CLINICAL AND THERAPEUTIC ASPECTS OF COLIFORM MASTITIS IN DAIRY COWS UNDER INTENSIVE MANAGEMENT

by

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Academic Dissertation

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1. LIST OF ORIGINAL ARTICLES


2. ABBREVIATIONS

<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BM SCC</td>
<td>Bulk milk somatic cell count</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony Forming Unit</td>
</tr>
<tr>
<td>CMT</td>
<td>California Mastitis Test</td>
</tr>
<tr>
<td>CNS</td>
<td>Coagulase-negative staphylococci</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclo-oxygenase</td>
</tr>
<tr>
<td>IM</td>
<td>Intramuscularly</td>
</tr>
<tr>
<td>IMM</td>
<td>Intramammarily</td>
</tr>
<tr>
<td>IMI</td>
<td>Intramammary infection</td>
</tr>
<tr>
<td>LIR</td>
<td>Lactational incidence risk</td>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>NSAID</td>
<td>Nonsteroidal anti-inflammatory drug</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>SCC</td>
<td>Somatic cell count</td>
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<tr>
<td>TMS</td>
<td>Trimethoprim-sulphadiazine</td>
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3. SUMMARY

Clinical mastitis caused by *Escherichia coli* is an important condition affecting lactating dairy cows. The thesis includes a study of clinical mastitis in large, high producing Israeli dairy herds. Thereafter, the effect of non-steroidal anti-inflammatory drugs (NSAIDs) and antimicrobials was evaluated in experimentally induced and field cases of clinical mastitis.

A four-year retrospective study was performed to determine clinical, bacteriological and epidemiological aspects of acute clinical mastitis in seven Israeli dairy herds. A total of 1124 clinical mastitis cases were detected by abnormal changes in the milk and udder with concurrent decrease of at least 25% in daily milk production. A total of 1190 quarters were affected with clinical mastitis in 1089 cows. The rear quarters had a higher incidence risk (64.7% of quarter cases) than the front quarters. The annual herd-year-incidence varied from 4.2 to 126.8 cases/100 cows/year. The whole-lactation incidence risk (LIR) was 20.8 per 100 lactations. LIR increased from the first to fifth lactation and then decreased. Most clinical mastitis cases were associated with coliform bacteria (60.2% of cases), environmental streptococci (18.6%), coagulase-negative staphylococci (8.7%) and samples from which no bacterial growth was detected (8.1%).

Most cases of clinical mastitis occurred in the early stages of lactation, with 51.4% of all cases, 52.3% of coliform cases and 54.6% of environmental streptococci mastitis cases occurring during the first 4 months of lactation. The median days in milk at diagnosis was 118 days. The incidence was lower in the dry summer months. The ratio of peak to low incidence was 1.62 with a calculated peak incidence in January.

The efficacy of the NSAIDs; ketoprofen, phenylbutazone and dipyone for the treatment of clinical mastitis was evaluated in a series of clinical field trials. A total of 684 clinical mastitis cases were treated with parenteral trimethoprim-sulphadiazine (TMS) only or in combination with NSAID. Recovery rate for cases treated by TMS only was 80.7%, compared to 89.4%, 91.2% and 92.6% for cases treated with TMS and dipyreme, phenylbutazone or ketoprofen, respectively. NSAIDs treatment significantly improved recovery of *E. coli* clinical mastitis, the OR for recovery of cases treated by TMS only compared to all other cases treated by TMS and dipyreme, phenylbutazone or ketoprofen was 0.44. Ketoprofen and TMS combination therapy was significantly better
than TMS only, TMS- dipyrone or TMS-phenylbutazone combination therapy. The OR for recovery of ketoprofen-TMS combination therapy compared to TMS only, was 3.00.

The effect of TMS in the treatment of coliform mastitis was evaluated by the association between in vitro susceptibility to TMS and the outcome of treatment. The recovery rate for the cases that were sensitive to TMS was 89.1% (147 of 165), and the recovery rate for the cases that were resistant to TMS was 74.6% (47 of 63). The OR for recovery of TMS treated cases associated TMS sensitive organisms compared to TMS resistant organisms was 2.75.

The efficacy of intramuscularly and intramammarily administered cefquinome was evaluated in experimental E. coli mastitis in dairy cows. Forty-seven multiparous, Israeli Holstein cows in early lactation were used, and 400 to 750 cfu of E. coli were infused into two healthy quarters of each cow. All cows developed typical signs of acute clinical mastitis by 12 to 16 h postinoculation. Parenteral cefquinome treatment, with or without intramammary cefquinome, significantly improved clinical recovery and return to milk production.

The bacteriological cure rates were significantly higher for cows in the groups treated with cefquinome than for cows in the control group treated with intramammary ampicillin and cloxacillin.

In this study the importance of coliform mastitis in Israeli dairy herds was demonstrated. NSAIDs were beneficial in the treatment of clinical mastitis and ketoprofen seems to be more so than phenylbutazone and dipyrone. Treatment with the antimicrobial TMS was found effective in field cases and cefquinome in experimentally induced mastitis.

However, more studies are required keeping in mind economical and public health aspects related to the use of these drugs in dairy cows.
4. INTRODUCTION

4.1. Acute clinical mastitis – aetiology and recent developments

Despite world-wide efforts, mastitis has remained economically the most important disease in dairy cattle (Wilesmith et al., 1986; Miller and Dorn, 1990; Miller et al., 1993). Based on the effects on productivity, international trade, animal welfare and zoonotic risk, mastitis was ranked highest, above all other infectious diseases such as salmonellosis, paratuberculosis and bovine virus diarrhoea (Wells et al., 1998). The implementation of effective measures for prevention and control of the disease has resulted in marked reduction and even eradication of subclinical mastitis caused by contagious organisms such as Staphylococcus aureus and Streptococcus agalactiae in many well-managed herds. Preventive measures of considerable impact have included improving of milking hygiene and technique, culling of chronically infected cows, post-milking teat dip and blanket dry cow antibiotic therapy.

In developed countries, social and economic changes as well as increased demand for high quality milk and prevention of environment pollution, have resulted in fewer and larger, high producing industrial dairy farms. Regulatory demand for low bulk milk SCC (BMSCC) has led to further reduction in subclinical mastitis (Schukken et al., 1992). However, increased individual cow’s milk production (Pösö and Mantysaari, 1996), lower BMSCC and post milking teat disinfection were found to be associated with increased risk for clinical mastitis (Miltenburg et al., 1996; Lam et al., 1997; Barkema et al., 1998; Elbers et al., 1998).

Experimental studies have also demonstrated the association of prechallenge low SCC and neutrophil activity with the severity of experimentally induced Escherichia coli mastitis (Shuster et al., 1996). This situation has resulted in a gradual switch from mastitis caused by contagious pathogens to clinical cases caused by environmental organisms (Bennett, 1990).

The problem of acute clinical mastitis caused by environmental pathogens has been addressed by many researchers over the years (Erskine et al., 1988; Hogan et al., 1989; Schukken et al., 1989; Gonzalez et al., 1990). In fact, clinical mastitis was found to become one of the most common diseases of dairy cows in many herds (Wilesmith et al., 1986; Erskine et al., 1988; Hogan et al., 1989; Gonzalez et al., 1990; Kelton et al., 1998). The incidence of clinical mastitis can vary between 5 to 110 cases/100 cows/year (Wilesmith et al., 1986; Hogan
et al., 1989; Erskine et al., 1998; Miltenburg et al., 1996; Kelton et al., 1998). Coliforms are probably the major etiologic organisms of clinical mastitis in many dairies (Gonzalez et al., 1990; Hogan et al., 1989; Eberhart et al., 1979; Jones and Ward, 1990; Miltenburg et al., 1996), but there are differences between countries in this respect (Pyörälä and Pyörälä, 1998; Waage et al., 1999).

Increase of environmental mastitis is of great economic importance due to loss of milk production, production of lower quality milk, culling and death of cows, cost of drugs and veterinary services and reduced reproductive efficiency (Cullor 1990; Barker et al., 1998; Loeffler et al., 1999). In the USA, it was estimated that the cost per clinical case amount to 107 US$ (Miller et al, 1993).

4.2. Pathogenesis of coliform mastitis

It is generally accepted that coliforms infect the bovine udder via the teat canal. The required infective dose is very low; as little as 50 CFUs infused into the teat canal consistently elicit severe clinical mastitis (Frost and Hill, 1982). In most cases the organisms probably do not adhere to the udder epithelium or invade the udder parenchyma. However, certain strains of E. coli are capable of epithelial adherence and invasion leading to chronic intramammary infection and recurrent clinical mastitis (Lipman et al., 1994; Döpfer et al., 1999). These strains of coliforms may also infect and invade the non-lactating dry cow udder remaining quiescent within the udder until calving, subsequently causing clinical mastitis in early lactation (McDonald and Anderson, 1981; Todhunter et al., 1991; Bradley and Green, 1999). Clinical mastitis can occur due to flare-ups of these quiescent infections at periods of stress or immunosuppression. However, the low prevalence of coliform IMI both in dry and late lactating cows (N.Y. Shpigel, unpublished data) compared with the high incidence of coliform mastitis occurring at various stages of lactation, indicate that this suggested mechanism is possible but of probable lower significance.

