FACTORS ASSOCIATED WITH BOVINE MASTITIS TREATMENT FAILURE

FAKTORI OD KOJIH ZAVISI EFIKASNOST TERAPIJE MASTITISA KOD KRAVA

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Effective mastitis control programmes rely on decreased risk of exposure to mastitis-causing organisms. Cows affected by mastitis are an obvious source of infection to herd mates (regardless of whether the pathogens were contagious or environmental in origin). Decreasing exposure risk by shortening or cessation of the shedding phase, of cows affected by intramammary infection (**IMI**), is a reasonable approach. This can be achieved by different means- temporary or permanent removal from supply, pre-term drying off and lactational or dry cow treatment. Mastitis treatment and its failure, with an emphasis on antimicrobial therapy, are the main focus of this paper. Pharmacokinetic properties of the used formulation are not discussed.

Bovine mastitis treatment failure is common despite an appropriate choice of antimicrobial. Current treatments of clinical mastitis during lactation often have a poorer cure rate than is predicted by in vitro sensitivity, especially in the case of *Staphylococcus aureus* which, as a chronic infection, is responsible for huge economic losses. Estimates of bacterial cure rate for *Staph aureus* mastitis during lactation mastitis fall between 25 and 50% (Sol et al., 2000). Antimicrobial resistance of mastitis-causing organisms (**MCOs**) is commonly not the precipitating factor in treatment failure (Constable and Morin, 2003; Pengov and Ceru, 2003; Hoe and Ruegg, 2005). The explanation should therefore be sought in terms of other factors, which also influence the outcome of therapy (Pengov and Ceru, 2003).

The successful use of antimicrobial drugs for mastitis treatment depends on the same basic principles that apply to all microbial infections: (1) Selecting an effective antimicrobial agent, (2) Attaining and maintaining therapeutic concentrations of the drug at the infection site for long enough, (3) Minimising local or systemic side effects of therapy, and (4) The administration of supportive, non-antimicrobial therapy when indicated (Ziv, 1980).

There are four major groups of factors associated with bovine mastitis treatment failure:

1. Management and iatrogenic factors,
2. Drug factors,
3. Mastitis-causing organism factors, and
4. Mammary gland factors.

Additionally, herd, cow and quarter level factors, can also affect results of the bovine mastitis treatment.

Management and iatrogenic factors

Many management and iatrogenic factors can be the reason for mastitis treatment failure. In the literature (Nickerson, 1987; du Preez, 1988; Soback, 1988; Tyler et al., 1992; Sol et al., 1994; du Preez, 2000; Prescott and Baggot, 1993; Gruet et al., 2001; Pyörälä, 2002b; Dingwell et al., 2003; Pengov and Ceru, 2003; Serieys et al., 2005; Schukken et al., 2007), the following factors are mentioned:

Inaccurate diagnosis

An important factor affecting the cure rate of intramammary infections in cows is the quality and precision of mastitis diagnostics.

In many countries, including New Zealand, mastitis diagnosis and antimicrobials treatment are available and left to the farm personnel. Treatment of bovine mastitis, in such cases, is often empirically based on a presumptive cause and prior historical efficacy. Wrong clinical diagnosis (*i.e.* over-diagnosing, teat canal infections, aetiological factors) leads to inappropriate therapy and, usually, to overuse of antimicrobials.

Relying on the farm personnel's perception of "how well" a particular treatment is working can be also misleading.

Delayed initial treatment

As with any infectious disease, to decrease the risk of failure, bovine mastitis treatment should commence as soon as possible after the clinical appearance. Researchers have found that immediately treating invasive mastitis delays progression of the disease (*i.e.* chronic form or abscess formation; du Preez, 2000; Schukken et al., 2007) and increases treatment success (Sol et al., 1994; Dingwell et al., 2003). The increased risk of failure is due to internationalisation of MCOs and changes associated with the inflammatory response.

Inadequate supportive treatment

Inflammatory change in the mammary gland can impair drug distribution. The use of supportive treatment (anti-inflammatory) can decrease swelling and provide better drug distribution. In cases where mastitis is associated with the "sick cow syndrome" it is essential to have the general condition (*i.e.* shock, toxaemia, dehydration, septicaemia) of the animal addressed. In some cases, such as coliform mastitis, the supportive treatment is a priority to the antimicrobial treatment of mastitis.

