Options for handling chronic subclinical mastitis during lactation in modern dairy farms

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ABSTRACT

Subclinical mastitis is the predominant form of mastitis in modern cow herds, and greatly affects dairy economics. The aim of the present study was to exploit available on-line computerized data to suggest a rational procedure that would enable effective treatment of infected udders. The cows were divided among five categories: no intervention, antibiotic treatment, drying-off specific quarter(s) with casein hydrolyzate, drying-off the whole cow and culling. The first step in the analysis was identification of the infected udder and the causative pathogen. The second step was to determine the sensitivity of the pathogen (mostly bacteria) to antibiotic treatment. Of the 62 high-SCC cows, 40 (64.5%) were cured. The highest cure was achieved in mammary glands infected with *Streptococcus dysgalactiae*, followed by those with *Staphylococcus Chromogenes*. No differences were found among cure of cows in their first to third lactation but significantly lower in lactations 4 and 5. When treatment was applied within a month from the estimated occurrence of the infection, the success was over 73%, whereas treatment after 3 months or more achieved significantly lower success. The average SCC towards the time of antibiotic treatment was ~1.5×10^6 cells mL^{-1}. At first milk testing, ~1 month after treatment, SCC was at a level of ~100,000 cells mL^{-1} and it remained at that level for the subsequent 3 months. The 17 cows that underwent drying-off of a single infected gland had SCC > 10^6 cells mL^{-1} for at least 3 months. Drying off of the secretion from the infected glands reduced the overall SCC to < 200,000 cells mL^{-1}. Milk yield from the uninfected three quarters decreased on average by ~9% during 30 days post-treatment. Treatment options of subclinical mastitis in a commercial dairy herd require early detection, identification of the bacterium and calculation of the economic benefit, taking into account the conditions and the value of the animal to the farmer.

Key words: Mastitis, Treatment, Casein hydrolyzate, Herd management

INTRODUCTION

Economic pressure drives modern dairy farmers to exert continuous efforts to maximize profitability. This is achieved by constant improvement of genetic selection, nutrition, and herd management. Modern dairy farms are characterized by high levels of computerized data acquisition, which provides on-line information on cows' milk yield, milk composition and body weight, and input on cows' behavior, such as step counts, lying duration, and rumination, all of which help farmers to reach their goals (Katz et al., 2007). A side effect of modernization is the increase in herd size to hundreds and even thousands of cows (Oleggini et al., 2003; Demircan & Binici, 2009). One outcome of this trend is the development of a concept that cow herd management and health control should be focused on the herd (Nir, 2003) rather than on the individual cow, as in traditional dairy farming. 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 (Katz et al., 2007).

Mastitis is the single most important factor that imposes economic burdens on dairy farms worldwide. It is estimated that mastitis infections affected 30% of dairy cattle and cost the EU dairy industry about €1.55 billion in 2005 (Hillerton & Berry, 2005) and the US industry US $2 billion (Losinger, 2005). Subclinical infection results in decreased milk yield, deterioration in milk quality, and increased risk of culling (Huijps et al., 2008; Leitner et al., 2011b), especially owing to its wide prevalence, reaching 20 to 40% of the udders in some herds (Hillerton & Berry, 2005; Pyörälä & Taponen, 2009). Many of the cows with subclinical chronic infection are not noticed because there are no recognizable symptoms, and the milk appears normal. Routine monthly milk recording including SCC is a practical procedure in many countries, which could serve as a basis for treatment decisions (Barlow et al., 2009; van den Borne et al., 2010b). However, the elapsed time between two such tests results in delay of the relevant information for a decision.

On-line computerized milking systems which provide measures of changes in milk yield and conductivity can help in the identification of subclinically infected cows soon after the occurrence of the infection (Maatje et al., 1992). Thus, in addition to the identification of visible clinical mastitis that system provides valuable information on new infection, not visible to the farmer, close to its occurrence, which could help in shortening the elapsed time till treatment.

