E. and Berry, E.A.: Treating mastitis in the cow - a tradition or an archaism. *J. Appl. Microbiol.* 98:1250-1255, 2005.
- Hogeveen, H., Huijps, K. and Lam, T.J.G.M.: Economic aspects of mastitis: New developments. *NZ Vet. J.* 59:16-23, 2011.
- Huijps, K., Lam, T.J.G.M. and Hogeveen, H.: Costs of mastitis: facts and perception. *J. Dairy Res.* 75:113-120, 2008.
- Losinger, W.C.: Economic impacts of reduced milk production associated with an increase in bulk-tank somatic cell count on US dairies. *J. Am. Vet. Med. Assoc.* 226:1652-1658, 2005.
- Botrel, M.A., Haenni, M., Morignat, E., Sulpice, P., Madec, J.Y. and Calavas, D.: Distribution and antimicrobial resistance of clinical and subclinical mastitis pathogens in dairy cows in Rhône-Alpes, France. *Foodborne Pathog. Dis.* 7:479-487, 2010.
- Pitkälä, A., Haveri, M., Pyörälä, S., Myllys, V. and Honkanen-Buzalski, T.: Bovine mastitis in Finland 2001 - prevalence, distribution of bacteria, and antimicrobial resistance. *J. Dairy Sci.* 87:2433-2441, 2004.
- Oliveira, L. and Ruegg, P.L.: Treatments of clinical mastitis occurring in cows on 51 large dairy herds in Wisconsin. *J. Dairy Sci.* 97:5426-5436, 2014.
- Ruegg, P.L.: Responsible Use of Antibiotics for Treatment of Clinical Mastitis. <http://articles.extension.org/pages/72958/responsible-use-of-antibiotics-for-treatment-of-clinical-mastitis>, 2016.
- Hertl, J.A., Schukken, Y.H., Welcome, F.L., Tauer, L.W. and Gröhn, Y.T.: Pathogen-specific effects on milk yield in repeated clinical mastitis episodes in Holstein dairy cows. *J. Dairy Sci.* 97:1465-1480, 2014.
- Shalloo, L., Dillon, P., Rath, M. and Wallace, M.: Description and validation of the Moorepark dairy system model. *J. Dairy Sci.* 87:1945-1959, 2004.
- Steenefeld, W., van Werven, T., Barkema, H.W. and Hogeveen, H.: Cow-specific treatment of clinical mastitis: An economic approach. *J. Dairy Sci.* 94 174-188, 2011.
- Bezman, D., Lemberskiy-Kuzin, L., Katz, G., Merin, U. and Leitner, G.: Influence of intramammary infection of a single gland in dairy cows on the cow's milk quality. *J. Dairy Res.* 82:304-311, 2015.
- Blum, S.E., Heller, E.D. and Leitner, G.: Long term effects of *Escherichia coli* mastitis. *Vet. J.* 201:72-77, 2014.
- Silanikove, N., Merin, U., Shapiro, F. and Leitner, G.: Milk metabolites as indicators of mammary gland functions and milk quality. *J. Dairy Res.* 81:358-363, 2014.
- Lavon, Y., Ezra, E., Leitner, G. and Wolfenson, D.: Association of conception rate with pattern and level of somatic cell count elevation relative to time of insemination in dairy cows. *J. Dairy Sci.* 94:4538-4545, 2011.
- Leitner, G., Merin, U. and Silanikove, N.: Effects of glandular bacterial infection and stage of lactation on milk clotting parameters: Comparison among cows, goats and sheep. *Int. Dairy J.* 21:279-285, 2011.
- Pyörälä, S. and Taponen, S.: Coagulase-negative staphylococci - Emerging mastitis pathogens. *Vet. Microbiol.* 134:3-8, 2009.
- Berry, E.A. and Hillerton, J.E.: The effect of selective dry cow treatment on new intramammary infections. *J. Dairy Sci.* 85:112-121, 2002.
- Johnson, A.P., Godden, S.M., Royster, E., Zuidhof, S., Miller, B. and Sorg, J.: Randomized noninferiority study evaluating the efficacy of 2 commercial dry cow mastitis formulations. *J. Dairy Sci.* 99:593-607, 2016.
