1. INTRODUCTION

Mastitis is the most common reason for antimicrobial treatment of dairy cows (Grave et al. 1999; Mitchell et al. 1998). Antimicrobials have been used to treat mastitis for more than fifty years, but consensus about the most efficient, safe, and economical treatment is still lacking. The concept of evidence based medicine has recently been introduced to veterinary medicine (Cockcroft & Holmes, 2003) and those principles should apply also to treatment of mastitis. The impact on public health should also be taken into account. The aim of this paper is to review current treatments of clinical mastitis during lactation and seek for evidence-based, best practice treatment recommendations for that common disease of dairy cattle.

2. PHARMACOKINETIC AND PHARMACODYNAMIC CONSIDERATIONS

Pharmacokinetics of an antimicrobial substance greatly affects its suitability for mastitis treatment. Penetration of a substance into milk when administered parenterally or absorption and distribution throughout the udder when infused intramammarily depends on its main pharmacokinetic characteristics. These are lipid solubility, degree of ionization, extent of binding to serum and udder proteins, and the type of the vehicle (Prescott et al. 2000). Weak organic bases tend to accumulate in milk in the ionized form after parenteral administration and reach in milk concentrations higher than those in blood. On the contrary, concentrations of weak acids in milk are much lower than in blood. Pharmacokinetic studies have mostly been carried out in healthy cows, which complicates the situation.

Pharmacodynamics of the substance is another aspect which should be considered. Susceptibility in vitro for the antimicrobial substance used is a prerequisite for treatment of infections, but efficacy in vivo does not guarantee efficacy in vivo. Antimicrobial resistance among mastitis pathogens has not yet emerged to a very high level, but geographical regions may differ in this respect (Lehtolainen et al. 2003; Erskine et al. 2004). The biggest problem is the widespread resistance of staphylococci, particularly S. aureus, to penicillin G (Güler et al. 2005; Olsen et al. 2006; Pitkälä et al. 2004). Coagulase-negative staphylococci tend to be more resistant than S. aureus and can
develop multiresistance (Pitkälä et al. 2004). Mastitis streptococci have remained susceptible for penicillin G, but emerging resistance to macrolides and lincosamides is possible (Erskine et al. 2004; Loch et al. 2005; Pitkälä et al. 2004). Antimicrobial susceptibility of coliform bacteria varies (Bengtsson et al. 2005; Lehtolainen et al. 2003; Morin et al. 1998), but antimicrobials are seldom beneficial in the treatment of coliform mastitis. Other environmental bacteria such as Enterococci are commonly resistant to several substances and respond poorly to all treatment (Pitkälä et al. 2004). Some studies have reported low correlation between cure rates of mastitis and results from susceptibility tests (Hoe & Ruegg, 2005). Poor relationship between treatment result of mastitis and in vitro susceptibility of the causing pathogen appears to be mostly typical for coliform mastitis (Pyörälä & Pyörälä, 1998; Pyörälä & Syväjärvi, 1987). The problem is that most breakpoint concentrations for in vitro susceptibility testing of animal pathogens have mainly been derived from human data (NCCLS, 2002). They do not take into account the pharmacokinetic aspects of ruminants and mammary gland. Using a β-lactamase test for determining resistance to penicillin G of staphylococci is one way to evade the problem (NCCLS 2002; Olsen et al. 2005). A novel approach to improve the accuracy of routine susceptibility tests of mastitis pathogens was recently suggested (Klement et al. 2005).

Selecting a substance with a low MIC (minimum inhibitory concentration) value for the target pathogens is preferable, in particular if antimicrobials are used systemically (Prescott et al. 2000; Ziv, 1980). Antimicrobials can be divided in concentration-dependent and time-dependent drugs; in the first group (e.g. aminoglycosides and fluoroquinolones) concentrations of several magnitudes of the MIC of the target organisms at the infection site increase the efficacy, in the latter group (e.g. penicillins and macrolides) the efficacy depends on the time, during which the concentration exceeds MIC but high concentrations do not increase efficacy (Prescott et al. 2000). The antimicrobial should preferably have bactericidal action, as phagocytosis is impaired in the mammary gland (Kehrli & Harp, 2001). Milk should not interfere with the activity. Activity of macrolides, tetracyclines and trimethoprim-sulphonamides has been shown to be reduced in milk (Fang & Pyörälä, 1996; Louhi et al. 1992).