The modern high producing dairy cow is known to suffer severe immunosuppression in the periparturient period. The negative energy and protein balance associated with high milk production, development of metabolic disturbances such as ketosis, and nutritional deficiencies were all demonstrated to affect the immune function of the dairy cow (Goff and Horst, 1997; Mallard et al., 1998). Various management factors affect the exposure of the lactating udder to the highly prevalent coliforms in the environment (Elbers et al., 1998). Under modern dairy farm conditions contamination and infection of the udder by
coliforms are probably highly prevalent. Most of these infections are aborted by the innate immune system of the udder culminating in the elimination of the invading microorganisms without any signs of disease. However, under certain conditions, the invading organisms start to proliferate in the milk spaces of the udder. Rapid bacterial proliferation is a prerequisite to disease induction and positively correlated with severity of clinical signs and recovery rate (Lohuis et al., 1990a). Bacterial counts in the milk of cows experimentally infected with coliforms peak between 12 and 24 hours after infection (Vandeputte-Van Messom et al., 1993; Van Werven et al., 1997). In severe cases bacterial counts probably peak later than in mildly affected mastitic cows. Bacterial growth rates were also higher in post parturient (Shuster et al., 1996) and older cows (Van Werven et al., 1997; Pyörälä and Pyörälä, 1998) and this increase was associated with disease severity. This course of events depends on the ability of the mammary cells to recognise the invading organism and the elaborated cascade of events induced by the concerted release of inflammatory mediators such as eicosanoids and cytokines. Cytokines such as tumor necrosis factor (TNF) -a, Interferon-g, interleukin (IL) -1 and IL-8 further activate mammary leukocytes and endothelial cells leading to adhesion and migration of vascular leukocytes into the mammary tissue and milk spaces (Adams and Shaw, 1994; Riollet et al., 2000). Activation and recruitment of vascular neutrophils is a key factor in the pathogenesis of coliform mastitis suggested to affect both severity and outcome of the disease (Shuster et al., 1996).

Proliferating and phagocytosed bacteria are known to release endotoxins and to elicit a local and systemic inflammatory response. The pathogenesis of coliform mastitis cannot be solely attributed to the local or systemic effects of endotoxins released by the organisms in the udder. Intramammary endotoxin infusion was reported to elicit an inflammatory and systemic response in cows made tolerant to systemic endotoxins (Verheijden et al., 1983). Furthermore, the disease induced by intramammary infusion of various doses of endotoxins is considerably different from field cases or experimentally induced coliform mastitis. Endotoxin induced mastitis is a milder disease where all infused animals completely recover irrespective of treatment. Comparing experimental intramammary infusion of endotoxins to bacterial infection, electron microscopic studies revealed a different pattern of polymorphonuclear leukocytes invasion and udder lesions (Hill, 1994). Furthermore, intramammary E. coli challenge increased the percentage of apoptotic circulating polymorphonuclear leukocytes while intramammary endotoxin challenge did not have any effect on polymorph apoptosis (Van Oostveldt et al., 2000).
The local and systemic signs in coliform mastitis are generally attributed to the effect of inflammatory mediators and modulators released in the udder. Systemic invasion of coliforms from the udder is not likely and bacteraemia has not been demonstrated in field cases (Powers et al., 1986) or experimentally induced coliform mastitis (Pyörälä et al., 1994; Shpigel, 1998). However, the occurrence of bacteraemia was reported in 32% of cows with severe or protracted clinical mastitis (Cebra et al., 1996).

Acute to peracute clinical mastitis, especially when caused by gram-negative organisms, is a typical septic condition (Bone, 1993; Eberhart et al., 1979; Eberhart, 1984). The systemic response to mammary infection and inflammation was extensively described in both field cases and experimentally induced mastitis models (Jones and Ward, 1989; Lohuis et al., 1988a; Lohuis et al., 1988b). This response includes fever, tachycardia, increased respiratory rate and leukopenia followed by leukocytosis. Some cows progress into severe sepsis associated with multi-organ dysfunction, hypoperfusion or hypotension. These cows may further progress into severe lactic acidosis, oliguric renal failure, severe depression, marked hypotension, septic shock and death (Dejong, 1987; Anderson, 1989; Radostits, 1961; Radostits, 1970).

The pathophysiology of gram-negative sepsis has been the subject of extensive research for the last century (Bone, 1993). It is generally accepted that lipopolysaccharides (LPS) elaborated by gram-negative microorganisms initiate a cascade of events leading to sepsis and septic shock (Bone, 1993). Indeed, intramammary infusion of LPS leads to the development of acute clinical mastitis which is clinically similar to the naturally occurring disease (Schalm et al., 1964; Carroll et al., 1964). The effect of LPS is mediated by release of inflammatory mediators, such as TNF-a, IL-1, IL-6, IL-8, eicosanoids, and by activation of factor XII (Hageman factor) (Lees, 1991; Van Miert, 1991). This is followed by a complicated cascade of release of inflammatory mediators and modulators intertwined with numerous feedback loops. These inflammatory mediators are responsible for both the systemic signs of sepsis and the local inflammatory process in the mammary tissue. The local and systemic inflammatory processes negatively affect milk production in both infected and uninfected quarters (Shuster et al., 1991).

Similar changes were described in experimentally induced coliform mastitis. Activity of TNF-a and levels of IL-1 and IL-8 in milk of infected quarters peaked 14 to 16 hours after challenge, coinciding with clinical signs and fever.
(Riollet et al., 2000; Shuster et al., 1996). However, no association was found between milk levels of cytokines and severity of the disease.

The eicosanoids are physiologically active metabolites of arachidonic acid, a normal constituent of cell membrane phospholipid. Binding of LPS to cell membranes activates the membrane-bound enzyme, phospholipase A₂, liberating arachidonic acid which is metabolised by two major enzyme systems, cyclooxygenase (COX) and lipoxygenase (Higgins, 1985a, b). The prostaglandins, prostacyclin and thromboxanes are the products of the COX pathway whereas the leukotrienes are synthesised following lipoxygenase enzyme activity. Several studies implicated the role of various eicosanoids, both prostanoids and leukotrienes, in the pathophysiology of experimentally induced and field cases of acute mastitis (Anderson et al., 1985; Anderson et al., 1986; Rose et al., 1989; Zia et al., 1987). Leukotrienes stimulates endothelial cells were shown to produce the phospholipid mediator, platelet-activating factor, that acts to trigger and immobilise vascular leukocytes leading to transvascular migration (Zimmerman et al., 1992).

4.3. Treatment of coliform mastitis

Although various preventive measures and management practices can be effective, there is still a great need for efficient therapeutic measures against acute and peracute clinical mastitis caused by coliforms. Currently these measures include the use of antimicrobials, anti-inflammatory agents, and supportive therapy (Ziv, 1992; Morin, 1999). Systemic and intramammary antimicrobials are widely used in the treatment of coliform mastitis (Eberhart et al., 1979). Clinical mastitis is still the most frequent reason for antibiotic use in lactating dairy cattle (Gardner et al., 1990; Meek et al., 1986; Guterbock, 1995). In a US study, 82% of the antibiotic residue violations were related to the treatment of mastitis (Reneau, 1993).

Even though mastitis has been treated with antimicrobials for decades, our knowledge about their efficacy is still very scarce. The assessment of mastitis treatments should include in vitro studies, experimentally induced mastitis studies and clinical field trials. In vitro studies and experimentally induced mastitis studies would demonstrate the therapeutic potential of various pharmaceuticals, define their mode of action and improve our understanding of the pathophysiology of acute mastitis. However, these studies never can finally prove the efficacy of these pharmaceuticals in the treatment of mastitis.
This problem was well addressed by Verschueren in 1992 who suggested that “It should be borne in mind that, whereas it is interesting and sometimes helpful to show how a product works, what remains essential is to show that it works”. Medical therapy should be based on the results of clinical field trials rather than on physiological reasoning. Clinical trials are clinical research studies designed to assess the efficacy of a treatment protocol by comparing its effects with those of another protocol in a comparable group of client-owned, naturally occurring clinical cases. A well designed clinical trial should be randomised, blind, and controlled. Special considerations should be taken to prevent bias and confounding, and to ensure proper randomisation, blocking, and statistical analysis (Schukken and Deluyker, 1995). The treatment of coliform mastitis is still highly controversial, and there is a great need for well designed clinical trials to substantiate appropriate treatment regimens.

4.3.1. Anti-inflammatory treatment

The non-steroidal anti-inflammatory drugs (NSAIDs) are known for many years for their anti-inflammatory, antipyretic and analgesic effects. These effects are mediated by the inhibition of the COX enzyme complex (Higgins, 1985a,b). COX has two isoforms, the COX1, which is constitutively expressed in most tissues and responsible for maintenance of various physiological processes, and the inducible COX2, a pro-inflammatory enzyme (DeWitt, 1991; Vane, 1994). Nearly all currently used veterinary NSAIDs are non-selective COX1 and COX2 inhibitors.

It was recently demonstrated in a rat model of inflammation that the acute early peak expression of COX2 which was pro-inflammatory was followed by a second, larger increase of COX2 expression which was anti-inflammatory. COX2 inhibitors inhibited inflammation at the early stage but significantly exacerbated inflammation later on at 48 hours (Gilroy et al., 1999).

