CLINICAL MASTITIS, DIRECT CULTURE AND THERAPY

By

Jørgen Katholm

Søndergade 37, Vivild, Denmark

Not Published

ABSTRACT.

This paper is written by a practitioner veterinarian.

The clinical symptoms of mastitis cases give little information on the pathogen involved.

For more than 18 years mastitis bacteria have been cultured directly from clinical cases of mastitis.

Different media have been used: Müller Hinton agar, Mannitol agar, SELMA dish and blood agar. The SELMA dish is found to be an excellent dish that gives even untrained bacteriologists a good differentiation of the different mastitis pathogens.

Today a set of blood agar dishes, one ordinary and one containing penicillin 0.1 i.u/ml, is used. It is cheap and the hemolysis is seen better than on the SELMA dish.

Staphylococcae mastitis is often connected with teat lesions and has a low cure rate.

Like staphylococcus, Streptococcus dysgalactiae is often connected with lesions of the teat. It might be found together with Actinomyces pyogenes.

For Streptococcus uberis no particular patterns have been observed. Like E. coli, Streptococcus uberis is referred to as environmental mastitis pathogens. I believe that immunosuppression is an essential factor when these cases occur endemically.

For Actinomyces pyogenes mastitis the systemic symptoms seem to be better controlled by tylosin therapy.

Yeast mastitis often gives a swollen quarter for more than one week. Antibiotic therapy is not effective, and the quarter is clinically normal after 2 - 3 weeks of milking.

In coliform mastitis CFU in milk is a valuable prognostic tool. When solitary colonies are found by direct culture of a milk sample in the homelab, the case is uncomplicated. When carpet growth is found, it corresponding more than 1 million CFU/ml, endotoxins are often found in the milk vein and prognosis is reserved. In these cases therapy must be intense and include frequent milking, fluids (NaCl 7% 4-5 ml/kg body weight or blood transfusion) , calcium at first visit, NSAIDs, pain relief epidural (1½ ml Morfine 20mg/ml +1 ml Xylacin 20mg/ml and isotonic NaCl ad 10 ml) and antibiotics.

This article is based on the following papers, which will be referred to in the text by their roman numerals I - VIII

I   Katholm J.: Acute mastitis in a Danish country practice. Frequency, Clinical parameters, bacteriological prevalence and resistance pattern. Dansk Veterinær Tidsskrift 1986, 69, 24, 1261-1265.

II   Katholm J.: Coliform Mastitis. Home cultivation and therapy. Dansk Veterinær Tidsskrift 1989, 72, 4, 171-177.

III   J. Katholm, Ø. R. Jepsen and N. Agger. Peracute coliform mastitis. Experiences from 70 cases in General Practice. International Conference on mastitis. St Georgen/Längsee Austria. 1989. 8 - 12.

IV   Katholm J.:Mastitis Diagnostics on SELMA agar. Dansk Veterinær Tidsskrift. 1990, 73, 57-60.

V   Katholm J. and Andersen P.H.: Acute coliform mastitis in dairy cows: Endotoxin and biochemical changes in plasma and colony-forming units (CFU) in milk.
Paper for application for the title "Kvægfagdyrlæge recognized by the Danish Veterinary Organisation" 1990. Not published.

VI   Katholm J. and P. H. Andersen:Acute Coliform Mastitis in Cattle. International Conference Mastitis:Physiology or Pathology, Gent, Belgium, 1990, 140.

VII   Katholm J. and Andersen P.H.: Acute Coliform Mastitis in Cattle: Endotoxin and biochemical changes in plasma and colony-forming units (CFU) in milk. The Veterinary Record, November 28, 1992, 513-514.

VIII   Katholm J. and Andersen P.H.: Severe coliform mastitis in Practice, Efficacy of a therapy regimen not including antibiotics. XX World Buiatrics Congress, Sydney Australia 1998, p. 261-264
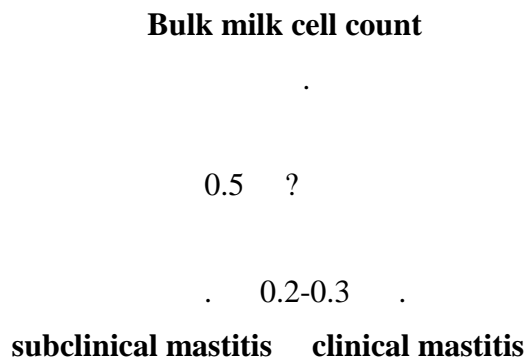
CLINICAL MASTITIS.

Mastitis is an inflammatory reaction of the mammary gland (IDF, 1987). Ordinarily, but not necessarily, mastitis reflects the presence of pathogenic bacteria in the milk compartment of the udder (Sandholm and Mattila 1986).

The incidence of acute clinical mastitis in Denmark is 0.55 per cow per year (Jensen 1987).

As seen in figure 1 there is a 0.5 correlation between bulk milk somatic cell count (SCC) and subclinical mastitis, and a 0.2-0.3 correlation between clinical and subclinical mastitis (Grommers et al. 1989). But the correlation between clinical mastitis and bulk milk SCC is not defined.

Figure 1: Correlation between bulk milk somatic cell count, subclinical mastitis, and clinical mastitis.(Grommers et al. 1989

Bulk milk cell count

.

0.5   ?

.   0.2-0.3   .

subclinical mastitis   clinical mastitis

In my opinion the correlation between bulk milk somatic cell count BMSCC and clinical mastitis is negative. Erskine et al. (1988) found that the incidence of clinical mastitis was higher in herds with low BMSCC (table 1).

Table 1:Herd incidence and percentage of clinical mastitis caused by various bacteria. (Erskine et al 1988).