3. INTRAMAMMARY TREATMENT

The most common route of the administration of antimicrobials in mastitis is the intramammary (IMM) route (Gruet et al. 2001). The advantages of this route are high concentrations of the substance achieved in the milk (Franklin et al. 1984, 1986; Moretain et al. 1989) and low consumption of the antimicrobial as the drug is directly infused into the diseased quarter. Disadvantages could be the uneven distribution of many substances throughout the udder (Ehinger et al. 2000; Ullberg et al. 1958), risk for contamination when infusing the product via the teat canal (Erskine, 2003), and possible irritation of the mammary tissue caused by the preparation. In addition, some earlier in vitro studies showed that antimicrobials may disturb phagocytosis when given IMM (Nickerson et al. 1986; Ziv et al. 1983), but clinical relevance of this finding is unknown.

Many IMM products seem to have appeared to the market without supportive scientific data on the efficacy. Intramammary products with combinations of two or even three antimicrobials were introduced due to suggested synergistic action and broad spectrum. The evidence of their efficacy against clinical mastitis is many times lacking and synergistic action has never been proven in vivo (Taponen et al. 2003a; Whittle & Hanlon, 1997; Ødegaard & Sviland, 2001). The idea of intramammaries containing a combination of antimicrobials can be regarded outdated. The requirements for authorization of veterinary drugs have changed and efficacy claims must now be supported by scientific data. Very few new IMM products have however been authorized recently and only one product (for subclinical mastitis) via the centralized procedure in the European Union (Anon., 2006).
The efficacy of IMM treatment varies according to the causing pathogen. Best therapy response has been shown for mastitis caused by streptococci and coagulase-negative staphylococci (Davis et al. 1975; Deluyker et al. 1999; Taponen et al. 2003). Efficacy in clinical mastitis caused by S. aureus or some environmental pathogens has been unsatisfactory. Clinical cure rates have been lower than 60% and bacteriological cure rates as low as 10-40% (Deluyker et al. 1999; Guterbock et al. 1993; Knight et al. 2000). It is evident that bovine mammary gland is in general a difficult target for antimicrobial treatment, which is reflected in the low response of IMM treatments (Craven, 1987).

4. SYSTEMIC TREATMENT

Systemic (parenteral) route of administration was introduced into mastitis therapy by Swedish and later Israeli authors (Ullberg et al. 1985; Ziv, 1980). It was suggested that systemic treatment would penetrate throughout the udder better and be more efficient than IMM treatment in therapy of mastitis. Systemic treatment of mastitis was widely adopted in the Nordic countries and this practice still continues (Ekman et al. 1994). However, the superiority of systemic treatment of mastitis over IMM treatment was never proven in comparative clinical trials.

Pharmacokinetics of antimicrobials with systemic administration into adult ruminants is problematic (Prescott et al. 2000). It is difficult to achieve and maintain therapeutic concentrations in milk or udder tissue. Intravenous administration would in general produce higher concentrations in milk, but it is often unpractical in field conditions. The slowly absorbed antibiotic preparations for intramuscular use are the worst choice in mastitis, because they do not generally produce therapeutic concentrations in milk or tissues (Blanchflower, 1983; Prescott et al. 2000). One additional problem for the practitioner is that dosage recommendations of many antibiotic preparations for adult cattle may be too low regarding pharmacological aspects, but residue studies have been carried out using the approved dosages. Repeated intramuscular injections of large volumes of antibiotics can be irritating and cannot be recommended from the animal welfare point of view (Kaartinen et al. 1999; Pyörälä et al. 1994a).