Some effects of NSAIDs, such as iron chelation, suppression of oxygen radical formation and lysosomal enzyme liberation, centrally mediated analgesic and anti-inflammatory effects and inhibition of bradykinin-induced oedema, may be attributed to other mechanisms than COX inhibition (Frey, 1992; Aruoma and Halliwell, 1988; Hiller and Willson, 1983; Brune et al., 1992; Twomey and Dale, 1992; Landoni et al., 1995b). For certain NSAIDs such as ketoprofen (Walker, 1980; Dawson et al., 1982; Kantor, 1986) and tolfenamic acid (Moi- lanen et al., 1988; Moilanen et al., 1989), lipoxygenase inhibition activity was
demonstrated in addition to the above mentioned effects. However, later studies in calves (Landoni et al., 1995a) and horses (Landoni and Lees, 1995) failed to show 5-lipoxygenase inhibitory activity for ketoprofen.

Although all NSAIDs are characterised as anti-inflammatory, analgesic and anti-pyretic drugs with a similar mode of action, there are considerable differences between these drugs. The various NSAIDs differ in their chemical structure, pharmacokinetics, anti-inflammatory, analgesic and antipyretic potency, prostanoid synthesis inhibition activity, antibradykinin activity, anti-platelet aggregation activity and toxicity (Higgins, 1985a; Frey, 1992; Insel, 1990). Various experimental procedures in small laboratory animals as well as in dogs, cattle and horses were used to evaluate the relative anti-inflammatory, antipyretic and analgesic potency, and the prostanoid synthesis inhibition activity of the NSAIDs (Higgins, 1985a; Higgins, 1985b; Mazue et al., 1982; Julou et al., 1976; Espinasse et al., 1992; Matsuda et al., 1983). Invitro cell systems were used to study the activity of NSAIDs to inhibit prostanoid synthesis (Higgins, 1985a; Higgins, 1985b; Matsuda et al., 1983). The anti-prostaglandin activity of flunixin meglumine (Anderson et al., 1986a) and tolfenamic acid (Deleforge, 1993) was also demonstrated in the udder in experimental endotoxin-induced mastitis.

Based on these studies flunixin meglumine, tolfenamic acid and ketoprofen can be classified as highly potent NSAIDs and phenylbutazone as a medium potency NSAID. Although there is very little information concerning the properties of dipyrone in farm animals, this drug is probably a potent analgesic and anti-pyretic but devoid of anti-inflammatory activity (Frey, 1992; Booth, 1982; Naylor et al., 1984; Tatsuo et al 1999). However, a more recent study indicated that COX2 inhibition could play an important role in the pharmacological effects of dipyrone and its active metabolites (Campos et al., 1999).

The effects of NSAIDs have been studied in experimental coliform and endotoxin-induced mastitis models. These NSAIDs have included flunixin meglumine (Anderson et al., 1986a; Anderson et al., 1986c; Anderson et al., 1989), carprofen (Lohuis et al., 1990b; Lohuis et al., 1991), and flurbiprofen (Lohuis et al., 1989). In these studies various clinical, haematological, biochemical and pathological parameters were found to be positively affected by the NSAIDs. Among the parameters measured were rectal temperature, heart rate, respiratory rate, anorexia, depression, udder and milk inflammatory changes. Inspite of all these beneficial effects no significant effect on milk
production, rate of return to production or survival could be demonstrated. This can probably be explained by the fact that most studies used the endotoxin model, and all animals recovered with rapid return to production in these experiments.

A survey from the U.S.A (Kopcha et al., 1992) demonstrated the extensive use of NSAIDs in food animal practice. Of the responding practitioners 93% reported using NSAIDs and dairy practitioners more so than beef practitioners. NSAIDs have been advocated for the treatment of coliform mastitis (Eberhart, 1984; Anderson, 1989; Lohuis, 1991). However, efficacy has not been proven and the U.S.A Food and Drug Administration has never approved mastitis claim for any of these drugs. Inspite of the ample experimental evidence for the potential efficacy of NSAIDs in the treatment of clinical mastitis, this has never been proven in a clinical trial. There is no evidence that NSAIDs can improve recovery and survival of mastitic cows and reduce the loss of milk production. In two published field trials, phenylbutazone or flunixin meglumine treatment did not alter the outcome of severe clinical mastitis in cows that also received antibiotic treatment (Dascanio et al., 1995, Green et al 1997).

4.3.2. Antimicrobial treatment

Field trials and trials with experimentally induced coliform mastitis have failed to prove the efficacy of antimicrobial treatment. Administration of some antimicrobial agents that were effective in vitro and were pharmacokinetically adequate did not improve clinical recovery, normalisation of haematological and biochemical parameters, or elimination of bacteria in cases of spontaneous or experimentally induced coliform mastitis (Jones and Ward, 1990; Erskine et al., 1992; Guterbock et al, 1993; Pyörälä et al, 1994; Wilson et al., 1996; Pyörälä and Pyörälä, 1998). In these studies antimicrobial treatments included parenteral and intramammary gentamicin, intramammary cepahpirin or amoxyclillin, parenteral trimethoprim-sulfadiazine or intramammary colistin sulphate, intramammary florfenicol, and parenteral enrofloxacin.

The rationale for this approach was also questioned on the basis of our present knowledge of the pathophysiology of coliform mastitis (Pyörälä et al, 1994; Erskine et al., 1991), which includes the spontaneous rapid drop of milk bacterial counts 8 to 24 h after infection and the risk of a massive release of bacterial endotoxins induced by antimicrobials (Hill et al., 1978; Pyörälä et al., 1994; Shenep and Mogan, 1984; Shenep et al., 1985).
Antimicrobial drugs are assumed to exert their beneficial therapeutic effect via bactericidal or bacteriostatic action. In addition to the antimicrobial effect, some drugs have been reported to affect the pathophysiological processes by other modes of actions. One example is the capability of polymyxins to neutralise bacterial endotoxins (Ziv et al., 1978), even though this effect has not been documented in vivo. Antimicrobials might also exert possibly detrimental effects by causing massive bacterial endotoxin release induced by drugs (Shenep et al., 1984; Shenep et al., 1985) and by interfering with phagocytic activity in the udder (Ziv et al., 1983; Nickerson et al., 1985; Lintner and Eberhart, 1990; Paape et al., 1990; Paape et al., 1991; Hoeben et al., 1998). Significant differences were reported in the capacity of antimicrobials to release endotoxins and other bacterial toxins and of some bacterial strains to spontaneously release these toxins (Hurley, 1992; Takahashi et al., 1997). Pharmacokinetic problems in the treatment of adult ruminants decrease the efficacy of many antimicrobials in the udder and thus the risk of provoking massive release of endotoxin may be unlikely (Prescott and Baggot, 1993). The clinical significance of both antimicrobial endotoxin inactivation and bactericidal-induced endotoxin release is yet to be proved for coliform mastitis. The controversy over the use of antimicrobial treatment for coliform mastitis is further heightened by a recent field study, showing that clinical and bacteriological cure rates were significantly higher in clinical mastitis cases caused by environmental streptococci or coliform bacteria when treated by intramammary administration of cephapirin and/or intravenous administration of oxytetracycline (Morin et al., 1998). The interpretation of that study is however problematic because data from two very different bacteriological groups, streptococci and coliforms, had been pooled.

The claim that treatment of coliform mastitis should primarily focus on removing endotoxins from the udder and counteracting their effect, is largely unproven. Frequent milk-out after oxytocin injections and anti-inflammatory treatment are commonly advocated for treatment of coliform mastitis. Oxytocin injection before milking was found as effective as intramammary cephapirin or amoxicillin for treatment of field cases of mild clinical mastitis (Guterbock et al., 1993).

Cefquinome is a broad spectrum, fourth generation cephalosporin with improved antibacterial activity against Gram-negative bacteria over the second and third generation cephalosporins (Sader and Jones, 1993). Cefquinome is resistant to b-lactamases produced by the majority of clinically important bacteria. Chemically, cefquinome is a new cephem; its zwitterionic structure can facilitate
rapid penetration across biological membranes including the porins of the bacterial cell wall. The in vitro activity of cefquinome against E. coli of bovine mastitis origin is comparable to, or better than, third generation cephalosporins; the MIC90 and MIC50 values have been reported to be 0.13 mg/ml with a low resistance rate (Schmid et al., 1994).

Sulphonamides and trimethoprim (TMS) combinations have been used for many years for the treatment of infectious conditions caused by susceptible organisms. Although efficacy has never been established in clinical mastitis trials, this combination is still frequently used in the treatment of clinical mastitis (Ziv, 1992). The combination is problematic in the ruminant as pharmacokinetics of the components markedly differs from each other (Prescott and Baggot, 1983; Kaartinen et al., 1999). It is evident that doses recommended by the manufacturers are quite low for producing therapeutic concentrations in the milk. From the other hand, the range of concentrations of the combination, which offers synergistic activity, is rather large (Bushby, 1980).
5. AIMS OF THE PRESENT STUDY

The aims of this study were:

1. To study clinical, bacteriological and epidemiological aspects of clinical mastitis in high yielding dairy cows under intensive management.