- Scherpenzeel, C.G.M., den Uijl, I.E.M., Van Schaik, G., Riekerink, R.O., Keurentjes, J.M. and Lam, T.J.G.M.: Evaluation of the use of dry cow antibiotics in low somatic cell count cows. *J. Dairy Sci.* 97:3606-3614, 2014.

21. Barlow, J., White, L., Zadoks, R.N. and Schukken, Y.H.: A mathematical model demonstrating indirect and overall effects of lactation therapy targeting subclinical mastitis in dairy herds. *Prevent. Vet. Med.* 90:31-42, 2009.
22. Steeneveld, W., Swinkels, J. and Hogeveen, H.: Stochastic modeling to assess economic effects of treatment of chronic subclinical mastitis caused by *Streptococcus uberis*. *J. Dairy Res.* 74:459-467, 2007.
23. van den Borne, B.H.P., van Schaik, G., Lam, T.J.G.M. and Nielen, M.: Therapeutic effects of antimicrobial treatment during lactation of recently acquired bovine subclinical mastitis: Two linked randomized field trials. *J. Dairy Sci.* 93:218-233, 2010.
24. Lago, A., Godden, S.M., Bey, R., Ruegg, P.L. and Leslie, K.: The selective treatment of clinical mastitis based on on-farm culture results: I. Effects on antibiotic use, milk withholding time, and short-term clinical and bacteriological outcomes. *J. Dairy Sci.* 94:4441-4456, 2011.
25. Lago, A., Godden, S.M., Bey, R., Ruegg, P.L. and Leslie, K.: The selective treatment of clinical mastitis based on on-farm culture results: II. Effects on lactation performance, including clinical mastitis recurrence, somatic cell count, milk production, and cow survival. *J. Dairy Sci.* 94:4457-4467, 2011.
26. McDougall, S., Parker, K.I., Heuer, C. and Compton, C.W.R.: A review of prevention and control of heifer mastitis via non-antibiotic strategies. *Vet. Microbiol.* 134:177-185, 2009.
27. Heymann, D.L.: *The world health report 2007: A safer future: global public health security in the 21st century.* Prentice, T., Reinders, L.T., (Eds). World Health Organization, 2007.
28. Leitner, G., Jacoby, S., Maltz, E. and Silanikove, N.: Casein hydrolyzate intramammary treatment improves the comfort behavior of cows induced into dry-off. *Livest. Sci.* 110:292-297, 2007.
29. Leitner, G., Koren, O., Jacoby, S., Merin, U. and Silanikove, N.: Options for handling chronic subclinical mastitis during lactation in modern dairy farms. *Isr. J. Vet. Med.* 67:162-169, 2012.
30. Shamay, A., Shapiro, F., Leitner, G. and Silanikove, N.: Infusions of casein hydrolyzates into the mammary gland disrupt tight junction integrity and induce involution in cows. *J. Dairy Sci.* 86:1250-1258, 2003.
31. Silanikove, N., Iscovich, J. and Leitner, G.: Therapeutic treatment with casein hydrolyzate eradicates effectively bacterial infection in treated mammary quarters in cows. In: H. Hogeveen, Ed., *Mastitis in Dairy Production: Current Knowledge and Future Solutions*, pp. 327-332. Wageningen Academic Publishers, Wageningen, the Netherlands. 2005.
32. Silanikove, N., Shapiro, F., Merin, U. and Leitner, G.: Tissue-type plasminogen activator and plasminogen embedded in casein rule its degradation under physiological situations: manipulation with casein hydrolysate. *J. Dairy Res.* 80:227-232, 2013.
33. Leitner, G., Jacoby, S. and Silanikove, N.: An evaluation of casein hydrolyzate in combination with antibiotic for bacterial cure and subsequent increase in milk yield in dairy cows. *BMC Vet. Res.* 7:3, 2011.