Very few substances have optimal pharmacokinetic and pharmacodynamic characteristics for systemic mastitis treatment. Even if the drug has ideal properties (Kaartinen et al. 1995; Sanders et al. 1992), the treatment results from clinical trials may still be disappointing, as in case of fluoroquinolones or macrolides. Commonly used broad-spectrum antibiotics, such as oxytetracycline and ceftiofur, have been tested for systemic mastitis treatment or prevention with no effect (Duenas et al. 2001; Erskine et al. 1996; Lents et al. 2002; Owens et al. 1999). Intramuscular ceftiofur does not attain therapeutic concentrations in the milk (Erskine et al. 1995). Published information on oxytetracycline is scant, but according to manufacturers’ data sheets, therapeutic concentrations can be achieved in milk using a dose of 10 mg/kg only against very susceptible bacteria (MIC \(\leq 1\) µg/ml). The same problem applies to trimethoprim-sulphonamide combinations, and very high dosing is necessary to reach therapeutic concentrations in milk (Kaartinen et al. 1999). Furthermore, the activity of oxytetracycline and trimethoprim-sulphonamides is decreased in milk (Fang & Pyörälä; Greko et al. 1999). Macrolides, which are narrow spectrum drugs with activity against Gram-positive bacteria only, would have ideal pharmacokinetics (Franklin et al. 1986). However they may have less favourable pharmacodynamics: they are bacteriostatic in action and milk interferes with their activity (Louhi et al. 1992). Good penetration into cells does not warrant intracellular killing of bacteria (Madgwick et al. 1989). These may be the reasons for the reported poor efficacy of macrolides in systemic treatment of clinical mastitis (Owens et al. 1999; Pyörälä & Pyörälä, 1998).

One substance used for systemic treatment is penicillin G, which as weak acid penetrates poorly into mammary gland. Due to the very low MIC values of susceptible organisms, therapeutic...
concentrations can be maintained in milk using the dose 20 mg/kg of penicillin G procaine (Franklin et al. 1984, 1986; Ziv & Storper, 1985). The claim dose however differs between countries (Hillerton & Cliem, 2002). Milk does not interfere with the activity of penicillin G (Louhi et al. 1992). Different systemic or combined regimens using penicillin G procaine have been tested in several trials (Funke, 1982; Jarp et al. 1989; Pyörälä & Pyörälä, 1998, Waage, 1997). Finally, the antimicrobial used for systemic treatment of mastitis must be approved for dairy cattle. The availability of substances on the market differs between countries. Currently many substances are used for that indication off-label and without proven efficacy (Zwald et al. 2004). For example, penicillin G procaine or fluoroquinolones are not approved for dairy cattle in USA.

5. WHICH ROUTE OF ADMINISTRATION SHOULD BE SELECTED?

The ultimate question is if the antibiotic should accumulate in the milk or in the udder tissue (Table I) (Erskine, 2003). This may depend on the infection: mastitis streptococci are known to stay in the milk compartment, but S. aureus bacteria can penetrate into udder tissue and cause deep infection.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Milk/ducts</th>
<th>Udder tissue</th>
<th>Cow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Str. Agalactiae</td>
<td>+++</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Other streptococci</td>
<td>+++</td>
<td>+</td>
<td>- -</td>
</tr>
<tr>
<td>S. aureus</td>
<td>+</td>
<td>+++</td>
<td>- -</td>
</tr>
<tr>
<td>CNS</td>
<td>+++</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Coliforms</td>
<td>+</td>
<td>- -</td>
<td>+++</td>
</tr>
</tbody>
</table>

Randomized comparative field trials using IMM vs parenteral treatment of mastitis with the same antimicrobial do not exist. In one study, IMM treatment was compared with combined treatment in experimental S. aureus mastitis. Different β-lactam substances were used, and no information about the penicillin susceptibility of the bacterial strain was available, so no conclusions can be drawn (Owens et al. 1988). In another study, treatment with parenteral penethamate hydroiodide was compared with IMM penicillin-dihydrostreptomycin treatment, and no difference was seen (McDougall, 1998). Penethamate is a more lipophilic penicillin G formulation and diffuses better than penicillin G procaine into milk (Ziv & Storper, 1985).

As conclusion, it seems that the only type of mastitis where systemic treatment would be clearly advantageous is mastitis caused by S. aureus. This is also logical from a theoretical point of view as S. aureus causes a deep infection and penicillin G may distribute more evenly into infection foci by the systemic administration (Erskine, 2003; Knight et al. 2000). S. aureus bacteria have many characteristics which make them difficult targets for antibiotic therapy (Sol et al. 2000). They can survive in the phagocytizing cells. In mastitis caused by penicillin-susceptible S. aureus best results were achieved using combination of systemic and IMM treatment with penicillin G for 5 days (Taponen et al. 2003b). On the other hand, completing 5-day IMM penicillin G treatment of mastitis due to penicillin-susceptible S. aureus for 3 days with systemic penicillin did not increase cure rates as compared with only one additional systemic administration (Waage, 1997). Cure rates for mastitis caused by penicillin-resistant isolates seems to be inferior to those of mastitis due to penicillin-susceptible isolates (Pyörälä & Pyörälä, 1998; Sol et al 2000; Taponen et al. 2003b; Ziv & Storper 1985). It is not known if this is due to pharmacologic problems of the drugs used, or virulence factors possibly linked to β-lactamase gene of the resistant isolates (Haveri et al. 2005).