The decision on 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 clinical mastitis needs to be justified with respect to the costs of medicine and milk loss (Steeneveld et al., 2007; Barlow et al., 2009; van den Borne et al., 2010b).

The control means that are currently available in the hands of veterinarians and farmers to enable them to handle mastitis during lactation include treatment with antibiotics, segregating the milk obtained from the infected quarter, obliterating the infected gland or culling the cow.

Recently, a new technique based on infusion of casein hydrolyzate (CNH) into treated glands was shown to be effective in drying-off milk secretion from the treated gland. This procedure imitates in an accelerated mode the process induced during mammary gland involution (Shamay et al., 2003; Silanikove et al., 2005a; Leitner et al., 2007, 2011a). In addition to the immediate improvement of milk quality, infusion of CNH forcefully activated the glandular innate immune system, an effect that was reflected in high cure rates of up to 90% (Silanikove et al., 2005b) and the gland reverts to full activity in the subsequent lactation (Shamay et al., 2003; Silanikove et al., 2005a; Leitner et al., 2007, 2011a).

The aim of the present study was to suggest a procedure that would enable veterinarians and farmers to exploit mastitis-control as close as possible to the occurrence of the infection by on-line computerized data acquisition or in the lesser preferred routine by the monthly test. The procedures considered were: no intervention, antibiotic treatment, drying-off milk secretion from an infected gland with CNH, drying-off the whole udder or culling.

MATERIALS AND METHODS

Study protocol

The study was carried out in a dairy herd of 200 lactating Israeli Holstein cows at the Agricultural Research Organization, the Volcani Center. The dairy parlor is equipped with an on-line computerized AfiFarm Herd Management (S.A.E. Afikim, Israel) data acquisition system that includes the AfiLab (S.A.E. Afikim, Israel) milk analyzer, which provides on-line data on milk gross composition (fat, protein and lactose) and milk conductivity (a measure of mastitis)(http://www.afimilk.com). The cows were milked three times a day, and the average milk yield (MY) in this farm throughout 2008 was ~11,500 L during 305 days of lactation. 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 170,000 to 220,000 cells mL^{-1}. Clinical udder infections were treated with antibiotics according to the herd veterinarian's decision. Daily on-line cow exception report (conductivity, MY and animal behavior) produced by the computerized system and the monthly routine milk recording served as the basis for identification of suspected cows. The cows identified by the on-line computer and cows with SCC > 200,000 cells mL^{-1} (monthly routine milk recording) were examined by quarter, for bacteriology, CMT and SCC (Leitner et al., 2006). If a bacterium was isolated accompanied with SCC > 200,000 cells mL^{-1}, an antimicrobial susceptibility test was performed in accordance with NCCLS guidelines (NCCLS, 1999) by means of commercially available disks – Dispens-O-Disc (Susceptibility Test System, Difco) or BBL Sensi-Disc Antimicrobial Susceptibility Test Discs (Becton Dickinson, MD, USA) – which were applied as recommended by the manufacturers. The plates were incubated at 30°C for methicillin (5 μg/disk), and at 37°C for other antibiotics: penicillin G (10 units/disk), erythromycin (15 μg/disk), cephalothin (30 μg/disk), neomycin (30 μg/disk), trimethoprim-sulfamethoxazol (1.25-23.75 μg/disk). The results were interpreted in terms of susceptibility or resistance, according to the manufacturers' recommendations.

Cows were treated with antibiotics except for cows that had only one quarter with SCC > 10^6 cells mL^{-1} for at least three months with or without isolated bacteria. The latter cows and cows that failed the antibiotic treatment underwent quarter drying-off with CNH.