		Strep. agalactiae	Staph. A	Coliform	Sterile	Incidence per month
Low BMSCC $\leq$150.000	(N=12)	0	2.2	43.5	28.6	4.23
High BMSCC >700.000	(N= 6)	41.5	18.3	8	8.8	2.91

This can be a real difference but it can also be a reflection of the owners of the well managed farms being more alert and therefore finding and treating mastitis cases quicker. These cases might be overlooked in the other farms and not treated because they self cure. Especially this might explain the big difference in E. coli mastitis. But in my opinion the difference in incidence is real and can be explained by cows with high BMSCC being more resistant to new acute infections, especially E. coli. Barkema et al. (1998) did not find significant higher incidence rate of clinical mastitis in herds with low bulk milk somatic cell count (BMSCC) $\leq$ 150.000 compared to two groups of herds with BMSCC between 150.000-250.000 and 251.000-400.000.

But they also found that clinical mastitis caused by gram negative bacteria occurred more often in herds in the low BMSCC category

A lot of misunderstandings between the national mastitis laboratories and the practitioner veterinarian in the beginning of the eighties were based on these simple facts. Herds pointed out by their SCC being higher than 500.000 did not correspond with the veterinarians opinion of the herd having a high incidence of clinical mastitis, and at that time the price differentiation did not motivate the owners and the veterinarians to endeavour to lower the prevalence of sub clinical mastitis.

Mastitis pathogens can more or less be divided into two groups: 1) those that need to live and multiply principally on and in the cow`s udder and are contagious, spreading from animal to animal by contamination during milking and 2) those that are present in the cow`s environment (Sandholm and Mattila 1986) (Table 2).

Table 2: Connection between origination of infection and pathogen.

Udder	Environment
Staphylococcus aureus	E. coli
Streptococcus agalactiae	Streptococcus uberis
Streptococcus dysgalactiae	Actinomyces pyogenes

It is important to know this difference. While the "5 point plan" (proper milking technique and a proper functioning milking machine, use of post-milking teat disinfection, dry cow treatment, treatment of clinical cases, and culling of chronically infected animals) will control the prevalence of contagious pathogens in dairy herds, the components of the "5 point plan" are less effective at controlling the enviromental pathogens (Smith and Hogan 1996)

In herds where a mastitis control routine based on post milking teat disinfection and drying off therapy is always used, the prevalence of intramammary infection falls to low levels because those that formerly caused most mastitis (i.e. <u>Streptococcus agalactiae</u>, <u>Streptococcus dysgalactiae</u> and <u>Staphylococcus aureus</u>) can bee limited or reduced to low levels. Clinical mastitis is reduced and the herd SCC falls, but the frequency of acute clinical mastitis due to coliform infection does not decline and in some herds may increase. (Bramley and Dodd 1984).

To control mastitis causes by the environmental pathogens the "5 point plan" must additionally address hygiene, nutrition, housing and cow comfort, air and water quality, antibiotic use, health monitoring, breeding policy, and cow characteristics such as immunologic competence, cow conformation (teat and udder) and milk production level (Schukken et al 1998)

The ability of veterinarians to differentiate the clinical symptoms of the different pathogens was investigated by (Funke 1983).(Table 3)

Table 3. The expected pathogen based on the clinical symptoms compared to the pathogen verified at the laboratory (Funke 1983).

Clinical diagnosis.		Pathogen found in laboratory in %							
agent	Number	Sterile	Staph.a	Stp.d	Stp.u	oth.stp	coli	co.p	mix.
Staph.	230	22	27	13	4	9	16	1	2
Strep.	88	26	22	15	10	8	7	-	2
Coli	27	11	18	18	-	-	38	-	4
Actino. p.	5	-	-	20	-	-	-	60	20
no guess	414	21	27	14	5	6	16	2	2
More pathog.	25	16	32	12	4	16	4	-	-
Total	789	21	26	14	5	7	15	2	2

The clinical diagnois <u>Staph. aureus</u> was confirmed in 27% of the cases. Streptococci was confirmed in 33% of the cases and Coliforms in 38% of the cases. Of 53 cases of coliform mastitis this diagnosis has been clinically diagnosed in 10 cases (19%).

This shows that the clinical diagnosis of the pathogen involved in clinical mastitis cases is of little value.

Until quick cow-side tests have been developed culture of bacteria is the only way to confirm the diagnosis.

DIRECT CULTURE - HOME LABORATORY.

As a massive infection by one agent often occurs in clinical mastitis, there are good possibilities for direct culture from a milk sample, taken under sterile conditions.

The sample is taken in a sterile tube held as horisontally as possible. I put the sample in my bag until return to the homelab. I shake the sample a little before dipping a sterile cotton pin in the sample.

The agar dish is stored with the bottom up in a closed plastic bag at 5°C. Before use the top of the agar dish is shaken for condensed water.

The cotton pin is then slowly moved across the agar area.

In the last 12 years I have used different agar types for direct culture.

Müller Hinton agar.

In the beginning the direct culture was made only to confirm that the pathogen found was not resistant to the antibiotic given to the cow.

The preferred agar for resistance evaluation is Müller Hinton agar. It was, therefore, natural to start with this agar. I used Müller Hinton agar in the years 1980 - 1983. Tablets with different antibiotics were placed directly on the dish. After incubation the inhibition zone for each antibiotic was measured. For untrained bacteriologists it is difficult to differentiate the mastitis pathogens on colony morphology alone, that is why I changed to mannitol agar in 1984.

The different pathogens can, to some extent, be typed by the resistance patterns.

Gram negative bacteria grow up to the penicillin tablet.

Penicillin resistant staphylococci often have a small zone around the penicillin tablet.

Staphylococci often grow better around and close to the Sulfa/Trim. tablet.

Streptococci are often found close to the neomycin and streptomycin tablets.

Yeast will grow all over the plate and is typically 1-2 days underway, an inhibition zone might be seen around the neomycin tablet.

Mannitol Agar.

Mannitol agar for direct culture was introduced in Denmark by Hanssen and Jepsen (1984).

It is important to know that it is a special agar with mannitol as indicator.

Mannitol agar bought in ordinary laboratories might contain 10 % salt, this agar is selective for staphylococci. This fact was the cause for some misunderstandings.

It is important to know that the inhibition zone is not always the same on the different agar types. The correct diameters on the mannitol agar are given by ROSCO A/S Tåstrupgårdsvej 30, DK-2630 Tåstrup, Denmark.