2. To evaluate the efficacy of non-steroidal anti-inflammatory drugs in the treatment of field cases of acute and peracute clinical coliform mastitis in high-producing dairy cows.

3. To assess the efficacy of certain antimicrobials in the treatment of coliform mastitis in high yielding dairy cows.

4. To analyse the effect of the in vitro sensitivity of coliform udder pathogens to the antimicrobial drug used in the treatment on the outcome of field cases of mastitis.
6. MATERIALS

6.1 Herds (Papers I, II, IV, V)

The material comprised seven herds located in the central part of Israel on the coastal plane of the Mediterranean sea. The herds were within the practice area of the Ambulatory Clinic of the Koret School of Veterinary Medicine, which provided a complete herd-health service and the herds were visited every other day. All clinical, reproduction, production and management data were computer recorded by the herd manager and the attending veterinarian. Each herd consisted of 50 to 300 milking cows (a total study population of about 1500 Israeli-Holstein cows). In five of the herds, cows were milked three times a day and the average annual milk production ranged between 9000 to over 10,000 kg per cow. Two herds were milking twice daily with an average annual milk production of about 8000 kg per cow. Cows were kept under a loose housing system and fed a total mixed ration. Dry cows were kept separately and fed high-quality wheat hay supplemented with lactating cows’ ration. Dry-cow therapy was not used with the exception of one herd where selective intramammary infusion was given to *Staphylococcus aureus*-infected cows. Once a month, individual cow and bulk tank milk was sampled and analysed for somatic cell count by the Central Laboratory for Milk Recording. A composite milk sample was obtained at least annually from every lactating cow and submitted for bacteriological examination.

6.2 Animals

6.2.1 Field studies (Papers I, II, IV, V)

All clinical cases of mastitis occurring from September 1989 through December 1993 in seven dairy herds described above were analysed retrospectively for clinical, bacteriological and epidemiological aspects (Paper V).

In the same herds, the NSAIDs treatment studies were conducted including field cases of clinical mastitis occurring from September 1989 to April 1993 (Papers I, II). The effect of the *in vitro* sensitivity of coliforms on the outcome of treatment was studied in field cases of clinical mastitis occurring from June 1991 to December 1993 (Paper IV).
6.2.2 Experimentally induced mastitis (Paper III)

Forty-seven clinically healthy, multiparous Israeli Black and White cows were used in this study. All cows were in early to mid lactation (range 41 to 155 d of lactation); mean milk production was at least 25 L/d. Potentially suitable cows were purchased from commercial dairy herds, brought to the experimental dairy herd at the Volcani Research Center (Bet Dagan, Israel), and were allowed 7 d for acclimatisation before the induction of experimental infection. Cows were kept under a loose housing system, milked three times a day, and were fed a total mixed ration. The milking equipment consisted of double-sided, three-stall autotandem milking parlour with automatic removal of milking units. The milking equipment was controlled by a computer with an electronic, autoidentification pedometer and milk conductivity monitoring system (Afimilk®; Special Agriculture Equipment Afikim, Kibbutz Afikim, Israel). All cows were also identified by freeze brand marks. Mammary glands were eligible for experimental infection only if the SCC of the foremilk was <400,000/ml (the SCC limit for healthy cows in Israeli conditions) and if major udder pathogens were not isolated from milk samples collected daily for 3 consecutive days prior to the day of infection. Milk SCC were measured with the Fossomatic 360 instrument (Foss Electric, Hillerød, Denmark) as described by the International Dairy Federation (IDF 1995).
7. METHODS

7.1 Experimentally induced mastitis (Paper III)

In each cow, two quarters were infused intracisternally with serum-resistant *E. coli* strain, which had earlier been used in experimental studies (Bramley, 1976). This strain was sensitive to cefquinome (MIC = 0.1 µg/ml) and ampicillin (MIC = 0.5 µg/ml) *in vitro*. The challenge inoculum was prepared as described in Paper III. The actual inoculum concentration was determined by plating on eosin methylene blue agar. Quarters were infused with bacteria immediately after the morning milking; the details of the procedure are given in Paper III.

7.2 Clinical examination of cows

In the field trials (Papers I, II, IV, V), clinical mastitis was defined by the diagnosis of abnormal changes (acute, local, and systemic) in the body, udder, and milk, with concurrent decrease of at least 25 % in daily milk production. Changes in the udder included pain, swelling, warmth, and abnormal appearance of milk. Clinical mastitis was initially diagnosed by trained dairy employee by the routine use of computerised on-line milk conductivity system and the strip cup method to detect abnormal milk from the cow’s udder quarter. All the cases were further examined and recorded by the attending veterinarian. Only lactating cows at least 2 days in milk were included in the present studies. Cows with clinical mastitis as a sequel to teat injury or any udder trauma were not included.

In the experimentally induced *E. coli* mastitis study (Paper III), systemic and local clinical signs were monitored throughout the study period. Rectal temperature, heart rate, respiratory rate, and rate of primary ruminal contractions were determined according to the study schedule. Clinical signs were graded as previously described (Anderson et al., 1986b).

7.3 Treatments

In the NSAIDs trial (Papers I, II) all clinical cases of mastitis included in this study were treated with a TMS preparation (Diaziprim Forte, Vitamed Ltd., Israel; 200 mg sulphadiazine sodium and 40 mg trimethoprim per ml). Each cow received a priming dose of 20 grams (30 mg/kg) sulphadiazine-Na and 4 grams (6 mg/kg) trimethoprim intramuscularly upon initial diagnosis. Additional
intramuscular treatments of 10 grams (15 mg/kg) sulphadiazine-Na and 2 grams (3 mg/kg) trimethoprim were given daily. The dose used was as suggested by the manufacturer of the TMS preparation.

The first part of the study (Sept. 1989 to Feb. 1991) included 205 cows with clinical mastitis treated with the TMS preparation only. This group served as historical control for the ketoprofen evaluation study which included groups of blind and non-blind contemporary control groups. It should be emphasised that the historical control group was severely biased since many cows with severe clinical signs were treated with additional drugs and were excluded.

The ketoprofen evaluation study (Paper I) was in two parts. In the first, the non-blind part of the study, 2 grams (3 mg/kg) of ketoprofen (Ketofen-RM528 10% solution, Rhone Merieux, France) was administered intramuscularly once daily as long as the antimicrobial treatment was continued. The treatment was randomized so that all odd-numbered cows were allocated to one treatment group, while even-numbered cows to the control group. The second part of the study was placebo-controlled and blinded, and consisted of ketoprofen-treated cows and control cows.

The third part of the study (Paper II) included two clinical trials. Initially the relative efficacy of phenylbutazone and dipyrone in the treatment of acute mastitis was studied. Thereafter the efficacy of dipyrone was evaluated in a randomised, blind, placebo-controlled trial.

In the first trial, cows were randomly treated, once daily intramuscularly, with either 20 grams (30 mg/kg) dipyrone (Vitalgin, Vitamed Ltd., Israel; 500 mg/ml dipyrone) or 4 grams (6 mg/kg) phenylbutazone (Phenylbutazone, Vitamed Ltd., Israel; 200 mg/ml phenylbutazone sodium) for the duration of the antimicrobial therapy. In the second trial, cows were randomly treated once daily intramuscularly, with either 20 grams of dipyrone or a placebo preparation. The placebo product was provided by Vitamed Ltd., Israel, and the blinding procedure was similar to that described in the ketoprofen study. Only three dairy farms were included in this part of the trial. The doses of preparations used were according to manufacturer’s recommendations.

A group of 253 cows, treated for clinical mastitis with the TMS preparation only, served as historical control for the phenylbutazone and dipyrone evaluation study. Again, this historical control group was biased since many cows with severe
clinical signs were treated with additional agents and therefore were excluded from the trial. This group included the 205 cows of the historical controls to be described in the previous study. The systemic daily treatment was repeated until milk production started to increase, to a maximum of five treatments. In addition to the injectable treatment, affected quarters were completely milked several times daily. Severely affected cows becoming recumbent due to hypocalcemia were treated intravenously with calcium infusion.

In the experimentally induced *E. coli* mastitis study (Paper III), cows were randomly allocated to four treatment groups, each including 12 cows. Four antimicrobial therapy regimens were used: 1) 75 mg of cefquinome (Cefquinome intramammary; Hoechst Veterinär GmbH, Wiesbaden, Germany) administered intramammarily three times at 12-h intervals, 2) 75 mg of cefquinome administered intramammarily three times at 12-h intervals and 1 mg/kg of cefquinome (Cefquinome suspension; Hoechst Veterinär GmbH) administered intramuscularly two times at a 24-h interval, 3) 1 mg/kg of cefquinome administered intramuscularly two times at a 24-h interval, and 4) 75 mg of ampicillin and 200 mg of cloxacillin (Gelstamp®; SmithKline Beecham, Tiergesundheit GmbH, München, Germany) administered intramammarily three times at 12-h intervals. The dosing of drugs was as recommended by the manufacturers. Treatment started 12 to 16 h after inoculation when clear clinical signs of acute mastitis became evident. Veterinarians and other attending personal were not aware about the allocation groups and treatments.