Cows treated with antibiotic were infused with a tube of Nafpenzal MC (180 mg Penicillin G (300000 I.U.), 100 mg Dihydrostreptomycin, 100 mg Nafcillin) or DC (300 mg Procaine benzylpenicillin (300000 I.U.), 100 mg Dihydrostreptomycin, 100 mg Nafcillin)(Intervet, Boxmeer, The Netherlands) daily for 3 days in all quarters, both infected and uninfected, together with 50 mL of 30 PEN and intramuscularly injected GENTAJECT (ABIC Biological Laboratories, Teva Ltd., Israel). Treatments were carried out after the midday milking and the treated cows were not milked at the evening milking. The milk was discarded for a few days in accordance with the instructions of Delvotest (DSM Food Specialties, Delft, The Netherlands), and was then tested daily for antibiotic residues till complete disappearance of inhibition.

Cows treated with CNH (prepared under Good Laboratory Practice conditions; Leitner et al., 2007) received one or two infusions of 10 mL of peptide concentrate of the CNH preparation, at ~7 mg mL^{-1}, into the infected quarter at the midday milking and were not milked at the evening milking. Milk yield per cow was calculated as daily MY at 30 days before treatment and 30 days from the 5^{th} day post- treatment. At the next and subsequent milkings, the remaining untreated quarters were milked normally. Dried-off cows received the routine treatment of the herd, i.e., Nafpenzal DC (Leitner et al., 2007). Food was offered ad lib in mangers located in the sheds.

Bacterial cure was defined as the non-appearance of the bacterium and SCC < 150,000 cells mL^{-1} in milk sampled from the treated quarter tested monthly for the first 100 days following treatment.

Statistical analysis

All statistical analyses were carried out with JMP software (SAS Institute, 2000). The effects of infection-causing agents *(Strep. dysgalactiae, Strep. uberis, Staph. aureus, Staph. chromogenes*), lactation number (1-5), time (1, 2, 3 months) of incidence of high SCC (> 200,000 cells mL^{-1}) and DIM (< 99, 100-200, > 200) on cure were compared separately by means of the chi-squared (χ^2) test. These results were further analyzed for the major effects – causative bacterial infection, lactation number, DIM and time (month) of incidence – with a nominal logistic test.

RESULTS

Overall, 62 cows with high SCC (> 200,000 cells mL^{-1}) and/or identified bacteria (Fig. 1A-D) were treated with antibiotics and the infected quarters of 17 cows were dried-off with CNH. The on-line system identified 22 of the 62 cows most of which had very low SCC (~30,000 cells mL^{-1}) at the last routine milk recording. All the bacteria tested were found to be sensitive to at least one of the antibiotic present in the drugs used. Of the 62 cows, 40 (64.5%) were cured. The highest cure rate was achieved in mammary glands infected with *Strep. dysgalactiae*, followed by those with *Staph. chromogenes*, with 81.8% (18/22) and 69.6% (16/23), respectively (Fig. 1A). Cure in cows infected with *Strep. uberis* was lower than 40% (2/5). Interestingly, 4 of the 12 cows without detected causative agent returned to normal milk production with low SCC after treatment. No differences were found among cure of cows in their first to third lactations – ~70% – whereas in older cows (lactations 4 and 5) the treatment success was significantly lower (25%)(Fig. 1B). No significant difference was found between success of treatment applied at the beginning of the lactation and that applied more than 100 days postpartum, but success in the later days of the lactation were lower (Fig. 1C). When treatment was applied within a month from the estimated occurrence of the infection, the cure was over 73%, whereas treatment after 3 months or more achieved significantly lower cure (46.9%)(Fig. 1D). Examination of all the above effects showed that causative agents were the major significant effects (P = 0.027), followed by the elapsed time between initiation of inflammation symptoms and application of treatment (P = 0.058).

Milk yield and SCC of the 40 cured cows are displayed in Fig. 2. The average SCC (Fig. 2A) towards the time of antibiotic treatment was ~1,500,000 cells mL^{-1}. At first milk testing, ~1 month after treatment, SCC was at a normal level (~100,000 cells mL^{-1}) and it remained at that level for the subsequent 3 months. On average, before treatment, milk yield was 35 kg d^{-1} (Fig. 2B). At the first milk testing after treatment, milk production increased slightly, and it remained at the new level for up to 3 months, despite the advancing in DIM which typically is associated with reduced MY.