After spreading the sample with the cotton pin, resistance tablets are placed on the agar and the dish is incubated bottom up at 37°C.

By the colour of the streptococcae colonies they could roughly be divided into two groups Str. dysgalactiae and Str. uberis.

The differentation criteria on the mannitol agar of the different bacteria are seen in table 4.

The home laboratory culture on mannitol agar was compared with results from the national veterinary laboratory, Aarhus (Paper I). Of 79 samples compared 76% gave exactly the same result. The 79samples were selected among 145 mainly on two criteria, cases where the typing on the mannitol agar was difficult and if there were more cases on one day.

Table 4. Bacterial diagnostics on mannitol agar. (Table IV).

Bacteria	agar colour	opacity	colony diameter	catalase +/-	KOH 3% +/-
Staph. aureus	Yellow	++	>1	+	-
Str. dysgal.	red	-	<1	-	-
Str. uberis	Yellow	+-	<1	-	-
E. coli	Yellow	+	>2	+	+
Yeast	Red/Yellow	+	1-2 Days	+	-
Act.pyogenes		-		-	-
Staph. epiderm	Red/Yellow	++	>1	+	-
Str. agalactia	Red	-	<1	-	-
Str. bovis	Yellow	+-	<1	-	-

SELMA dish.

In 1988 a new petri dish was introduced i Sweden by Jonsson and Ekman 1988, and sold through NOVO diagnostics Malmø.

This plate was sold in Denmark by NOVO Diagnostics Copenhagen. I have used this type of dish in the years 1989-1991. This dish is now sold by Kruuse A/S.

The SELMA dish is a SELective Mastitis Agar. It consists of three agars. I: Blood agar with aesculin. II: Mannitol agar with salt (selective for staphylococci). III: McConkey agar (selective for gram negative bacteria).

The sampling, storing and spread onto the agar is the same as mentioned earlier.

The diagnosis of the bacteria, which I now find most important, is more specific on the SELMA dish, than on the mannitol agar. Streptococci grow on I. Staph. grow on I and II. E. coli grow on I and III. On the other hand there is no sensitivity test for the gram negatives, but this is practically unimportant as I will mention later.

The differentiation criteria on the SELMA dish of the different bacteria are seen in table 5.

Table 5: Bacterial Diagnostics on the SELMA Dish (+ = growth) (Table V).

Bacteria	I Blood Agar	II Staphylo-coccus agar	III Gramnega-tive agar
Staph. aureus	+ (clear and diffuse hemolysis)	+ yellow agar	-
Staph. (other)	+	+	-
Str. agalactiae	+(beta-hemolysis)	-	-
Str. dysgalact.	+(alpha hem.green)	-	-
Str. uberis	+(aesculin pos.)	-	-
Actinomyces pyogenes	+ (hem. 2 days)	-	-
E. coli	+	-	+ red and red agar
Klebsiella	+	-	+ red
Pseudomonas	+ smell	-	+
Yeast	+	+/-	-

Table 6: Distribution of the Mastitis pathogens from 75 cases directly diagnosed from the SELMA Dish and number of verified diagnoses from National Veterinary Laboratory (N.V.L.). (Table IV)

Pathogen	SELMA	N.V.L.
Staphylococcus aureus	20	18
Streptococcus dysgalactiae	20	16
Streptococcus uberis	6	3
Actinomyces pyogenes	11	10
Escherichia coli	15	14
Sterile	3	3
TOTAL	75	64

If Str. agalactia can be excluded there is a good differentiation between Str. dysgalactiae and Str. uberis. Str. uberis is aesculin positive and with a UV-light the colonies and the agar will be dark.

The agar is thick and the hemolysis is weak so it is difficult to find Str. agalactiae.

Staphylococci can be differentiated into Staph. aureus which is double hemolytic on agar I and yellov on agar II and other staphylococci. The staphylococci can be tested for penicillinase production by a nitrocifin disk placed on the colonies on agar II, and incubated for 15 min. If the disk turns red, it produces penicillinase.

E. coli can be differentiated from other gram negatives by the red colonies and red agar on agar III. After culture in for about 4-6 hours coliform bacteria can be seen on the agar plates.

A. pyogenes grow on the blood agar, I. They can be seen as pinpoints after 24 hours but better after 48 hours. This is a clear advantage over the Mannitol agar because not all A. pyogenes can be diagnosed by the smell of the secretion.

On the other hand yeast is difficult to see on the SELMA dish and that is a disadvantage.

I have tested this SELMA dish and the results are seen in table 6. For more details, see Paper IV.

The diagnosis on the SELMA dish was verified in 85% of the cases.

Blood agar.

In the beginning of 1992 Kvægbrugets Laboratorium in Ladelund introduced a set of blood agar dishes, one without penicillin and one with 0.1 i.u. penicillin, and the price was half the price of the SELMA dish. The dishes can only be stored for 3 weeks but the price was a great advantage and I have used these dishes since then.

The bacteria are recognized by their growth, haemolysis, smell and colonial morphology.

This bacteriology is known to all veterinarians but I will summarize a few characteristics.

Staphylococcus aureus.

Will be recognized by a double haemolysis
The penicillin resistant strain will grow on both dishes.

Coagulase negative staphylokokkus (Micrococcus).

The colonies are bigger than streptococcus and catalase positive.

Streptococcus dysgalactiae.

A weak green colour in the agar around the colony is often seen.
A top is often seen in the middle of the colony.

Streptococcus uberis.

Is recognized by the aesculin breakdown (colonies are brown in UV-light). For the mucoid type the aesculin breakdown will not be seen until after 2 days.

Haemolytic streptococcus.

Might be B-streptococcus and shall therefore always be sent for typing by an official laboratory.

Streptococcus faecalis.

Is aesculin positive like Streptococcus uberis but Streptococcus faecalis grow on a dish with 0.1 i.e. penicillin/ml and a dish with 1.0 i.e penicillin/ml
Streptococcus lactis looks like Streptococcus fæcalis but they do not grow on a 1.0 i.e. penicillin/ml dish. (Veterinærdirektoratet 1992)

Actinomyces pyogenes.