**7.4 Assessment of recovery**

In the field trials (Papers I, II, IV) the outcome of every case was categorised into one of the following groups: **recovered**, cows that returned to at least 75 % of the pre-mastitis daily milk production; **blind quarter**, cows in which lactation ceased in the affected quarter for the duration of the present lactation; **culled**, cows that died, were salvage slaughtered, culled or did not return to at least 75 % of pre-mastitis daily milk production. A previously infected quarter was counted as having a new clinical infection when the cow was categorised as recovered after the previous episode, regardless of the time elapsed between the episodes.

In the experimentally induced *E. coli* mastitis study (Paper III) recovery was assessed by the bacteriological cure of the infected quarters, the return to the pre-infection levels of milk production, and blood chemistry and clinical parameters.
7.5 Sample collection

In the field trials (Papers I, II, IV, V), a milk sample was aseptically taken from the affected quarter(s) of all cases prior to treatment as recommended (Brown et al., 1982). Samples were frozen immediately and transported twice weekly to the laboratory for bacteriological examination. Freezing of milk samples has been shown to have minor effect of the recovery of pathogens from the samples (Dinsmore et al., 1992); in addition, more than the standard volume of milk (0.03 ml) was used in culturing the milk samples later.

In the experimentally induced E. coli study (Paper III), duplicate quarter milk samples were aseptically collected from all quarters of each cow for bacteriological culture at 6 h after infection, immediately pre-treatment, and 7 and 14 d post-treatment. At the time of clinical examinations, CMT was recorded using a five-point scale (Schalm, et al., 1971).

In the same study, jugular blood samples were collected as described in Paper III. Whole blood was analysed for total white blood cell (WBC) counts, and haematocrit. Serum samples were analysed for total calcium, total serum protein, aspartate serum transaminase, urea, creatinine, inorganic phosphorus, and sodium. The biochemical analysis was determined enzymatically by use of an automated analyser (Selective chemistry analyser; Kone, Espoo, Finland).

7.6 Bacteriological methods (Papers I, II, III, IV, V)

Bacteriological methods described earlier (Barnes-Pallesen et al., 1987) were used in all studies. A volume of 0.03 ml of milk was cultured. Gram stain and culture characteristics were used for primary identification for all isolates. All staphylococci with a b-haemolytic pattern, a positive coagulase reaction and a positive CAMP reaction were presumed to be Staphylococcus aureus. Streptococci were identified by haemolytic patterns, CAMP reaction and hydrolysis of esculin on esculin blood agar, and typing for Lancefield serological grouping. Coliform bacteria and other Gram-negative bacteria were identified using culture characteristics on MacConkey agar, growth in triple sugar iron agar, urease, catalase, oxidase and indole production. Corynebacteria and Arcanobacterium (previously: Actinomyces) pyogenes were identified using culture characteristics on blood agar, motility and catalase and urease production.
Bacterial suspensions were inoculated into Mueller-Hinton agar plates (Mueller-Hinton Agar, Difco laboratory, Detroit, MI, USA) and antimicrobial susceptibility tests were performed on all organisms by use of antimicrobial-impregnated disks (Dispens -O-Disc, Difco Laboratory, Detroit, MI, USA). The *in vitro* disc sensitivity tests were interpreted by the NCCLS standards (NCCLS 1983). For *E. coli*, a diameter of the clear zone of 16 mm or above was considered sensitive for TMS (Paper IV).

### 7.7 Statistical analysis

For Paper V, incidence risks of clinical mastitis were computed by dividing the number of occurrences of clinical mastitis during a defined period by average number of lactating cows during that period. The association between parity and clinical mastitis incidence was measured by ratio of risks and their significance was estimated by chi-square and the extended Mantel-Haenszel procedure (Breslow and Day, 1980). Data were stratified in the Mantel-Haenszel procedure on herds and type of cultured organism (defined as environmental streptococci, coliforms and all others) and the odds ratios (OR) and 95% confidence intervals (CI) were calculated. The Pearson chi-square test was used to separately analyse the association of clinical mastitis quarter location with type of cultured organism or parity. The seasonal variation of clinical mastitis incidence was tested with Edwards's test corrected for changing size of the population at risk in different months (Edwards, 1961). Significance was set at two tailed 0.05 for all tests.

For the evaluation of treatments, recovered cows were considered as treatment success and blind quarters and culled cows as treatment failure (Papers I, II, IV). In the field trials (Papers I, II) evaluating the efficacy of NSAIDs, the study population was divided into two groups; one group included cases where Gram-negative organisms were isolated and the second group all other cases including Gram-positive organisms, negative culture and non-sampled cases. The odds ratios of recovery were evaluated for ketoprofen treatment group versus non-ketoprofen treatment group and for the phenylbutazone treatment group versus the dipyrdione treatment group. All treatment groups were also compared to no treatment historical control groups. These ratios and their 95% confidence intervals were estimated using a logistic regression model adjusted for farm and type of infecting organism (i.e. defined as either Gram-negative or not Gram-negative) (Breslow and Day, 1980).
Multivariate logistic regression was used to evaluate the effect of sensitivity to TMS on treatment outcome (Paper IV). Several statistical models were fitted to the data to adjust for confounding associated with NSAID treatment, days in lactation, parity, herd, and type of infectious organism (defined as \textit{E. coli} or not \textit{E. coli}). The model that best fitted the data was chosen by use of likelihood ratio test statistics (Hosmer and Lemeshow, 1989). Values of \( P \leq 0.05 \) were considered significant. Using the model with the best fit, the odds ratio of recovery and 95\% confidence intervals were calculated for cases affected by organisms that were sensitive to TMS versus cases affected by organisms that were resistant to TMS (Breslow and Day, 1980).

In the experimentally induced mastitis (Paper III) quarter bacteriological infection rates and cure rates among treatment groups were compared by chi-square tests. Treatment differences among all other parameters were tested by least squares ANOVA; data were blocked by day of challenge (SAS User’s Guide: 1985). Differences among treatment means were determined using Duncan’s multiple range test. For all comparisons, \( P < 0.05 \) was considered to be significant.
8. RESULTS

8.1 Clinical mastitis in Israeli dairy herds (Paper V)

During the 4-year study, 1124 cases of acute clinical mastitis were detected in lactating cows. The annual incidence of clinical mastitis varied widely between herds and years from 4.2 to 126.8 cases/100 cows at risk per year. The lactational incidence risk (LIR) for the whole period was 20.8 cases per 100 lactations. The LIR of clinical mastitis increased from first to fifth lactation and then decreased (Table 1).

Table 1. Parity distribution of cows affected by clinical mastitis and all calving cows during the study period and the lactational incidence risk (LIR) for each parity group.

<table>
<thead>
<tr>
<th>Parity</th>
<th>Mastitis N</th>
<th>Mastitis %</th>
<th>All calvings N</th>
<th>All calvings %</th>
<th>LIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>251</td>
<td>21.1</td>
<td>1755</td>
<td>33.7</td>
<td>14.3</td>
</tr>
<tr>
<td>2</td>
<td>246</td>
<td>22.7</td>
<td>1254</td>
<td>24.1</td>
<td>19.6</td>
</tr>
<tr>
<td>3</td>
<td>239</td>
<td>22.0</td>
<td>895</td>
<td>17.2</td>
<td>26.7</td>
</tr>
<tr>
<td>4</td>
<td>168</td>
<td>15.5</td>
<td>613</td>
<td>11.8</td>
<td>27.4</td>
</tr>
<tr>
<td>5</td>
<td>112</td>
<td>10.3</td>
<td>383</td>
<td>7.4</td>
<td>29.2</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>3.9</td>
<td>188</td>
<td>3.6</td>
<td>22.3</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>2.0</td>
<td>84</td>
<td>1.6</td>
<td>26.2</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>0.5</td>
<td>28</td>
<td>0.5</td>
<td>17.9</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0.0</td>
<td>7</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>1085</td>
<td></td>
<td>5207</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After controlling for the farm and cultured organism, the odds ratios (OR) and 95% confidence intervals (CI) for a clinical mastitis case for different lactations were calculated (Paper V). The proportions of microorganisms were calculated for quarter milk samples of 978 cows affected by clinical mastitis. The most prevalent microorganisms isolated were *Escherichia coli* (51.2%), *Streptococcus dysgalactiae* (10.7%), CNS (7.7%), *Staphylococcus aureus* (6.4%) and *A. pyogenes* (4.9%).

Because cows could have multiple infections in one quarter and/or several quarters infected, the proportional prevalences of microorganisms were also calculated for cow cases. Most clinical cases were associated with coliform bacteria (60% of cases), environmental streptococci (19%), coagulase negative staphylococci (9%) and samples from which no bacterial growth was detected
(8%). A total of 1190 quarters was affected with clinical mastitis in 1089 cows. A single quarter was affected in 93% (1008/1089) of the cows, and two quarters in 6% (70/1089). No significant association could be demonstrated between type of cultured microorganism, defined as environmental streptococci, coliforms and all others, and udder quarter location.

Most cases of clinical mastitis occurred in the first few months of lactation, with 51.4% of all cases, 52.3% of coliform cases and 54.6% of environmental streptococci mastitis cases occurring during the first 4 months of lactation. The median number of days in milk at diagnosis was 117.5 days.

Some seasonal variation was seen in the occurrence of clinical mastitis. The monthly distribution of clinical mastitis in the years 1990-1993 is presented in Figure 1. The years differed from each other but a significant difference in the seasonal incidence was seen, with a lower incidence in the summer months.