The 17 cows that underwent single quarter drying-off by CNH were in various lactations (1-4), DIM (60-480), empty or pregnant, with MY of 23-55 kg d^{-1}, were infected with various bacteria – *Strep. dysgalactiae, Strep. uberis, Staph. aureus, Staph. chromogenes, E. coli* or no identified causative agent. All the 17 cows had one infected gland with SCC > 10^6 cells mL^{-1} for at least 3 months, which forced the farmer to milk the infected glands separately because the other three glands were free of bacteria. Several cows had undergone a failed antibiotic treatment. After treatment the infected glands were not milked, and during the following 5 to 15 days the treated quarters atrophied, with disappearance of pressure, swelling and pain. Figure 3 presents the mean changes in SCC and MY, at the cow level, before and after treatment. Drying off of the secretion from the infected glands and cessation of milking were reflected in reduction of the overall SCC to < 200,000 cells mL^{-1}. Milk yield from the uninfected three quarters decreased on average by ~9% during 30 days post-treatment. The largest decrease in milk yield was recorded for cows close to partum, which had high yields, whereas almost unchanged yields were recorded later in the lactation, from cows producing ~30 kg d^{-1}. Eight of the cows treated with CNH had proceeded into their next lactation at the time of closing the study. At drying off, the cows were treated with antibiotic in the three active glands. All the cows delivered healthy calves, and in the subsequent lactation, seven of the eight treated glands functioned normally, with no bacteria being isolated. In the udder of one cow which was infected with *E. coli*, the treated gland remained nonfunctional. The SCC during the first 100 days of the subsequent lactation in all the quarters, including the treated glands, was lower than 100,000 cells mL^{-1}, except for one cow which was re-infected in one of the untreated glands.

DISCUSSION

The aim of the study was to devise a procedure as a management tool to cope with subclinical mastitis during lactation utilizing on-line computerized data. The underlying idea was to exploit the on-line computerized data available in modern farms, particularly those related to milk yield, and information related to SCC, e.g., conductivity, etc., in combination with the data yielded by the routine monthly testing of individual cows' milk, as described in Materials and Methods (Sec. 2.2.). Then, data on the value of the cow to the owner, such as lactation number, days in milk (DIM), MY (daily and throughout the lactation) and reproduction status, together with data on the number of infected quarters, times of infection, identities of bacterial isolates and their sensitivity to antibiotics, formed the basis for the treatment decisions by the herd veterinarian and the owner.

The results clearly indicate that without isolation/identification of the agent causing the infection, antibiotic treatment is not justified. This situation is different from that presented by cases of clinical mastitis, where time of treatment is crucial (Hillerton & Berry, 2005), therefore the decision on use of a certain drug depends on the major causative bacteria isolated in the herd at that point of time. In cases of chronic infection, it is crucial to isolate the causative bacterium and to test its sensitivity to any given antibiotic before selecting a treatment. It should be noted that not every isolate means infection. Thus, it was suggested that "true" infection may be regarded as such only if there is an increase in SCC (due to inflammation), and/or a change in the distribution of leukocytes in the milk with or without the isolation of the causative agent (Leitner, 2010). Moreover, it is crucial to insure that there is an infection because there are many reports on spontaneous cure of subclinical mastitis, 20-50% (Taponen et al., 2006; van den Borne et al., 2010b). Isolation of bacteria without an increase in SCC and/or a change in cell distribution cannot be classified as mastitis, thus repeated culturing, as advised by Berkama et al. (2006), is the preferred methodology. According to these definitions spontaneous cure found in our previous studied in cows and sheep was < 5% (Chaffer et al., 1999; Shwimmer et al., 2008).

The present results also indicated that although a bacterium may be found sensitive to the drug in the laboratory, it was not always eliminated in vivo, as in the case of *Strep. uberis*, and as reported by Barlow et al. (2009). Thus, it is important to test which drug is suitable for each herd and, if the probability of cure is low as the case in *Staph. uberis*, to take that into account in making the decision.