Will not be seen until after 2 days of incubation. A sharp haemolysis will be seen. They often grow along with Streptococcus dysgalactiae.

Coliform.

Will grow on both dishes and there will be a characteristic smell when the dish is opened.

<u>**Yeast**</u>.

Might look like micrococcus colonies but yeast often grows more slowly and often 2 days are needed before fine colonies can be seen. They will grow on both dishes.

<u>**Sterile samples.**</u>

Morin and Constable (1998) found that episodes of clinical mastitis with no bacterial growth from milk were similar to gram-negative mastitis episodes, but significantly different from gram-positive mastitis episodes. However, cows with clinical mastitis and no bacterial growth from milk had significantly lower heart rates, higher rumen contraction rates, and a lower frequency of gland enlargement than cows with gram-negative mastitis, indicating milder clinical disease.

Zorah et al. (1993) found that 51.2% of whey samples, from quarter milk samples from cases of clinical mastitis in which no bacterial pathogen had been isolated by standard culture techniques, antigens to <u>E. coli</u> were detected by an ELISA test.

<u>Differentiation of the Bacteria.</u>

On all the agar types the bacteria might be differentiated by their growth and colonial morphology. As an aid in the differentiation a few simple tests are of great value.

Catalase test.

Is used to differentiate between streptococci which are negative and staphylococci and <u>E. coli</u> which are positive.

A colony is placed on an object glass with a needle. A drop of 3% hydrogenperoxide is placed on the colony. If small bubbles are seen the test is positive (staph. or coli).

KOH - test.

This test is a quick test to be used instead of gram staining. The gram negative bacteria will be test positive.

If the catalase test is positive this test can be used to differentiate between staphylococci and coliforms. One drop of 3% KOH is placed on the object glass. A bacterial colony is taken with a needle. The needle is then dipped at the edge of the drop and lifted several times ½ cm above the drop. If the test is positive (gram negative bacteria) a thin slimy string is lifted along with the needle.

Microscopy.

With gram staining a higher certainty of the diagnosis can be achieved.

A quick impression of the bacterial shape is achieved with nigrosin staining. A bit of a bacterial colony is dissolved in a drop of water placed on an object glass. This drop is mixed with a drop of nigrosin 5% and spread over the object glass. After drying microscopy gives a good impression of the bacterial shape.

Summary.

Direct culture gives the veterinarian an excellent opportunity to correlate the bacteriological diagnosis with clinical symptoms. This led to the obvious correlation between the numbers of coliform bacteria after direct culture and the severity of clinical symptoms of cows suffering from coliform mastitis.

CLINICAL MASTITIS, SYMPTOMS AMD THERAPY.

I my practice I use direct cultur in about 80%-90% of all the mastitis cases I treat. To get an impression of the distribution of pathogens in one area it is necessary to collect milk samples from all mastitis cases for a period of time. I have done this in 3 periods as can be seen in table 7. One should remember that farmers in Denmark are not allowed to treat mastitis. The frequencies then reflect all the clinical mastitis cases in the area in that period, of course influenced by the difference in the farmers' wish to have cows treated.

Table 7. The distribution of pathogens from 3 periods where all clinical mastitis cases were cultured.

Year	1985	87-88	1990
Agar type	mannitol	mannitol	SELMA
No of cases	145	246	75
pathogen			
Staphylococcus aureus	21	19	27
Other staphylococci	1	4	
Streptococcus uberis	17	10	8
Streptococcus dysgalactiae	26	23	27
Actinomyces pyogenes	1	6	14
Escherichia coli	12	20	20
Yeast	2		
Sterile	20	18	4

It is seen that the most frequent mastitis pathogens are Staph. aureus, Strep. dysgalactiae, Strep. uberis and E. coli. This is not surprising.

An important question is whether it is possible to differentiate the mastitis pathogens from the clinical symptoms of a mastitis case. This was done in the trial from 1985. The results are seen in table 8 and 9.

Table 8: The distribution in percentage of the most important pathogenic bacteria for each of the examined clinical parameters. (Paper I).

Clinical Symptoms	Staph.	Strep dys.	Strep Uberis	E.Coli	Yeast	sterile	Total
Oedema	17	17	14	31	0	17	20
Lesion	35	31	12	4	0	23	18
Temp. > 40°C	16	21	21	26	10	0	13
Catarrah. secret.	20	28	19	7	2	21	88
Serous secret.	24	0	18	35	0	6	12

The clinical parameters are of little value, that is why the direct culture is so important. There is a tendency for E. coli mastitis to be related to oedema and temp. > 40°C and staphylococcus and streptococcus dysgalactiae to be frequent when lesions are found on the teat.

Table 9: The distribution in percentage of the most important pathogenic bacteria related to time of Infection in the lactation period. (Paper I).

	Staph.	Strep dys.	Strep uberis	E.Coli	Yeast	sterile	Total
0 - 1 weeks p.p.	28	34	3	9	0	16	22
2 - 4 - p.p.	14	21	14	36	0	14	10
5 - 20 - p.p.	18	25	25	11	5	11	39
> 20 - p.p.	25	21	17	4	0	25	17
Dry period	17	22	17	0	0	33	12

In table 9 it is seen that E. coli is most frequent in the period 2 -4 weeks after calving. And that no E.coli is found in the dry period.

From these observations it is seen that there are few guidelines for the practitioner, and Funke (1983) (Table 3) found that it is not possible for the practitioner to differentiate the different mastitis pathogens by the clinical symptoms.

The willingness of the cow to eat, the duration of the mastitis case and for how long a period there have been clots in the milk will give some guidelines, as mentioned later.

As a standard treatment today all lactating cows are milked thoroughly after injection of 10 i.u. of oxytocin vet.

Cows expected to suffer from mastitis caused by staphylococci and streptococci are then treated with 10 mill i.u. of penethamate hydriodide BAN (Leocillin® vet) i.m. and 1 mill i.u. of penethamate hydriodide BAN (Leocillin® vet) or a combination of 75 mg ampicillinum natrium DLS and 201 mg cloxacillinum natrium (Ampiclox® vet) DLS i.m.m.