Duration of treatment

Field trials with commercial antimicrobial products have demonstrated higher cure proportions with extended mastitis treatment (Oliver et al., 2003; Oliver et al., 2004; Owens et al., 1997; Deluyker et al., 2005). This is due to extended contact time of the antimicrobial with the MCOs. Extended treatment periods, also allow better antimicrobial distribution throughout the gland. However, the benefits of extended therapy have also some drawbacks, including: price of the antimicrobial, discarded milk, and labour, increased risk of introducing super infection through repeated intramammary treatment and increased risk of inhibitory substances grades. The benefits should be weighed against the drawbacks when deciding if extended therapy is a profitable option.

Improper dose

The success of anti-infective treatment depends on the time and drug concentration. Thus, lower dose than required or parenteral administration in dehydrated animal can lead to drug concentrations below required MIC at the site of infection.

Improper route of administration

The drugs used for bovine mastitis treatment can be administered intramammarilly or systemically. The systemic treatment is generally parenteral, to avoid the disturbances of the ruminal and gastro-intestinal micro flora. Following intramammary administration a high concentrations of the antimicrobial in the milk compartment of the mammary gland is expected to be achieved using smaller amounts of the active substances as the drug is administered

straight to the infection site (Gruet, 2001). Intramammary route of administration is advocated for treatment of mild cases of mastitis, associated with no sick-cow syndrome (Erskine et al., 2003). Parenteral therapy has been advocated for treatment of bovine mastitis cases where a significant oedema is present. This may impede the distribution of intramammary administered antimicrobials (Funke, 1961; Rasmussen, 1966; Ziv 1980). Additionally, parenteral treatment should be used when the cow's general health is impaired (Ziv, 1992; Erskine et al., 2003). Pharmacokinetics of the active are important for both routes of administration, in particular associated with parenteral therapy that additionally requires the administration of higher doses of the antimicrobial substances as it will be distributed throughout the cow's body before reaching the mammary gland.

Insertion of infusion cannula

Studies have shown that full cannula insertion through the teat canal can reduce the effectiveness of mastitis treatment. Research has shown that, in some instances, full insertion the intramammary cannula can transfer microbes from the teat end and the keratin lining of the teat canal into the teat cistern. Nickerson, (1987) showed new IMIs introduced in such a way can be reduced by over 50% through partial insertion of the cannula (only into the distal 2-3mm of the teat canal).

Trauma of the teat end or the teat canal is easily colonised by MCOs, particularly staphylococci, which can reinfect the gland at any time. Partial insertion can minimise teat canal and keratin plug damage, maintaining the integrity of the teat canal, an important part of the cow's defence system.

Super infection

Super infection can occur during intramammary treatment if the cannula is contaminated or there is poor sanitation of the teat end prior to treatment. Often, the secondary IMI caused by newly introduced organisms, result in worse clinical implications than if no intramammary treatment was administered at all, particularly if the new MCOs are *Pseudomonas aeruginosa, Candida sp,* and *Nocardia sp.*

Reinfection

Reinfection of treated quarters is a common explanation for bovine mastitis treatment failure in practice and efficacy research. The mechanisms promoting reinfection were listed by Sandholm et al (1990), and they can be summarised as:

a) Treatment or mammary defence mechanisms not sufficiently effective. Thus, an inoculum of MCO has remained.
b) Trauma of the teat canal or teat end. This will be discussed later.
c) Rising of the MCOs. In the upper parts of the mammary gland antimicrobial penetration is poor and cytotoxic effects incite local inflammatory reaction.
d) Increased susceptibility of the gland to re-infection. This will be discussed later.

Achieving clinical but not microbial cure

In many cases, especially with staphylococcal (e.g. *Staph aureus*) and some streptococcal (e.g. *Strep uberis*) infections, mastitis treatment results in a clinical cure while a microbial cure is not achieved. At present, diagnostic methodologies for bovine mastitis are not technically rigorous and a revision of clinical case to a subclinical state is often mistakenly considered as a cure. Achieving clinical but not microbial cure can be result of short duration of treatment or improper route of administration, resulting in low antimicrobial concentrations or required concentrations at shorter than needed time periods. Depression of the multiplication phase of the MCO can also leave a proportion of the microbial population refractory to antibiotic.