The second lesson that arises from this study concerns the importance of identifying cows with subclinical mastitis as soon as possible after the bacteria enter the gland: as found in other studies, the longer the duration of infection, the lower is the probability of cure (Steeneveld et al., 2007; Barlow et al., 2009; van den Borne et al., 2010b). Using on-line computerized milking devices helps in achieving this task.

The third conclusion is that older cows are more difficult to treat, probably because of more prolonged colonization of the bacteria in the gland tissue, and lower functionality of the immune system (Barkema et al., 2006).

Today, drying-off a gland with a substance that permanently destroys the gland is an option, although it reduces the value of the cow because of decreased milk yield in the current and subsequent lactations. Nevertheless, this treatment would improve the overall SCC in the bulk milk tank to an extent that would increase the premium quality of the milk, and thereby raise its price. However, with a new drug such as the CNH, which is currently under development as a veterinary drug, a cow can be treated in one or more of the glands during lactation, with minimal milk loss during the treatment (no withdrawal time), improved milk quality and consequently reduced SCC. Milk yield of the 3 remaining glands can increase after treatment due to compensation (White et al., 1937, in cows; Leitner et al., 2008, sheep and goats). Moreover, in many cases the CNH treated gland returns to full functionality in the following lactation.

The treatment principles outlined in this study may be used effectively like in a decision-tree analysis (Fig. 4) and as suggested elsewhere (Pinzón-Sánchez et al., 2011). Furthermore, as all the recorded data is computerized, a potential future application would be to use a decision-tree analysis as a framework of rules for treatments and to convert these rules into a computerized algorithm that would resolve the problem of the most appropriate treatment decision automatically.

By taking into account all the considerations evaluated in this study, it could be concluded that it is important in any decision making procedure to calculate economic constraints, taking into account parameters such as price of milk and costs of drugs used, including that of the loss of milk during and after the treatment, until the milk is fit for human consumption (Pinzón-Sánchez et al., 2011; Steeneveld et al., 2011; van den Borne et al., 2010a). It is important to reemphasize that repeated testing, although expensive, is a minor expense compared to the cost of antibiotic treatment.

Therefore, young cows, at the beginning of their lactation and producing high volumes of milk may be expected to cover the costs/losses imposed by the treatment during their productive life. Use of on-line data acquisition was shown to be an effective tool to reach these goals, and the potential availability of CNH in the future will increase the availability of efficient treatments that will be available at the hand of veterinarian and farmers to fight mastitis.

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Figure legends

Figure 1. Effects of antibiotic on cure as influenced by: bacterial sensitivity to the antibiotic (Fig. 1A); cow's age (lactation number; Fig. 1B); lactation stage (days in milk; Fig. 1C); and infection persistence (months of high SCC before treatment; Fig. 1D). Letters above bars with no common superscript differ significantly ($P < 0.05$).

Figure 2. Effects of antibiotic treatment on SCC (Fig. 2A) and milk yield (Fig. 2B), before and after treatment.

Figure 3. Effects of drying specific quarter with casein hydrolyzate (CNH) on SCC (Fig. 3A) and milk yield (Fig. 3B), before and after treatment. Milk yield per cow was calculated as daily milk yield at 30 days before treatment and 30 days from the 5^{th} day post treatment.

Figure 4. A schematic procedure for applying treatment options in cases of subclinical mastitis. The cows were designated to five categories: no intervention; antibiotic treatment; drying-off quarter/s; drying-off whole cow; culling. The first step in the analysis is to identify the infected udder and the causative pathogen. The second step is to determine the sensitivity of the pathogen (usually a bacterium) to antibiotic treatment. The justification for antibiotic treatment would also depend on the cow's age, because older cows will respond poorly to the treatment. An additional important factor is stage of lactation and days in milk – antibiotic treatment is costly because of the costs of treatment and of milk withdrawal. Thus, cows in late lactation most likely would not cover the cost of treatment by their subsequent output. If the cow does not meet the criteria for antibiotic treatment because of insensitivity of the pathogen, it is possible to dry off the infected quarter. If, however, the cow is in late lactation, her dry-off may be accelerated. If a given cow does not meet all of these criteria, a decision to cull her might be reached.