In infections of the milk compartment such as streptococcal mastitis, there is probably no advantage of the systemic administration. For example concentration of penicillin G in milk remains after
systemic administration 100-1000 fold lower than when given IMM (Franklin et al. 1984; Moretain et al. 1989). Based on the results from separate studies, cure rates in streptococcal mastitis using IMM treatment are equal or even better than using systemic administration (Davis et al. 1975; Deluyker et al. 2005; Hillerton & Kliem, 2002; Pyörälä & Pyörälä, 1998; Taponen et al. 2003a; Waage 1997).

In severe mastitis due to coliform bacteria, parenteral administration of antimicrobials has been suggested, due to the risk for bacteremia (Wenz et al. 2001). Generally, the efficacy of the antimicrobial treatment in coliform mastitis has been questioned as cure rates have been as high with or without antimicrobials or with drugs inefficient in vitro (Jones et al. 1990; Pyörälä et al. 1994b; Pyörälä & Syväjärvi, 1987). In serious E. coli mastitis with heavy growth of bacteria in the udder, use of systemic antimicrobial treatment can be beneficial, especially during the puerperal period when the defence mechanisms of the cow are compromised. Enrofloxacin, ceftiofur and cefquinome have shown efficacy in experimental or clinical trials (Dosogne et al. 2002; Erskine et al. 2002; Rantala et al. 2002; Shpigel et al. 1997). These substances belong to groups particularly important in human medicine, and animal use should be as restricted as possible (OIE 2006). So far there is no evidence about releasing massive amounts of endotoxin by using bactericidal antimicrobial treatment in E. coli mastitis (Dosogne et al. 2002).

For a true comparison of local, systemic and combined antimicrobial treatment of mastitis, scientific studies should conducted where:

- the bacterial isolates would be tested to be in vitro susceptible for the used antimicrobials,
- doses which would maintain therapeutic concentrations in the udder would be used systemically, and,
- the group size would be high enough for statistical comparison by bacterial species.

6. DURATION OF TREATMENT

One reason for poor cure rates in mastitis therapy is probably the short duration of standard treatments. Unfortunately, many IMM preparations have very short treatment lengths in the approved claims. Scientific studies comparing different durations of treatment of clinical mastitis are very few. Some authors have recently built up models to study economical aspects of treatment of subclinical mastitis which possibly could also be used for clinical mastitis (Swinkels et al. 2005a, b). Mastitis caused by S. aureus is the biggest challenge in mastitis therapy (Knight et al. 2000). Some earlier studies have reported on better efficacy of a long treatment in S. aureus mastitis (Funke, 1982; Pyörälä & Syväjärvi, 1988; Ziv & Storper, 1985), and more recent studies have confirmed this (Deluyker et al. 2005; Pyörälä & Pyörälä, 1998). Also mastitis due to Streptococcus uberis has been shown to benefit from long duration of treatment (Milne et al. 2002; Oliver et al. 2005). Regarding other Gram-positive pathogens than S. aureus such as coagulase-negative staphylococci and other streptococci, treatment can probably be shorter both from efficacy and economical points of view (Deluyker et al. 2005, Pyörälä & Pyörälä 1998). This has been shown for subclinical mastitis by Swinkels et al. (2005a,b). All mastitis treatment should be evidence based i.e. the efficacy of each product and treatment length should be demonstrated in scientific studies.

7. CONCLUSION

Treatment of clinical mastitis should be evidence based and take national (Table II) and international prudent use guidelines into account (Anon., 2003; OIE, 2005; FVE 1999).