34. Silanikove, N., Shapiro, F., Shamay, A. and Leitner, G.: Role of xanthine oxidase, lactoperoxidase, and NO in the innate immune system of mammary secretion during active involution in dairy cows: manipulation with casein hydrolyzates. *Free Radic. Biol. Med.* 38:1139-1151, 2005.
35. Oleggini, G.H., Ely, L.O. and Smith, J.W.: Effect of region and herd size on dairy herd performance parameters. *J. Dairy Sci.* 84:1044-1050, 2001.
36. Demircan, V. and Binici, T.: Effect of herd size on sustainability of dairy production. *Asian J. Anim. Vet. Advances* 4:60-65, 2009.
37. Nir, O.: What are production diseases, and how do we manage them? *Acta Vet. Scand.* 44:S21-S32 (Suppl. 1), 2003.
38. Katz, G., Arazi, A., Pinsky, N., Halachmi, I., Schmilovitz, Z., Aizinbud, E. and Maltz, E.: Current and near term technologies for automated recording of animal data for precision dairy farming. *J. Anim. Sci.* 85:377 (Suppl. 1), 2007.
39. Maatje, K., Huijsmans, P.J.M., Rossing, W. and Hogewerf, P.H.: The efficacy of in-line measurement of quarter milk electrical conductivity, milk yield and milk temperature for the detection of clinical and subclinical mastitis. *Livest. Product. Sci.* 30:239-249, 1992.
40. Sørensen, L.P., Bjerring, M. and Løvendahl, P.: Monitoring individual cow udder health in automated milking systems using online somatic cell counts. *J. Dairy Sci.* 99:608-620, 2016.
41. Leitner, G., Jacoby, S., Frank, E. and Shacked, R.: Options for Handling Mastitis during Lactation in Modern Dairy Farms. *Isr. J. Vet. Med.* 69:141-145, 2014.
42. Leitner, G., Krifucks, O., Merin, U., Lavi, Y. and Silanikove, N.: Interactions between bacteria type, proteolysis of casein and physico-chemical properties of bovine milk. *Int. Dairy J.* 16:648-654, 2006.
43. NCCLS: Performance standards for antimicrobial disk and dilution susceptibility tests of bacteria isolates from animals. Approved Standard M31-A, Wayne, PA, USA. 1999.
44. SAS Institute.: *JMP Statistics and Graphics Guide, Version 5.* SAS Institute Inc., Cary, NC, USA. 2000.
45. Pinzón-Sánchez, C., Cabrera, V.E. and Ruegg, P.L.: Decision tree analysis of treatment strategies for mild and moderate cases of clinical mastitis occurring in early lactation. *J. Dairy Sci.* 94:1873-1892, 2011.
46. van den Borne, B.H.P., Halasa, T., van Schaik, G., Hogeveen, H. and Nielen, M.: Bioeconomic modeling of lactational antimicrobial treatment of new bovine subclinical intramammary infections caused by contagious pathogens. *J. Dairy Sci.* 93:4034-4044, 2010.
47. Fogsgaard, K.K., Løvendahl, P., Bennedsgaard, T.W. and Østergaard, S.: Changes in milk yield, lactate dehydrogenase, milking frequency, and interquarter yield ratio persist for up to 8 weeks after antibiotic treatment of mastitis. *J. Dairy Sci.* 98:7686-7698, 2015.
48. Barkema, H.W., Schukken, Y.H. and Zadoks, R.N.: Invited Review: The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. *J. Dairy Sci.* 89:1877-1895, 2006.
49. Taponen, S., Simojokia, H., Haveria, M., Larsen, H.D. and Pyörälä, S.: Clinical characteristics and persistence of bovine mastitis caused by different species of coagulase-negative staphylococci identified with API or AFLP. *Vet. Microbiol.* 115:199-207, 2006.
50. Hamann, J. and Reichmuth, J.: Compensatory milk production within the bovine udder: effects of short-term non-milking of single quarters. *J. Dairy Res.* 57:17-22, 1990.
51. Hortet, P. and Seegers, H.: Loss in milk yield and related composition changes resulting from clinical mastitis in dairy cows. *Prevent. Vet. Med.* 37:1-20, 1998.