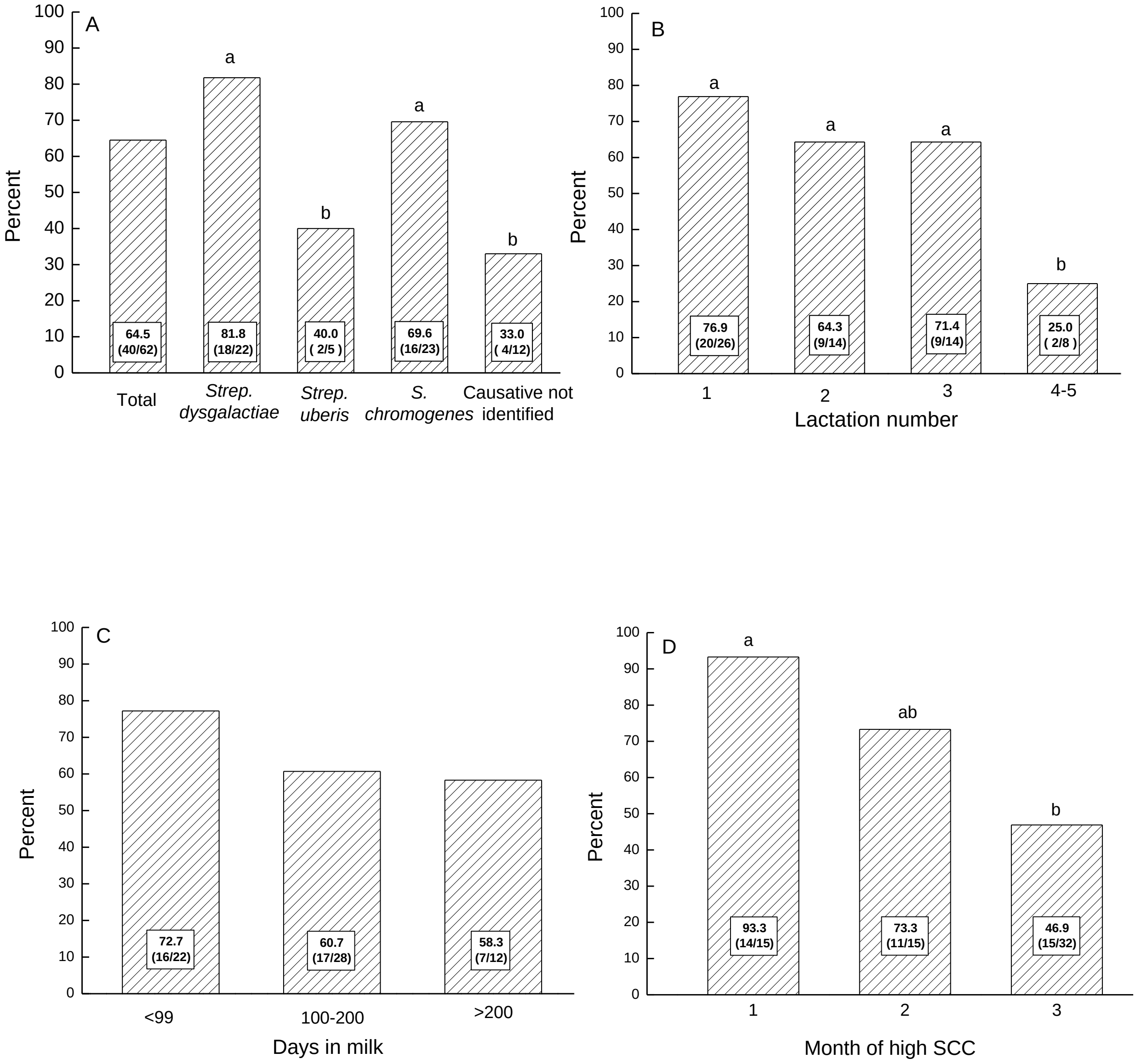
A
Percent
a
a
b
b
64.5 (40/62)
81.8 (18/22)
40.0 (2/5)
69.6 (16/23)
33.0 (4/12)
Total
Strep. dysgalactiae
Strep. uberis
S. chromogenes
Causative not identified
B
Percent
a
a
a
b
76.9 (20/26)
64.3 (9/14)
71.4 (9/14)
25.0 (2/8)
1
2
3
4-5
Lactation number
C
Percent
72.7 (16/22)
60.7 (17/28)
58.3 (7/12)
<99
100-200
>200
Days in milk
D
Percent
a
ab
b
93.3 (14/15)
73.3 (11/15)
46.9 (15/32)
1
2
3
Month of high SCC