Selection of cows for treatment

The probability of a cure can be estimated prior to treatment. There are many factors associated with cure proportions, but in general the following factors are most important: age, lactation stage, causative organism, quarter location and level of ICSCC prior to treatment initiation. For example, Schukken et al (2007) provide following calculations:

Scenario 1. Older cow, treated at 150 days in milk, infected with *Staph aureus* in a hind quarter with a SCC of 2,000,000 cells/mL has approximately 1% chance of cure.
Estimated probability to cure = (1/1[1 + exp (-1 x (0.40-1.25-1.05-1.53-0.95))] = 1%

Scenario 2. Heifer, treated at 220 days in milk, infected with *Staph aureus* in a front quarter with a SCC of 500,000 cells/mL has approximately 61% chance of cure.
Estimated probability to cure = (1/1[1 + exp (-1 x (0.40))] = 61%
(*Calculations reproduced with permission from the author – 11th April 2007*)

A similar predictive model is described by Bradley et al (2005) to aid in the decision to treat sub-clinical mastitis.

The fact that the cow factors can be used to predict the probability to cure is vastly underused by pharmaceutical companies, farmers and veterinarians when selecting candidates for treatment as opposed to culling (Erskine, 2006; Schukken et al., 2007).

Drug factors

Many antimicrobial, vehicle or formulation factors are associated with mastitis treatment failure and discussing these will be out of the scope of this paper. Most of the factors from this group are due to the pharmacokinetic characteristics of the active and the effects of the formulation on these properties. In the literature (du Preez 1988; Sandholm et al 1990; Daley et al 1992; Tyler et al 1992; Prescott and Baggot 1993; du Preez 2000; Erskine et al 2003; Serieys et al 2005) the following factors are mentioned:

• Improper antimicrobial selection • Short half-life of the drug • Inadequate local tissue concentration • Side effects of the drug • Other factors that will lead to inactivation of the antimicrobial *in vivo* or *in vitro*	• Low bio-availability • Weak passage of drug across the blood-milk barrier • High degree of milk and serum protein binding • Antagonism of concurrently used antibiotics

Factors related to mastitis-causing organism factors

Tissue invaders or intracellular location

Tissue invading organisms, such as coagulase-positive staphylococci, become walled off in the udder parenchyma by thick fibrous scar tissue, deep-seated abscesses. They can also gain refuge within the acid phagolysosomes of macrophages and neutrophils. Similarly, some strains of *Strep uberis* seek refuge in the mammary gland epithelial or secretory cells (Tamiselvam et al., 2006). Additionally, chronic *Staph aureus* infections pose therapeutic problems through promotion of localised avascular scar tissue, meaning that parenteral therapy probably provides little benefit (du Preez, 1988; Erskine et al., 1993; du Preez, 2000; Erskine et al., 2003). Therapy may kill the organisms that are not walled off, but at a later date, the organisms within the scar tissue can break out, multiply, cause additional damage to the udder secretory tissue and promote further formation of scar tissue. Consequently, when antimicrobial treatment is administered, antimicrobials cannot reach the MCOs and failure may occur even when the organisms are sensitive to the antimicrobial used (du Preez, 1988).

Microbial dormancy and metabolic state

Mastitis-causing organisms are most susceptible to antimicrobials during their logarithmic growth phase. Non-multiplying organisms are not sensitive to most antimicrobials. All microbial populations contain some organisms that are not in the active growth phase, which therefore survive. Bacteria exposed to antimicrobial may be inhibited from growth and can remain so for some time after the termination of therapy (Soback, 1988; Francis, 1989; du Preez, 2000; Erskine et al., 2003). Low multiplication rates of organisms are seen within phagocytes. According to Prescott and Baggot (1993), this is particularly true for *Staph aureus* infections.

"L" form of mastitis-causing organisms

Sometimes certain organisms develop an acapsular "L" form that is contained only in a cell membrane. Such L-forms are not susceptible to antimicrobials such as penicillins and cephalosporins that attack the cell wall even when the drug concentrations and contact time are sufficient (du Preez, 1988; Owens et al., 1988; du Preez, 2000; Erskine et al., 2003).

Microbial mechanisms that overcome antimicrobial effects in milk

Microbes have survived many years due to their ability of rapid acclimatisation to the environmental conditions. Pathogenic organisms escape antimicrobial factors by capsule or slime formation, receptor-mediated absorption of host proteins into microbes, interference with phagocyte function, leukocidin production or production of enzymes capable of digesting antimicrobials. Other mechanisms include adherence of bacteria to tissue linings that results in avoidance of the wash-out effect of milking, upward flotation of microbes with cream, and an increase in microbial replication rate (Sandholm et al., 1990; Barrio et al., 2000; du Preez, 2000; Vasi et al., 2000).

Short lived mastitis-causing organisms in the mammary gland

Some MCOs, particularly coliforms, are short lived in the mammary gland. Thus, the antimicrobial therapy may be of secondary importance relative to immediate supportive treatment of general condition (*i.e.* endotoxic shock).