Cows expected to suffer from Actinomyces pyogenes mastitis are treated with Tylosinum NFN (Tylan® vet) 10 mg/kg i.m and 600 mg i.m.m. (Katholm, 1988).

Cows expected to suffer from Coliform mastitis are treated with 200mg Sulfatroxazole and 40 mg trimethoprim (Potrox® vet)/15 kg i.v. amd 250 mg cefoperazonum NFN (Pathozone® vet and Cefotron® vet) i.m.m. Depending on the clinical symptoms these cows are additionally treated with fluids, NSAID´s, epidural pain relief , and calcium borogluconate.

Retreatments are based on the bacteriological findings after direct culture.

STAPHYLOCOCCAL MASTITIS

Traumatized tissue at the teat end or teat canal seems to form a predilection site for S. aureus which typically is a wound pathogen (Wadström, 1987). Staphylococcal mastitis is typically associated with teat canal colonization, teat canal irritation and inflammation. (Kaartinen and Jensen 1988). Colonization sites of the teat end give S. aureus a chance to adapt to milk. After adaptation, the bacteria can be propelled to upper parts of the milk ducts and survive there. (Sandholm et al. 1991).

Acute S. aureus mastitis occurred relatively more often if the teat injury was chronic than if it was an acute injury. (Waage, 1991a).

If the inflammatory response and associated PMN influx is delayed or is absent for the first few hours of infection, usually in early lactation, a very severe form of the disease occurs. Bacterial growth is uninhibited, _ toxin produces dramatic tissue damage and oedema, occlusion of blood supply finally resulting in necrosis and gangrene. (Hill, 1991).

Excretion of bacteria and cells in sequential milk samples from a case of staphylococcal mastitis can alter greatly (Bramley, 1991)

S. aureus can survive inside the PMN where they are protected from the effect of antibiotics. They can also live intracellular in macrophages and udder epithelial cells. Besides, tissue injuries and occluded milk ducts can reduce the concentration of the antibiotic at the infection site (Johnsson and Holmberg 1988)

The self cure rate of S. aureus mastitis within a 3-week period is approximately 25% (Sandholm et al., 1991).

Østerås (1991) found that the self cure in the dry period, of subclinical staphylococcal mastitis prior to drying off was 44%.

Funke and Strandberg (1991) found no difference in the bacteriological cure rate of clinical S. aureus mastitis treated for 3 or 5 days with penethamate hydriodide BAN (Leocillin®). The bacteriological cure rate after 3 days' therapy was 59%.

Pyörälä and Pyörälä 1998 found that a longer duration of treatment resulted in better bacteriologic cure rates in S. aureus mastitis (42% for 64 cows treated for 5 days vs 29% for 91 cows treated for 3-4 days). However, the difference was not significant.

Jarp et al. (1989) found the bacteriological cure rate of S. aureus mastitis to be 48.9% for cows treated with one im. injection of a combination of procaine penicillin and dihydrostreptomycin (Streptocillin®) followed by one i.m.m. treatment daily per infected quarter for four days. For cows treated three days with penicillinprocaine (Penovet®) im. the bacteriological cure rate was 47.3% and for cows treated five days with penicillin procaine im. the bacteriological cure rate was 59.5%. There was no statistically significant difference between the three regimens. If a normal cell count was requested the cure rates were 40.6%, 27,3% and 46.8%, respectively five days' treatment being statistically better than the three

days treatment. In severe mastitis caused by S. aureus the difference between the three regimens was very small.

Waage (1997) found no difference in cure rates of S. aureus in a clinical trail with two therapy regimes. Regimen 1. Benzylpenicillin prokain 15 mill iu im. at day 1. Regimen 2 benzylpenicillinprokain 15 mill iu im.in three days. Both regimes intramammary treatment with 200,000 iu benzylpenicillin prokain and 250 mg dihydrostreptomycin sulphate.

Sandholm et al. (1991) found that the comparison of suppressive effects of antibiotics on bacterial growth of S. aureus in milk and iso-sensitest broth (Oxoid CM 473) clearly shows that in most instances several times more antibiotic is required for milk to reach an equipotent bacterial inhibition as compared with iso-sensitest broth cultures. There were two exceptions to the rule , one with G-penicillin and another with streptomycin.

Parenteral treatment with spiramycin and erythromycin has been claimed to have a pharmacokinetic advantage over many other antibiotics. Calculations based on the pH partitioning hypothesis and supporting experimental data show that lipophilic, basic antibiotics concentrate from blood to the more acidic milk phase due to ion trapping. This pharmacokinetic advantage seems to be opposed as the milk phase protects S. aureus bacteria from these antibiotics (Sandholm et al. 1991).

Waage (1991b) found that the proportion of penicillin resistant strains of S. aureus varied with the clinical condition of the quarter. In quarters with acute mastitis and disturbed demeanour 96.6% was sensitive to penicillin, in subclinical mastitis 87.7% was sensitive to penicillin and in quarters with normal cell count 85.2% was sensitive to penicillin.

Own observations.

In table 8 it is seen that 35% of all mastitis cases with teat lesions was infected with S. aureus.

There is a great variation in the number of bacteria found after culture but there has been no obvious correlation with clinical symptoms and cure.

Clinical diagnosis might be suspected if there have been clots in the milk for several days, if the cow refuses to eat concentrate but still is eating the other feedstufs. If the temperature is elevated and the infection is more than 14 hours old, if there is a chronic stricture of the teat canal.

I recommend the owner to have these cows re-treated with 10 i.u. of penethamate hydriodide BAN (Leocillin®vet) i.m. and 1 i.u. of penethamate hydriodide BAN (Leocillin®vet) or a combination of 75 mg ampicillinum natrium DLS and 201 mg cloxacillinum natrium (Ampiclox®vet) DLS i.m.m. If the staphylococcus is resistant to penicillin I recommend to have the cows re-treated two days with spiramycinum INN (spiramycin® vet) 320,000 i.u./20 kg i.v. and a combination of 75 mg ampicillinum natrium DLS and 201 mg cloxacillinum natrium (Ampiclox® vet) DLS i.m.m. each day.