Figure 1

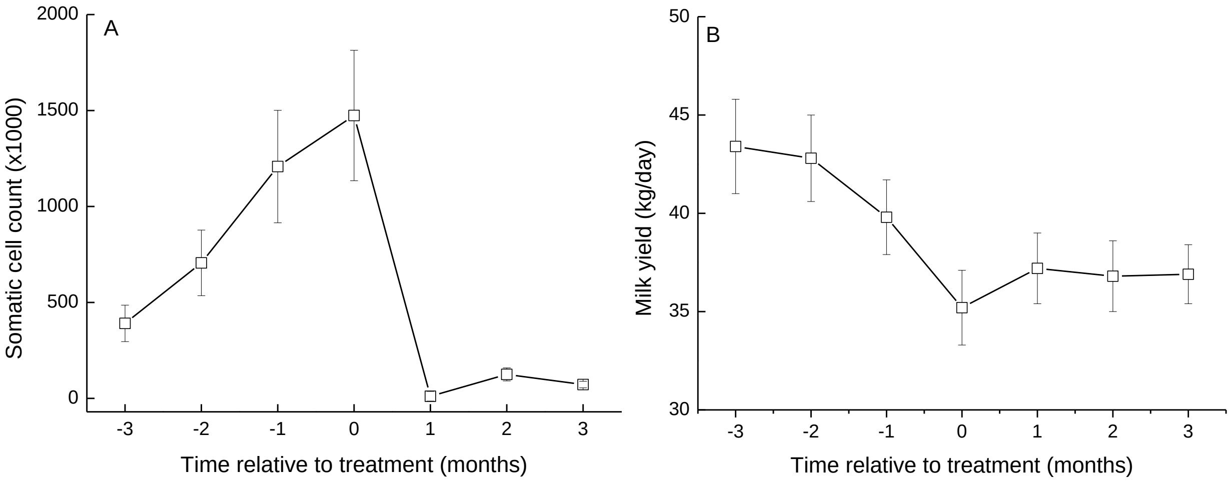
A
2000
1500
1000
500
0
Somatic cell count (x1000)
-3
-2
-1
0
1
2
3
Time relative to treatment (months)
B
50
45
40
35
30
Milk yield (kg/day)
-3
-2
-1
0
1
2
3
Time relative to treatment (months)