![Figure 1. Monthly incidence of 1124 cases of clinical mastitis in Holstein dairy cows in 7 Israeli herds in the years 1990 to 1993.](image)

### 8.2 Effect of NSAIDs in the treatment of clinical mastitis (Papers I, II)

The effect of NSAIDs treatment on the outcome of all clinical mastitis cases in Papers I and II was summarised and analysed and presented in Table 2. The most frequent aetiological pathogen were coliforms (mostly *E. coli*) cultured in 56% to 59% of cases in the various treatment groups (Table 2).
Recovery rate for cases treated by TMS only or TMS in conjunction with NSAIDs are shown in Table 2. NSAIDs treatment significantly improved recovery of clinical mastitis., the OR for recovery of cases treated by TMS only compared to all other cases treated by TMS and dipyrone, phenylbutazone or ketoprofen was 0.50 (CI= 0.32-0.77, P=0.0015).

**Table 2.** Comparison of therapeutic success of treatment of all clinical mastitis cases in the various treatment groups. All NSAIDs treatments were in combination with TMS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Paper</th>
<th>Cows</th>
<th>Pathogen</th>
<th>Cure rate</th>
<th>OR (95%CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Coliforms</td>
<td>Strep. CNS</td>
<td>Other</td>
<td></td>
</tr>
<tr>
<td>TMS</td>
<td>I,II</td>
<td>313</td>
<td>59</td>
<td>7</td>
<td>4</td>
<td>81</td>
</tr>
<tr>
<td>Dipyrone</td>
<td>II</td>
<td>209</td>
<td>56</td>
<td>13</td>
<td>3</td>
<td>28</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>II</td>
<td>66</td>
<td>58</td>
<td>9</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>I</td>
<td>96</td>
<td>58</td>
<td>10</td>
<td>4</td>
<td>27</td>
</tr>
</tbody>
</table>

Ketoprofen and TMS therapy was significantly better than TMS only, TMS-dipyrone or TMS-phenylbutazone combination therapy.

Similar results were obtained after analysis of the data for *E. coli* clinical mastitis cases (Table 3). NSAIDs treatment significantly improved recovery of *E. coli* clinical mastitis, the OR for recovery of cases treated by TMS only compared to all other cases treated by TMS and dipyrone, phenylbutazone or ketoprofen was 0.44 (CI= 0.24-0.79, P=0.0066).

**Table 3.** Comparison of therapeutic success of treatment of *E. coli* clinical mastitis cases in the various treatment groups. All NSAIDs treatments were in combination with TMS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Paper</th>
<th>Cows</th>
<th>Cure rate</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMS</td>
<td>I,II</td>
<td>181</td>
<td>81</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Dipyrone</td>
<td>II</td>
<td>113</td>
<td>89</td>
<td>2.0 (1.0-2.0)</td>
<td>0.05</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>II</td>
<td>34</td>
<td>91</td>
<td>2.5 (0.7-8.6)</td>
<td>0.15</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>I</td>
<td>54</td>
<td>93</td>
<td>3.0 (1.0-8.8)</td>
<td>0.047</td>
</tr>
</tbody>
</table>

Ketoprofen and TMS therapy was significantly better than TMS only, TMS-dipyrone or TMS-phenylbutazone combination therapy. The OR for recovery of ketoprofen-TMS combination therapy compared to TMS only, was 3.00 (CI=1.02-8.84, P=0.0469).
These results were further substantiated by the double blind, placebo-controlled field trial (Paper I). Recovery rate for clinical mastitis cases associated with Gram-negative udder pathogens treated with TMS only was 63.6% compared to 92% for TMS-ketoprofen combination therapy.

8.3 Effect of antimicrobials for treatment of clinical mastitis with special emphasis on coliforms (Papers III, IV)

8.3.1 Trimethoprim-sulphadiazine combination treatment

The efficacy of the antimicrobial combination TMS in the treatment of coliform clinical mastitis was demonstrated in this data set analysis. The recovery rate for the cases that were sensitive to TMS was 89% (147 of 165), and the recovery rate for the cases that were resistant to TMS was 75% (47 of 63). The OR for recovery of TMS treated cases associated with TMS sensitive organisms compared to TMS resistant organisms was 2.75 (CI=1.29-5.85, P=0.0089).

Since some cows were treated with TMS-NSAID combination (197 cases) while others received TMS treatment only (31 case), the additive effect of this treatment combination could be evaluated. The recovery rates for the group treated with NSAID and the group treated with TMS only were 87% (172 of 197) and 71% (22 of 31), respectively.

The odds of recovery for cases treated with NSAID was significantly higher than one with an odds ratio of 2.76 and 95% confidence interval of 1.12 to 6.79 (P=0.0269). The additive effect of both TMS sensitivity and NSAIDs treatment on outcome resulted in a higher recovery rate of 91% (Table 4)

**Table 4.** Comparison of the therapeutic success of NSAIDs for coliform mastitis associated with organisms that are sensitive to TMS *in vitro* and organisms that are resistant to TMS *in vitro.*

<table>
<thead>
<tr>
<th>TMS Sensitivity</th>
<th>NSAID treatment</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (%) (n/n)</td>
<td>Yes (%) (n/n)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>60 (6/10)</td>
<td>77 (41/53)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>76 (16/21)</td>
<td>91 (131/144)</td>
<td></td>
</tr>
</tbody>
</table>
8.3.2 Cefquinome treatment

The adequacy of the *E. coli* induced mastitis model used here was demonstrated by the fact that all cows developed severe, acute clinical mastitis. The changes in the various clinical, biochemical and haematological parameters are presented in Paper III and here in Figures 2-4. The mean clinical mastitis score (CMS) and CMT peaked around 24 h post-inoculation without a significant difference between treatment groups until d 2 postinoculation for CMS and d 7 for CMT (Figure 1 in Paper III). The difference in CMS seen during the study indicated faster return to normal of the udder and elimination of systemic signs of clinical mastitis in the cows treated with cefquinome; many of the control cows were still affected by clinical mastitis at the end of the study. Body temperature, heart rate, and respiratory rate peaked around 12 to 24 h after quarter inoculation without a significant difference among treatment groups (Figure 2). These changes were associated with decreased total WBC, serum calcium, and phosphate (Figures 3 and 4), and with changes in some other blood parameters.

Daily milk production declined to its lowest at 24 h postinoculation for the cefquinome parenteral treatment groups while declining further for the cefquinome intramammary treatment group and the control group, reaching its lowest on d 2 (Figure 2 in Paper III). Thereafter, milk production increased rapidly for the cefquinome parenteral treatment groups; milk production was significantly lower for the cefquinome intramammary treatment group and the control group. Milk production of cows in the control group was statistically significantly lower than the parenteral only cefquinome group from d 2 after infection until the end of the study.

The *E. coli* induced mastitis model was also assessed by the bacteriological infection rates 6 h after infection and immediately before antibiotic treatment (Table 5). The difference in bacteriological cure rates between cefquinome intramammary group and the control group (\( P = 0.042 \)), cefquinome intramammary and injectable group and the control group (\( P < 0.001 \)), and cefquinome injectable group and the control group (\( P = 0.042 \)) was statistically significant. The differences between the groups treated with cefquinome were not statistically significant.
Figure 2. Mean rectal temperature (top panel) and mean heart rate (bottom panel) in experimentally induced *Escherichia coli* mastitis in 47 cows before and after treatment with cefquinome administered intramammarily (u), cefquinome administered intramuscularly and intramammarily (n), cefquinome administered intramuscularly (?), and ampicillin and cloxacillin administered intramammarily (l).
Table 5. Bacteriological infection rates and cure rates in infused quarters among the various treatment groups. Cows were sampled 6 h after infection, immediately before antibiotic treatment, and 7 and 14 d after the end of the antibiotic treatment.

<table>
<thead>
<tr>
<th>Treatment and route of administration</th>
<th>Infected quarters</th>
<th>Recovered quarters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%) (no./no.)</td>
<td>(%) (no./no.)</td>
</tr>
<tr>
<td>Cefquinome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intramammary</td>
<td>96 23/24</td>
<td>83 19/23a</td>
</tr>
<tr>
<td>Intramammary and injectable</td>
<td>88 21/24</td>
<td>95 20/21a</td>
</tr>
<tr>
<td>Injectable</td>
<td>96 23/24</td>
<td>83 19/23a</td>
</tr>
<tr>
<td>Ampicillin and cloxacillin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intramammary</td>
<td>100 22/22</td>
<td>55 12/22b</td>
</tr>
</tbody>
</table>

a,b Rates with different letters differ (P < 0.05).

Figure 3. Mean primary rumen contractions (top panel) and mean white cell count (bottom panel) in experimentally induced *Escherichia coli* mastitis in 47 cows before and after treatment with cefquinomme administered intramammarily (u), cefquinomme administered intramuscularly and intramammarily (n), cefquinomme administered intramuscularly (?), and ampicillin and cloxacillin administered intramammarily (l).
Figure 4. Mean total serum calcium (top panel) and mean serum inorganic phosphorus (bottom panel) in experimentally induced *Escherichia coli* mastitis in 47 cows before and after treatment with cefquinome administered intramammarily (u), cefquinome administered intramuscularly and intramammarily (n), cefquinome administered intramuscularly (?), and ampicillin and cloxacillin administered intramammarily (l).
9. DISCUSSION

Unknown or poor case definition combined with reporting or submission bias are common problems affecting field studies. It was suggested by Bartlett et al. (1986) that these problems “can best be eliminated by having an investigator live on each of the study farm and observe every animal each day”. The design and conduct of the field studies presented in this study enabled us to minimise such inaccuracies. The present analysis included only acute, severe clinical mastitis cases as defined; milder and more chronic cases, although examined by the attending veterinarian, were not included.