STREPTOCOCCAL MASTITIS

Self cure for clinical streptococcal mastitis is found to vary from 15-67% (Åstrøm 1988).

Streptococcus dysgalactiae.

Streptococcus dysgalactiae was isolated from 27% of quarters with acute mastitis where the teat had an acute injury. If the teat had a chronic injury or no injury the bacteria were found in 11% of the quarters (Waage 1991a).

Grønborg-Pedersen (1991) found that Streptococcus dysgalactiae was the most commen bacteria isolated from heifers suffering from mastitis prior to and around calving.

Østerås (1991) found that the self cure in the dry period of subclinical Streptococcus dysgalactiae mastitis prior to drying off was 60%. And with antibiotic treatment the cure rate was 100%.

Own observations.

I found infections with Streptococcus dysgalactiae to be most common in the first week after calving (Paper I)

Like staphylococcus, Streptococcus dysgalactiae is often connected with lesions of the teat. (Paper I) It might be found together with Actinomyces pyogenes. (Paper IV)

Streptococcus uberis.

Bacterial populations in litter have been shown to be key risk factors for coliform and Streptococcus uberis infection (Bramley, 1982).

Streptococcus uberis is the most common bacteria found in new infections in dry cow (Johnsson and Holmberg 1988).

Thomas et al. (1990) found that 1,3 and 6 days after experimental infection with Streptococcus uberis organisms may be located free or phagocytosed, predominantly in the alveolar lumen. However, only a small proportion of neutrophils appear to be able to take up the bacteria. They found that the neutrophil response in experimental infection is profound but adherence to epithelium is not an integral part of the response. It would appear that before any substantial invasion of the tissue by bacteria can occur some breach in the ductular or glandular epithelium is required. Neutrophil polymorphs with their armoury of tissue damaging chemicals and enzymes could be the mediators of such damage.

According to the bacteriae colonal morphology Streptococcus uberis can be divided into a mucoid type that is aesculin positive after 1-2 days and a non-mucoid type that is aesculin positive after 1 day.

Own observations.

Like E. coli, Streptococcus uberis is referred to as environmental mastitis pathogens.

When Streptococcus uberis is found in more cases of clinical mastitis emphasize must be put on clean environment especially the straw bedding. To crowded straw areas for dry cows and a to crowded straw mattress for milking cow has been found as reasons for the infections. 6-7 m^2 pr cow is needed. (Figure 2). In tied stall a more intensive cleaning of the bedding solved the problem with mastitis in newly calved heifers.

Figure 2: The distribution of Streptocuccus uberis mastitis on a monthly basis in the year 1997 from a herd where the cows were held on a straw bedding.
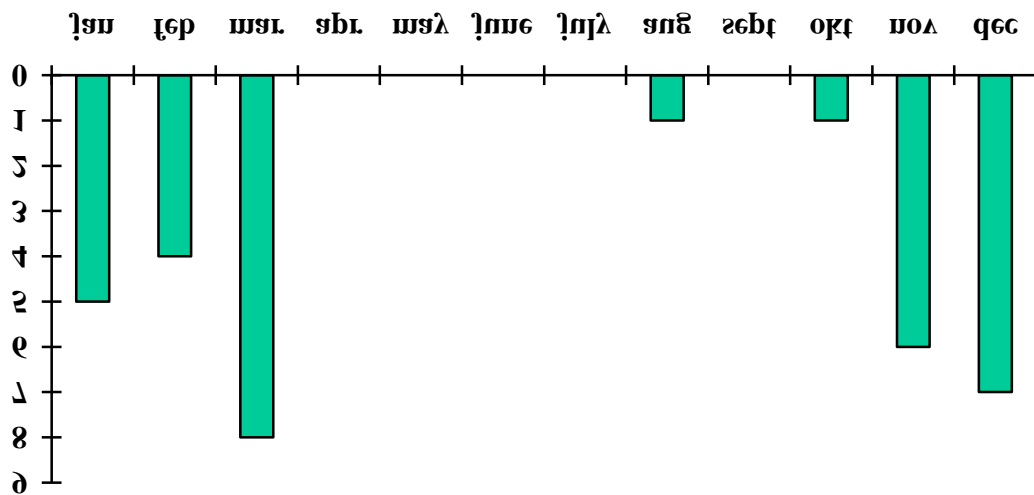
It is seen in figure 2 that the mastitis cases occurs in the winter period where the cows are housed the whole day. The problem was solved by reducing the numbers of cows on the straw area.

Figure 3: The distribution of Streptocuccus uberis mastitis on a monthly basis in the year 2000 from the same hers where the cows were held on a straw bedding until august and thereafter moved to a new area with sand in the stall.
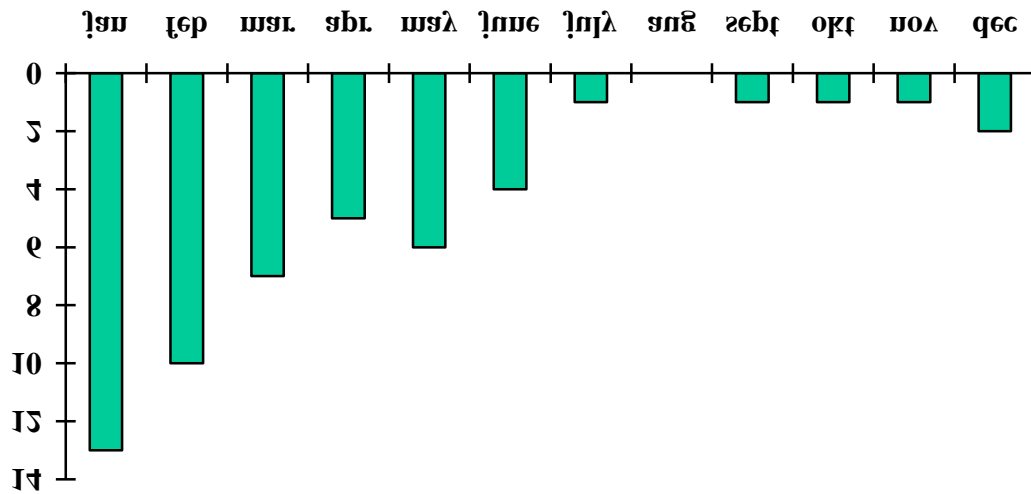
ACTINOMYCES PYOGENES MASTITIS.