Figure 2

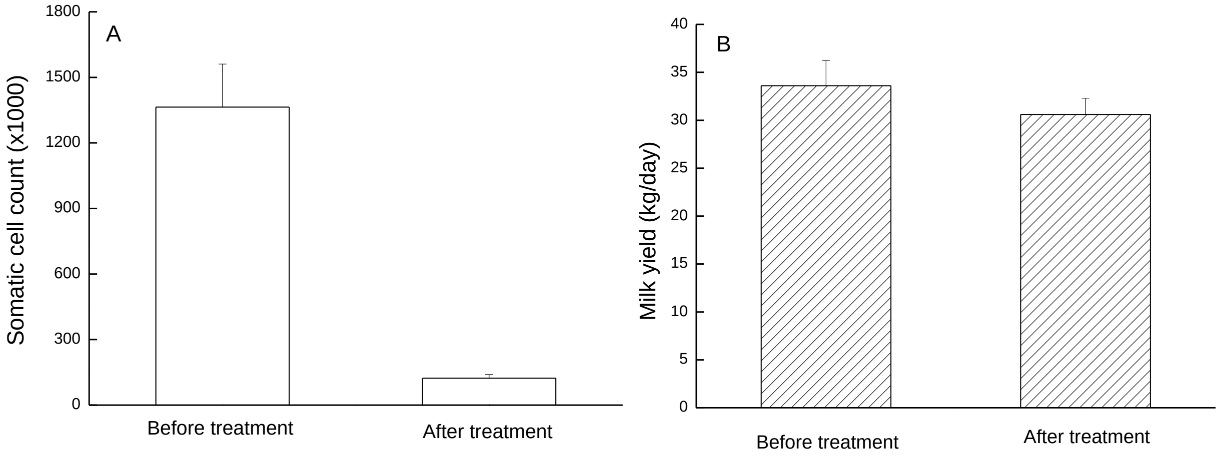
A
Somatic cell count (x1000)
1800
1500
1200
900
600
300
0
Before treatment
After treatment
B
Milk yield (kg/day)
40
35
30
25
20
15
10
5
0
Before treatment
After treatment

Figure 3

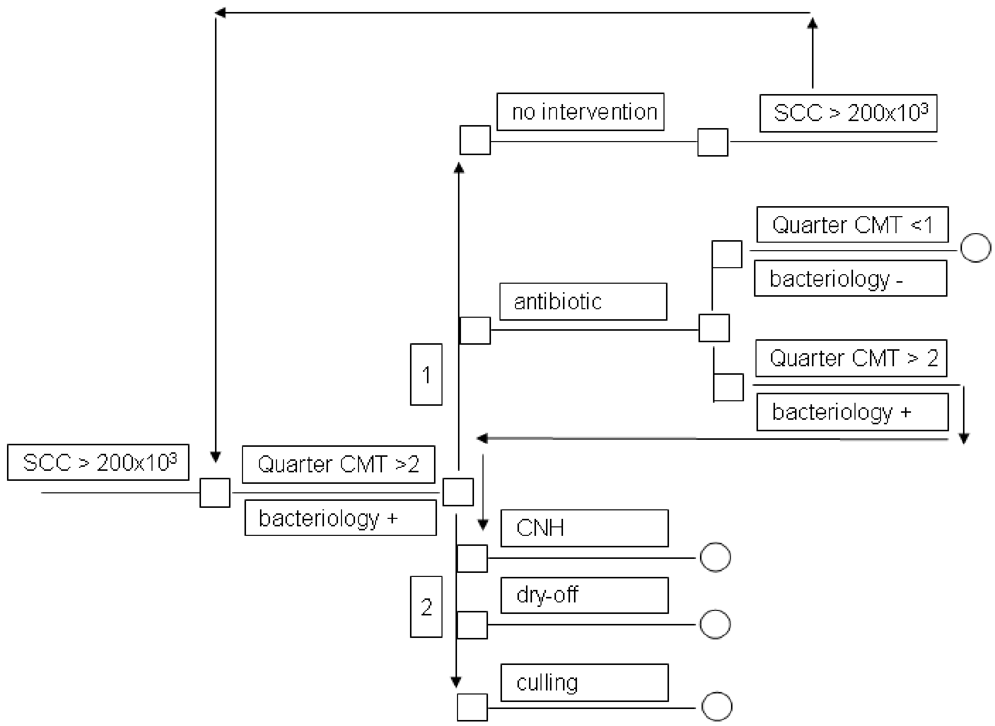
no intervention
SCC > 200x10³
Quarter CMT <1
bacteriology -
antibiotic
Quarter CMT > 2
bacteriology +
1
SCC > 200x10³
Quarter CMT >2
bacteriology +
CNH
2
dry-off
culling

Figure 4