Drug tolerance and resistance

Drug tolerance and resistance are usually species or strain characteristics. The widespread use of antimicrobials in mastitis treatment has raised the question of antimicrobial resistant strains of mastitis-causing organisms flourishing in preference to susceptible strains. Selection for resistant organisms may necessitate use of a different antimicrobial (du Preez, 1988; Soback, 1988).

However, even though, widely varying results of the efficacy of antimicrobial treatments have been reported, there is no real evidence that antimicrobial resistance poses an emerging crisis in bovine mastitis pathogenesis and treatment (Erskine 2006).

Selecting an ineffective antimicrobial agent, such as penicillin to treat β-lactamase-producing *Staph aureus* or *Bacteroides fragilis* (du Preez, 1988; Sandholm et al., 1990; Malinowski et al., 2002; Erskine et al., 2003) can result in treatment failure.

It is the opinion of the author that antimicrobial sensitivity testing should be used to make "herd profiles", regarding resistance. The choice of inappropriate drug, should not be an excuse for bovine mastitis treatment failure, particularly when antimicrobial sensitivity/resistance testing is readily available. The "herd profile" can have limitted merit in herd affected with environmental mastitis due to the diverse source of MCOs.

Mammary gland factors

Poorer uneven distribution and physical obstruction

In all cases of mastitis, oedema and inflammatory products to a certain extent obstruct the diffusion of antimicrobials by compression or blockage of the milk duct system, (Ziv, 1980; du Preez, 1988; Soback, 1988; Francis, 1989; Sandholm et al., 1990; Ziv, 1992; du Preez, 2000; Malinowski et al., 2002; Erskine et al., 2003), as does extensive necrosis (Prescott and Baggot 1993; du Preez, 2000) of the affected area of the gland and abscess formation (du Preez, 1988; Sandholm et al., 1990; McKellar, 1991; Daley et al., 1992; Erskine et al., 2003). The diffusion of antimicrobial solutions throughout the gland is impaired and for this reason it is often very difficult to bring antimicrobials into contact with MCO, particularly by intramammary route. It has been proposed that a systemic administration may overcome these problems (Ziv, 1980; du Preez, 2000).

Udder tissue necrosis

Mastitis which causes udder tissue necrosis leads to a poor blood supply of the affected areas and consequently a decreased redox potential that favours anaerobic MCOs. There is no effective passage of drugs into necrotic avascular udder tissue.

Teat canal infection (TCI)

Standard methods of antimicrobial administration into a mastitic quarter or for dry cow therapy do not necessarily eliminate TCI. TCI serves as a potential source of organisms for infection of the mammary gland parenchyma. After antimicrobial treatment the existing TCI may be a source of a new IMI or re-infection.

Trauma

Trauma predisposes the quarter to infection or re-infection due to decreased mammary gland defence power. Trauma of the tea canal and teat end was discussed in the management and iatrogenic factors associated with bovine mastitis treatment failure.

Adverse effects of drugs

Action of the antimicrobial or the vehicle can decrease the mammary gland defence powers. Drugs may exert direct effects on the phagocytic efficacy of polymorph-neutrophils (**PMN**) (Francis, 1989; Daley et al., 1992; Pyörälä, 2002a). Antimicrobials or formulated excipients can alter the oxidative burst activity of bovine PMN. Research has found that cloxacillin has no effect and enrofloxacin increases PMN activity. Neomycin, lincomycin, dihydrostreptomycin, doxycycline, oxytetracycline, danofloxacin, penicillin, ceftiofur, spiramycin, erythromycin and chloramphenicol reduce the activity (Hoeben et al., 1998; Paape et al., 2003). Because intracellular MCOs are not sensitive to antimicrobial action, dysfunctional PMNs may serve as a constant reservoir of protected MCO. Mammary gland tissue can be irritated by the active drug or additives, such as vehicle or thickeners. Irritation will exacerbate the inflammatory process and will further weaken drug distribution.

Herd, cow and quarter factors

An important part of the bovine mastitis strategy to minimise the use of antimicrobials is to refrain from treating cases with a poor prognosis. As discussed previously, the probability of cure can be estimated and is significantly influenced by cow and quarter factors, such as: age or parity, quarter number and location, days in milk, number of positive samples and colony-forming units prior to treatment and SCC levels before treatment initiation.

Schukken et al (2007) produced a table of a cow, quarter and pathogen factors affecting the cure rate of treatment trials for *Staph aureus* mastitis (Table 1).