Own observations.

For Actinomyces pyogenes mastitis the systemic symptoms seem to be better controlled by tylosin therapy (Katholm 1988).

YEAST MASTITIS

Own observations.

Yeast mastitis often gives a swollen quarter for more than one week. Antibiotic therapy is not necessary. The quarter is clinically normal after 2 - 3 weeks of milking.

COLIFORM MASTITIS.

Recommended principles for treatment of mastitis caused by gram negatives (all coliforms).

Summary.

Clinical diagnosis is confirmed at cow side with Limast® test. Exact diagnosis is based on result of direct culturing.

The majority of coliform mastitis cases are relatively mild and self limiting.

Around 20% of clinical cases treated by the veterinarian in Denmark are coliform mastitis.

In experimental trails it is found that the outcome of the disease may be predicted from the oxygen burst of blood neutrophils prior to infection, and that cows with high numbers of <u>E.coli</u> in secret from infected quarters showed greater losses in milk production.

This corresponds with the authors clinical finding where CFU at direct culturing seems to be more decisive for the prognosis than different types of treatment. Cases with severe symptoms show carpet growth at direct culturing indicating CFU higher than 10^7/ml.

Treatment is based on supportive treatment. Stripping out, calcium, 7% sodiumcloride, isotonic fluids, antiinflammatory drugs, pain relief and antibiotics.

Background.

Treatment of coliform mastitis is a constant challenge to cattle practitioners. There is a great variation in the outcome of the disease. Most cases are cured by the cow it self, a few cows die. These facts make every case an interesting case. To reduce cases of coliform mastitis in a herd a lot of prophylactic actions must be taken, e.g. balancing feed intake, hygiene, ventilation, selenium or E-vitamin supplementation, and vaccination. This paper will solely deal with treatment of a cow with a coliform infection in the udder.

The diagnosis is based on the well known clinical symptoms and I stress the importance of a pronounced drop in milk yield and total loss of appetite.

Veterinarians who do not make direct culture often think that they can point out cows suffering from coliform mastitis. From table 10 it is seen that 40% of the coliform diagnoses based on clinical symptoms will be wrong. In paper III we found that 33% of the clinical coliform diagnoses were other types of mastitis especially <u>Staphylococcus aureus</u>. In table 10 it is also seen that 14% of all mastitis cases not clinically suspected to be coliform, were coliform mastitis. Meaning that 63% (31 out of 49) of all cases of coliform mastitis is overlooked if selection is based on clinical symptoms. These findings were based on a selection for coliforme mastitis cases in a clinical trial and therefore more selective than usual. As Funke (1983) showed in table 3 under normal clinical conditions only 38% of the clinical cases selected as coliform were right.

Table 10. The number of cows with coliform mastitis in proportion to clinical suspicion. Paper II.

	Total	Coliform mastitis
Clinical diagnosis:coliform	30	18 (60%)
Clinical diagnosis:Not coliform	216	31 (14%)

Total	246	49 (20%)	

If the cow side test for endotoxins (Limast® test) is included after the clinical diagnosis there will be only few cases of Actinomyces infections where the clinical diagnosis of coliform mastitis does not correspond with the result at culturing. Still around 60% of all coliform mastitis cases is not found by that procedure. (Table 11)

Table 11: Relations between microbial diagnosis and clinical diagnosis for 464 cases of clinical mastitis treated in 1996, including a cowside test for endotoxin.

	Microbial diagnosis (no of cows)		
	Coliform+	Coliform-	Total
Total Clinical diagnosis (no of cows)			
Coliform+	28	1	29
Coliform-	44	391	435
Total	72(16%)	392(84%)	464

The clinical symptoms vary with the time of infection and therefore one should always try to estimate the time of infection by asking for the time since last normal milking.

In the mild cases the quarter will be swollen for a few hours and after 12 hours of infection the milk will turn very creamy with big clots.

In more severe cases the cow stops eating anything and looks depressed. Most characteristic of these coliform mastitis cases is the dramatic drop in milk yield which might be from 20 to 2 l per milking. Temperature will be elevated up to 41°C in the first 10 hours but then it will drop to subnormal around 38°C.. There will be subcutaneous oedema in the affected quarter, and maybe in the other quarter at the same side and subnormal temperature of the skin over the pelvic region. There might be diarrhoea but characteristically the milk will appear normal in the first 12 hours after infection. It will be a little thinner if the milk is examined carefully in a milk cup. Most veterinarians have experienced thinking that the cow suffers from a severe digestional disease and not until next visit to the cow to realise that is

was an overlooked coliform mastitis case. After 12 hours the secretion might be more serum like or it might turn creamy.

A cow side test for endotoxins in milk (Limast®) is now available (Waage et al. 1994). The test is easy to use and very specific. Test positive cases are coliform mastitis with few exeptions. The detection limit of the test is 10^4 CFU/ml, so quite a few cases may not be diagnosed with the test.

Table 12: Growth on SELMA dish, the number of CFU in the milk, the concentration of endotoxins and clinical status 14 days after first examination in cows with coliform mastitis[1].