Antimicrobials should not be available for the farm personal but treatment decision and drug selection should be made by veterinarians. Treatment should be targeted towards the causing

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bacteria whenever possible. In acute situations it is not possible, but treatment must be initiated based on herd data and personal experience. In clinical mastitis, rapid bacteriological diagnosis would facilitate the proper selection of the antimicrobial. Selective diagnostic media are available in many countries, which allow rapid (over-night) diagnosis (e.g. Selma selective agar, SVA, Uppsala, Sweden; ColiMast, ICP, Auckland, New Zealand). Recently, also a commercial, rapid PCR-based test for mastitis diagnostics has been launched to the market (PathoProof, Mastitis PCR Assay, Finzymes Diagnostics, Espoo, Finland). When the bacteriological diagnosis is available, the treatment can be re-evaluated and targeted towards the specific pathogen (Leslie & Keefe, 1998).

Table II. **Recommendations for antimicrobial treatment of mastitis in Finland** (Anon., 2003)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Species</th>
<th>Drug of choice</th>
<th>Alternative</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococci</td>
<td><em>Str. agalactiae</em> <em>dysgalactiae</em> <em>uberis</em></td>
<td>Penicillin G</td>
<td>IMM treatment preferable</td>
<td></td>
</tr>
<tr>
<td>Enterococci</td>
<td></td>
<td>According to susceptibility testing</td>
<td>Prognosis for bacteriological cure poor</td>
<td></td>
</tr>
<tr>
<td>Staphylococci</td>
<td><em>S. aureus</em> CNS</td>
<td>Penicillin G</td>
<td>Combination treatment in <em>S. aureus</em> mastitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>β-lactamase −</em></td>
<td>Cloxacillin</td>
<td>Macrolides Lincosamides</td>
<td>Local and/or systemic treatment depending on the drug used</td>
</tr>
<tr>
<td></td>
<td><em>S. aureus</em> CNS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>β-lactamase +</em></td>
<td>Macrolides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coliforms</td>
<td><em>E. coli</em></td>
<td>No antimicrobials</td>
<td>Enrofloxacin</td>
<td>Antimicrobials necessary in serious cases and during puerperal period</td>
</tr>
</tbody>
</table>

In principle, use of narrow-spectrum antimicrobials is preferable. First choice for mastitis due to streptococci and penicillin G susceptible staphylococci are β-lactam antibiotics, preferably penicillin G. Broad-spectrum antimicrobials such as 3rd or 4th generation cephalosporins should not be used as first alternatives for mastitis, as they may increase emergence of broad-spectrum β-lactam resistance (Batchelor et al. 2005; Mayer et al. 1994). Systemic treatment is recommended in clinical mastitis due to *S. aureus* (in combination with IMM treatment) and severe coliform mastitis. Duration of treatment should be extended in mastitis caused by *S. aureus* and possibly *S. uberis*. Treatment results should be monitored using CMT and with bacteriological culturing when necessary (contagious mastitis).

8. **SUMMARY**

This paper focuses on antimicrobial treatment of clinical mastitis during lactation. The recommendations how to treat clinical mastitis are based on published clinical studies and knowledge on pharmacokinetics and pharmacodynamics of the antimicrobial substances. Treatment of mastitis should be evidence-based and take prudent use guidelines for antimicrobial treatment of animals into account. Before treatment, an aseptic milk sample should always be taken for bacteriological examination. Diagnostic tests are available in many countries, which allow a rapid bacteriological diagnosis. In principle, use of narrow-spectrum antimicrobials is preferable and recommended in the prudent use guidelines. If the causing agent is susceptible for penicillin G, it should be used in the treatment. In mastitis due to *S. aureus*, resistance to penicillin G may be a problem. Mastitis caused by streptococci and coagulase-negative staphylococci is recommended to be treated intramammarily and the cure rates are generally high. *S. aureus* mastitis due to penicillin-susceptible strain can be treated with concomitant intramammary and parenteral treatment to increase therapy response. In coliform mastitis, parenteral antimicrobial treatment is recommended for cows with severe clinical signs and high number of bacteria in the milk. The duration of antimicrobial treatment is normally from 3 to 5 days and the longest in mastitis caused by *S. aureus*.
or *S. uberis*. Treatment response should be followed-up using CMT and bacteriological culturing when necessary.

9. **KEY WORDS**

Mastitis, clinical, treatment, antimicrobials, intramammary, systemic, prudent use.

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