Table 1. Cow, pathogen and treatment factors affecting cure in treatment trials of clinical or subclinical *Staphylococcus aureus* mastitis during lactation or the dry period.

	Deluyker et al., 2005	Deluyker et al., 2005	Dingwell et al., 2003	Osteras et al., 1999	Sol et al., 1994	Sol et al., 1997	Sol et al., 2000
Lactation stage	Lactation	Lactation	Dry period	Dry Period	Dry period	Lactation	Lactation
Cow factors:							
Parity	Decrease	Decrease	NS[1]	Decrease	Decrease	Decrease	Decrease
DIM[5]	Increase	NS	NA	NA	NA	Increase	Decrease
Rear vs. front quarters	NR[3]	Decrease	Decrease	Decrease	Decrease	Decrease	NS
SCC	NR	Decrease	Decrease	Decrease	Decrease	Decrease	Decrease
Number of pos. samples	NR	NR	Decrease	NR	Decrease	Decrease	NR
Number of CFU	Decrease	NS	Decrease	NR	NR	NR	NR
Number of quarters	NR	NR	NS	Decrease	Decrease	NR	NR
Pathogen factor:							
Penicillin resistant	NR	NR	NR	Decrease	NR	Decrease	Decrease
Treatment factor:							
Duration	Increase	Increase	NA	NA	NA	NR	Increase

[1]Not significant, [2]Not applicable. [3]Not reported. [5]Days in milk.

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In general, higher parity, higher SCC and increased numbers of positive samples or colony-forming units prior to treatment are associated with lower cure. Additionally, cure rates will be lower with increased number of quarters and when the rear quarters are affected.

Significantly lower treatment success in older cows or cows of higher parity (described by Sandgren et al 2006; Pyörälä and Pyörälä 1998, Sol et al 1994) is likely to be due to the mammary gland morphology and decreased general resistance to infectious diseases. Older cows are more likely to have experienced clinical or subclinical IMIs and have changes in mammary gland morphology that lead to decreased cure rates. Older cows, in general have larger volume of the mammary glands compared to heifers. The volume of the affected tissue is

also greater when more than one quarter is affected, or when rear quarters are affected. Mammary gland volume can influence the drug distribution, and subsequently the concentrations of the drug at the target site will be affected.

Cow's general condition

Sick or dehydrated cattle will not distribute systemically administered drugs at normal rates and will have prolonged tissue and milk levels due to reduced circulation and elimination of drugs (Rasmussen, 1966).

Previous mastitis history

Cows that have suffered previous mastitis are with increased risk of re-occurrence and have generally lower treatment success rates, due to the changes in the mammary gland defence mechanisms and probability of chronic changes in the udder with impaired vascularisation and consequently, impaired drug distribution.

Herd level

Additional factors that have been associated with bovine mastitis treatment failure include some herd level factors such as hygiene (Sol et al., 1994), bulk milk somatic cell count level (Østerås and Edge, 2000), number of new infections as judged by increase in somatic cell count (Østerås and Edge, 2000) and observed prevalence of *Staph aureus* in the herd. In herds with high prevalence of *Staph aureus* infection the risk of new intramammary infections or reinfection is higher.

Conclusion

Bovine mastitis treatment failure is a common problem in the clinical practice. It can occur associated with the following factors: Management and iatrogenic factors; Drug factors; Mastitis-causing organism factors; and Mammary gland factors. Problems associated with any of these groups may result in inappropriate choice and inadequate concentration of antimicrobial at the site of infection or in adequate concentration maintained for shorter time than required. Additionally, herd, cow and quarter level factors, can also affect results of the bovine mastitis treatment. Treating of cases with prognosis will have increased risk of treatment failure.

Veterinarians should have an active role in the education of the farmers in the treatment and management of bovine mastitis. Management and iatrogenic factors can be easily influenced. One of the main objectives must be early treatment initiated as soon as signs of the disease become apparent. The length of treatment should be accordingly to the speed of recovery. Extended therapy protocols are designed to maintain antimicrobial levels in milk greater than the minimum inhibitory concentration for a period that extends beyond the lifespan of neutrophils, aiming to kill organisms that seek a refuge into the white blood cells. Veterinarians' role in the improvement of the drug storage conditions is also important. It is imperative to treat younger cows on time, and prevent damage to the secretory tissue, resulting in increased susceptibility of repeats. Additionally, veterinarians can influence the choice of antimicrobials, based on the *in vitro* susceptibility testing. The choice of inappropriate drug should not be an excuse for bovine mastitis treatment failure, particularly when antimicrobial sensitivity/resistance testing is readily available.

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