The incidence risks and lactational risks for clinical mastitis found here are very similar to those previously reported (Bartlett et al., 1986; Bartlett et al., 1992; Erskine et al., 1988; Gröhn et al., 1990). The increased risk of clinical mastitis with parity (Gröhn et al., 1990), the quarter distribution (Gonzalez et al., 1990) and marked seasonal incidence (Erskine et al., 1988; Gonzalez et al., 1990) conform with previous reports. The marked seasonal incidence seems to link clinical mastitis with the colder and rainy winter season in Israel. In this data set, looking at various meteorological parameters, the monthly incidence of clinical mastitis was statistically significantly associated only with mean monthly millimeters of rain (N.Y. Shpigel, unpublished data). The median number of days in milk at diagnosis of clinical mastitis found in this study was considerably longer than those previously reported (Gröhn et al., 1990). This difference should most probably be attributed to the fact that only the first diagnosis of mastitis in each lactation was considered in this study. However, this might also be due to selection or reporting bias which occurred in previous studies where farmers tended to be more concerned with post partum cows while those in advanced lactation were overlooked. The extremely high proportion of coliform mastitis, 60.2% of cases, is probably unprecedented. This proportion might actually be even higher considering the possibility that freezing of milk samples may reduce the number of coliform isolates and increase the number of CNS isolates (Schukken, et al., 1989). Furthermore, it was suggested that most negative samples (8.1% in this study) could in fact be coliforms (Erskine et al., 1988; Gonzalez et al., 1990; Smith 1983).

Once the major importance of E. coli as the causative agent of clinical mastitis was established, the selection of adequate treatment can be discussed. Although efficacy of any pharmacological treatment should be proven in well designed clinical trials, the rational of such treatment is always based on patho-
physiological, pharmacodynamic and pharmacokinetic reasoning. Previously published studies and the data presented in this study have improved our understanding of the pathophysiology of coliform mastitis. However, these studies did not resolve the dispute around the use of antimicrobials and NSAIDs in this condition. In order to optimise the antimicrobial treatment it should be based on the best available pharmacodynamic and pharmacokinetic data, although we should remember that for most NSAIDs their anti-inflammatory effects last longer than would be expected from the plasma drug disposition. Unfavourable results of mastitis treatment might be explained by an inadequate use of antimicrobials and NSAIDs. Intramammary antimicrobial therapy is probably inadequate for the treatment of severe coliform mastitis. When this route was used, unfavourable results were observed in all previously published studies and also in the cefquinome study here. This can probably be explained by the inability to achieve adequate drug levels in the milk space of the severely inflamed and oedematous udder in this condition. Although largely untested, milk concentrations of some drugs are probably markedly elevated and activity may be affected, in severe coliform mastitis upon break down of the blood-udder barrier and influx of blood components into the udder and milk (Gips et al. 1995; DeGraves et al., 1996).

The observation that NSAID treatment at some stages can exacerbate inflammation (Gillroy, et al., 1999) clearly demonstrates the possibility that different drug administration protocols may lead to dramatic different outcome. Likewise, to the author’s knowledge, all but two previously published studies failed to demonstrate the efficacy of antimicrobial therapy in coliform mastitis. The exceptional study (Morin et al.,1998) showed significantly higher clinical cure rate at one time point after treatment for coliform mastitis treated with antimicrobials. However, it was not defined how many of the causing strains in each group were in vitro resistant to the drugs used; also the number of coliform cases (E. coli pooled with other coliforms) was low. The authors suggested that the “aggressive” and higher dosage protocol used in the study might have made the difference; however, using oxytetracycline concomitantly with cephalosporin might be considered as an antagonistic treatment (Prescott and Baggot, 1993). In the other study (Pyörälä et al., 1996), enrofloxacin plus NSAID treatment showed better efficacy over NSAID treatment only in a severe model of experimental E. coli mastitis; the number of cows was very small in that study.
NSAIDs might also affect the susceptibility of some bacteria to various antimicrobials. Phenylbutazone was shown to increase the in vitro sensitivity of *Bacillus stearothermophilus var. calidolactis* to aminoglycosides and macrolides (Nouws et al., 1995). Phenylbutazone is also known to interact with the same carrier system utilised in the tubular secretion of penicillin and other weak organic acid drugs like sulphonamides (Nierenberg, 1987). The clinical significance of these observations is still unknown. In the present studies, the combination of treatment of TMS sensitive organism and NSAID treatment resulted in higher recovery rates compared to either one alone.

Based on the known pathophysiology of mastitis and the pharmacodynamics and pharmacokinetics of NSAIDs, it was hypothesised that a potent NSAID such as ketoprofen may be effective in the treatment of clinical mastitis in dairy cows. In pharmacokinetic studies in normal cattle (DeGraves et al., 1996) and goats (Musser et al., 1998) ketoprofen was found to have short elimination half-life and non-detectable milk levels. However, milk levels of ketoprofen markedly increased in acute clinical mastitis (DeGraves et al., 1996). Serum concentrations do not correlate with anti-inflammatory activity that is lasting longer than measurable serum concentrations (Landoni et al., 1995a; Sams et al., 1995). These properties renders ketoprofen a very favourable NSAID for use in food animals due to low risk of drug residues.

Most of the available data on NSAIDs is related to experimental coliform or endotoxin-induced mastitis. In this study the efficacy of ketoprofen was investigated in clinical cases, the majority of which were associated with Gram-negative udder pathogens. The eligibility criteria for admittance to the trial included the limiting criterion of reduced daily milk production in combination with typical clinico-pathological changes in milk and udder. Therefore, the study population included only cases of peracute and acute clinical cases. Assessment of the response to treatment was based on production parameters. Most previous experimental or field clinical trials based their assessment on clinical, biochemical or haematological parameters. Many of the experimental studies have also used non-practical treatment schedules. In field cases, by the time of diagnosis and treatment, most of these parameters are either back to normal or considerably deviated from early values. This is especially true when using the endotoxin-induced mastitis model. For example, in field cases of coliform mastitis, by the time of treatment body temperature may often be back to normal.
The results of the ketoprofen study re-emphasised the importance of adequate control groups. The historical controls were not included in the statistical analysis, however, it should be noted that recovery rates for historical and non-blind control groups were similar while considerably lower in the placebo controls. As mentioned previously the historical control population was biased by the exclusion of severely affected cows where treatment deviated from the study protocol. A similar bias was encountered in the non-blind, non-placebo controlled part of the study, and may explain the smaller odds ratio relative to the estimate in the blinded part of the study. This problem was avoided in the second part of the study by the use of a blind, placebo-treated, control group. Clinical cases were randomly allocated to the various treatment groups. Negative, non-treated controls were not included for ethical reasons, although the use of positive, treated controls has in fact been advocated (Dohoo, 1987). The ketoprofen trial complied with well established criteria to be considered in clinical trials of mastitis therapy (Dohoo, 1987). Results from both parts of this study indicated that ketoprofen is an effective treatment, that it may increase the odds ratio of recovery by as much as seven and can be recommended as adjunctive therapy in the treatment of clinical mastitis due to coliform bacteria in dairy cows.

Phenylbutazone and dipyrone are both pyrazolon-derivative non-steroidal anti-inflammatory drugs (Booth, 1982). The anti-inflammatory, antipyretic and analgesic properties of phenylbutazone were demonstrated in laboratory animals (Mazue et al 1982) and in the horse (Lees and Higgins, 1985). The pharmacokinetics and toxicity of phenylbutazone has been studied in the cow (Lees 1988; Martin et al., 1984; De Veau et al., 1998). Contrary to ketoprofen, phenylbutazone has a very long elimination half-life (over 40 vs. 0.5 hour) but similarly, low milk levels are expected in normal cows where only 0.33% of the total dose was recovered from milk. The dosage regimen used in the present study was 6-7 mg/kg once daily for 2-3 days in most cases. This is a relatively high dose and accumulation and prolonged metabolism and elimination are expected. However, side-effects, toxicity or any adverse reactions were not observed at any stage of the study.

Contrary to phenylbutazone, the pharmacokinetic, pharmacodynamic and toxicologic properties of dipyrone in the bovine or other ruminants are largely unknown. Pharmacokinetic data is available from human studies in which dipyrone and its active metabolites were found in milk in higher levels than in plasma (Zylber-Katz et al., 1986; Levy et al., 1995). Breast milk levels were
non-detectable 48 hours after a single oral dose, and it is unknown if this data can be extrapolated to the milking cow. The dosage regimen used in the present study was 30-40 mg/kg once daily for 2-3 days in most cases. In sheep a dose of 88 mg/kg dipyrene effectively blocked the febrile response and partially blocked the depression induced by intravenously administered endotoxin (Naylor et al., 1984). This dose will amount to about 10 grams dipyrene per cow, half the dose used in the present study. Inspite of the high dose and the unknown pharmacokinetics of the drug, side-effects, toxicity or any adverse reactions were not observed at any stage of the study. Phenylbutazone and the other pyrazolone-derivative NSAIDs have been prohibited in the European Union for food animal use from the beginning of 1998 as no maximum residue limit values could have been set for them (Anonymous, 1997).

The effect of flunixin meglumine and phenylbutazone on acute toxic mastitis was evaluated in two field studies (Dascanio et al., 1995; Green et al., 1997). Flunixin meglumine in combination with antimicrobial and fluid therapy did not improve clinical recovery or return to milk production in toxic mastitis (Green et al., 1997). In that study all animals were recumbent or severely weak in extreme condition of sepsis. Based on experimental and clinical observations, it is fairly certain that these animals were treated at an advanced stage of the disease. Antimicrobial and NSAID treatment of severely septic cows at an advanced stage of coliform mastitis is probably ineffective and will not improve survival and return to production. Once again, the importance of early diagnosis and treatment was demonstrated.

The loss of milk associated with acute toxic mastitis was not significantly different among flunixin meglumine, phenylbutazone or saline treated cows (Dascanio et al., 1995). This lack of difference might be attributed to the single NSAID treatment application and the small number of animals included.

Full pharmacokinetic data of cefquinome in lactating cows is not available. The dosage regimen employed in the cefquinome study (Paper III) was based on in vitro MIC studies and pharmacokinetic studies in steers and cows (Hoechst Roussel Vet, unpublished data).

Pharmacokinetic studies in normal lactating sows showed that effective milk levels can be obtained after once daily intravenous administration of 1mg/kg or intramuscular administration of 2 mg/kg cefquinome (Von Block, 1996).
The local and systemic signs observed in the cefquinome study were similar to those described by others using a similar, experimentally induced *E. coli* mastitis model (Hill et al., 1978; Lohuis et al., 1990a; Pyörälä et al., 1994) and could not be differentiated from natural, field cases of acute coliform mastitis. The severity of the systemic involvement and of the sepsis that developed in the infected cows was clearly indicated by the various clinical, biochemical and haematological parameters described previously. No significant differences could be detected among the different groups from the beginning of the trial until the commencement of antimicrobial therapy. Cows in the control group further deteriorated clinically after the commencement of treatment and developed more severe local and systemic signs of clinical mastitis; those differences were present until the end of the study. Although the cows treated with cefquinome developed a comparable level of disease after the intramammary infection, as indicated by the various clinical, haematological and biochemical parameters, those cows showed a more rapid and complete response to treatment. Cows treated with cefquinome either intramuscularly or intramammarily returned more rapidly and completely to pre-infection milk production with similar disappearance of mastitic changes in the udder and abnormal deviations of the clinical, haematological, and biochemical parameters.

Treatment with cefquinome resulted in better bacteriological cure compared with the control group. The combination of intramuscular and intramammary cefquinome treatment resulted in the highest bacteriological cure rate. However, this difference was not significant when compared with the other cefquinome groups and should be reevaluated in larger studies in which more quarters are involved. Treatment for the control group resulted in a bacteriological cure rate of 55% (12 of 22 infected quarters); this cure rate was very close to the spontaneous bacteriological cure rate reported in coliform mastitis at 14 d postinfection in one USA study (Guterbock et al., 1993). However, spontaneous cure rate may in general be higher if less severe coliform cases are included, as was seen in Paper IV where cure rate of 75% was achieved in the group in which mastitis was caused by organisms resistant to the antibiotic used. Use of different criteria for cure makes comparison of studies difficult, as outcome from mastitis may vary if it is based on bacteriology, detection of inflammation, or recovery of milk production in the cow. In the field studies presented here, taking bacteriological follow-up samples had not been practical, but production data was easily achievable in the computerized management systems of the herds.
As previously indicated, results of field trials and trials with experimentally induced coliform mastitis that have been published thus far failed to prove the efficacy of any antimicrobial therapy. Furthermore, none of these trials were able to show the advantage of one antimicrobial treatment over the other. In the present study, negative, untreated controls were not included for ethical reasons. Nevertheless, the recovery rates and clinical responses achieved in the control group in the present trial were probably similar to what would have been found without treatment.

In the cefquinome study, the advantage of one antimicrobial therapy over the other was clearly indicated. This difference was indicated in terms of return to milk production, disappearance of clinical signs of acute mastitis, and return to normal of various haematological and biochemical parameters. This study clearly supported the efficacy of cefquinome in the treatment of bovine coliform mastitis and indicated that cefquinome therapy can improve recovery and survival of mastitic cows and reduce the loss of milk production. Of course, it must be kept in mind that this trial was carried out using an experimental mastitis model. The results remain to be confirmed in field studies of naturally occurring coliform mastitis cases. Furthermore, treatment of mastitis with an advanced broad-spectrum antimicrobial such as cefquinome must also be evaluated from economical and public health points of view.

Pharmacokinetic properties of sulphadiazine-trimethoprim in normal lactating dairy cows were recently published (Kaartinen et al., 1999). Furthermore, in vitro studies indicated the increased susceptibility of E. coli to TMS in mastitic milk when compared to normal milk or culture broth (Fang and Pyörälä, 1996). Based on the above cited information, effective antimicrobial activity against E. coli can be achieved in the udder after once daily intramuscular injection of 48 mg/kg TMS. This dose is considerably higher than previously recommended (25 mg/kg; Ziv 1992).

In the ketoprofen, phenylbutazone and dipyrone trials (Papers I, II) the effect of NSAID on recovery was studied and discussed above. Ketoprofen, phenylbutazone and dipyrone, combined with TMS, improved recovery and return to production of field cases of coliform mastitis when compared with cases treated with TMS only. These cases formed part of the TMS study as one group of cases treated with NSAID, and this treatment was included in the statistical model as an expected confounder of recovery. The effect of NSAID treatment on recovery found in the TMS study was, as expected, very similar to
that found in the ketoprofen, phenylbutazone and dipyrone studies described above.

The efficacy of TMS treatment could not be evaluated in the NSAIDs studies since all cases were treated with TMS. This study provided a unique opportunity to compare the effect of antimicrobial therapy with a virtual negative control group (cases associated with TMS resistant coliforms). The TMS therapy probably did not have any effect other than the antimicrobial effect. Therefore, cows were randomly allocated into the treatment and control groups by virtue of sensitivity to TMS of the infecting organism. Whether infection by strains that are sensitive or resistant to TMS is associated with other factors such as age, days in lactation, or herd which may confound recovery, is unknown. Some of these possible confounders were evaluated in the statistical analysis. There is a possibility that organisms that are sensitive to TMS induced a milder form of the disease and, hence, a higher recovery rate. In fact, the opposite might be true as the drug-sensitive strains of Gram-negative organisms are known to differ in their capacity to release endotoxins, either spontaneously or upon exposure to bactericidal antimicrobial drugs (Shenep and Mogan, 1984; Shenep et al., 1985). Therefore, if indeed endotoxin release induced by drugs is of any clinical significance in coliform mastitis, we would have expected a lower rate of therapeutic success for the group of strains that were sensitive to TMS. The antimicrobial therapy frequently has been opposed because of the rapid spontaneous drop of bacterial counts 8 to 24 h after infection (Hill et al., 1978; Pyörälä et al., 1994). Erskine et al. (1991) argued that most treatments under field situations are administered too late and may even be detrimental because they may further induce endotoxin release. The very high management standards of the participating herds and the use of computerised in and on-line electrical conductivity measurement systems probably facilitated early diagnosis of clinical mastitis. The inclusion criteria of the present studies ensured that only severe cases were included and treated for clinical mastitis.

Spontaneous cure rates are probably extremely high in mild cases of coliform mastitis and antimicrobial treatment is not necessary. Inclusion of mild cases of coliform mastitis in the field trials may dilute the possible effect of antimicrobial treatment to undetectable levels, and may explain the discrepancy between the results presented here and those published by other authors.
10. CONCLUSIONS

Clinical mastitis was found to be one of the most important diseases affecting Israeli dairy cows with mean lactational incidence risk of 20.8 per 100 lactations. Most clinical mastitis cases were associated with coliform bacteria.

The NSAIDs ketoprofen, phenylbutazone and dipyrone showed beneficial effects in the treatment of field cases of clinical mastitis. Ketoprofen was found superior to the other two.

Antimicrobial treatment may be efficient in the treatment of coliform mastitis in high yielding dairy cows. Trimethoprim sulphadiazine combination improved cure rates in field cases of coliform mastitis, if the mastitis causing bacterial strain was in vitro susceptible to this combination.

Cefquinome was found effective in the treatment of experimentally induced serious E. coli mastitis. The drug improved clinical recovery, return to milk production and bacteriological cure rates.

The question about the ultimate significance of antimicrobial treatment in coliform mastitis still remains open and more studies both in experimental models and in the field are needed.
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Nahum Yehuda Shpigel
12. REFERENCES


NCCLS. 1983. Performance standards for antimicrobial disk susceptibility tests. M2-T3 National committee for clinical laboratory standards, Villanova, PA USA.