No.	Direct culture	CFU/ml day 0	Endotoxin EU/ml	CFU/ml day 1	Endoxin EU/ml	Clinical status day 14
1	C	$2*10^9$	2.88	$1.5*10^7$	0.82	blind quarter
2	S	$6*10^4$	0.84	$1.3*10^4$	n.d.	Healthy
3	S	$8*10^4$	n.d.	$1*10^3$	n.d.	Healthy
4	C	$6.5*10^7$	4.32	$8.7*10^7$	15.36	Dead
5	C	$2.0*10^7$	n.d.	$1.1*10^5$	n.d.	Healthy
6	C	$1.8*10^9$	n.d.	$4*10^6$	4.30	Healthy
7	C	$5*10^7$	1.08	$2.8*10^5$	1.54	Healthy
8	C	$2.7*10^7$	n.d.	$1.6*10^3$	n.d.	Healthy
9	S	$1.1*10^6$	n.d.	$2.1*10^5$	n.d.	Healthy

[1]Endotoxin was not detected in control cows.; n.d.=endotoxin concentration < 0.02 EU/ml; C = carpet growth; S= isolated colonies

Culturing a milk sample from cases of coliform mastitis is still essential for the diagnosis.

The clinical symptoms at coliform mastitis are due to host responses to endotoxin. In paper IIV we found endotoxins in the blood from the milk vein in cows with coliform mastitis. The cow with the highest endotoxin level died and the other survived.(Table 12).

Endotoxin is able to affect many host cells and organs probably by integrating in the cell membrane. In the milk compartment of the udder, the macrophages are primarily responsible for the inflammation alarm. Polymorphonuclear leucocytes are attracted to the site by the cytokines and other mediators, and they release various mediators, such as active oxygen products, proteases, leukotrienes and prostaglandins. Activated cells damage invading pathogens as well as host tissue. A moderate increase in the quantity of mediators is essential for the defence, but excessive increase leads to adverse reactions and finally to shock. (Sandholm and Pyörälä 1995).

There are two important factors that affect the outcome of the disease within the gland, namely, the speed of mobilisation of neutrophils after infection and the opsonic activity of the milk against the strain of E. coli infection in the gland (Hill 1981).

Cows can be classified as moderate and severe responders to experimentally induced E. coli mastitis, based upon the reactive oxygen species-generating capacity of their blood neutrophils before infection (Vandeputte-van Messom et al. 1993).

Colony Forming Units (CFU).

Pyörälä et al. (1994) found that significant positive correlation's existed between bacterial counts, endotoxin concentrations in milk and clinical signs at the acute stage of the infection.

Hill (1981) found that that the ultimative severity of the mastitis appears to be directly proportional to the number of viable bacteria in the milk 10-12 hours after infection when peak numbers are usually attained. In a gland which will rapidly eliminate the bacteria the numbers rarely exceed 10^3/ml, whereas in a gland where the bacteria will not be removed the number is often between 10^6 and 10^7.

It was therefore natural to investigate if the colonial density on the agar plate could be correlated to CFU and to try to find the CFU where isolated colonies changed to carpet growth. This was done in paper V. Table 12 gives a clear impression of carpet growth being correlated to CFU higher than 10^6.

In paper III it was investigated if coliform mastitis cases with carpet growth were clinically more severe and had a bad prognosis.

In table 13 the cases are divided into two groups according to the colonial density after direct culture. It is worth mentioning that the mild coliform mastitis cases are not included in this trail.

Table 13: Colonial density, no. of cases, mean no. of days from calving to outbreak, no. of cases with supplementary treatment and clinical cure rate.

Colony density	No. of cases	Days from calving to outbreak	No of cases with suppl. treatment	Clin. cure rate (%)
Low	47	110.6	7	94
High	23	63.2*	11**	48***

* $p < 0.05$ (Student`s t Test) ** $p < 0.01$ (Chi-square Test)

*** $p < 0.001$ (Fisher`s exact Test). Paper III

It is seen that the clinical cure rate is significantly reduced in the cases where the direct culture showed carpet growth. Cows with severe clinical symptoms had supplementary treatment with calcium borogluconat and sodium salicylate. The cases with carpet growth had significantly more cases with severe clinical symptoms.

The clinical cure see table 15 are clearly different in the two groups as also found in an earlier investigation (Katholm et al. 1989).

The CFU/cm^2 at direct culture at first visit is a valuable prognostic tool. The lower the better prognosis. Lohuis et al. (1990) found in 11 experimentally infected cows a positive relation between area under curve of counts of E. coli up to 125 hours post inoculation, and losses in total milk production 21 days post inoculation. Cows with high numbers of E.coli in secret from infected quarters showed greater losses in milk production.

Erskine et al. (1889) found that peak bacterial concentration is highly correlated with milk losses resulting from infection.

Now and then you can be in doubt whether it is a question of impurity of the milk sample if there are only 1 - 2 colonies on the dish, but Eberhart (1984) as well as Smith et al. (1985) determine the importance of evaluating even one E. coli colony as a positive result if 0.01 ml has been applied for incubation. Smith et al.(1985) found three or less colonies in 58.2% of the samples incubated with 0.01 ml milk.

Endotoxin and mediators.

It is still discussed whether the systemic disease symptoms during coliform mastitis are caused by absorption of endotoxin from the udder or the formation of endogenous inflammatory mediators, such as Interleukines, Tumour-necrosis factor and probably others within the udder and their subsequent release into the circulation, (Lohuis 1990).

It is seen in table 12 that there is endotoxin in plasma from the milk vein even in cases where the quarter was clinically restored after 14 days.

The cow that died had the highest endotoxin values and the cow that had a destroyed quarter had the next highest endotoxin values.

Hakogi et al. (1989) found high concentrations of endotoxin in the milk from cows with acute mastitis where Gram-negative bacteria were isolated, whereas no endotoxins were found in plasma from the jugular vein. In gangrenous mastitis due to Gram-negative bacteria the endotoxin concentration was very high in both milk and plasma.

 The immune status of the herd.

It is difficult to evaluate the different treatments of coliform mastitis as the cow's own immune system is the most important factor for the outcome of a coliform mastitis case.

Hill (1981) found that the severity of the mastitis which followed after experimental infection depended on the speed at which neutrophils were mobilised in the gland and the opsonic activity of the whey within the gland. The PMN migration before infection was correlated indirectly with high susceptibility to experimental E. coli mastitis (Lohuis et al. 1990).

Severe cases of coliform mastitis are often a problem within one to two months in a herd and then disappear. This can easily be explained by a reduced immune status of the cows for this period of time.

Through depletive and suppressive effects on leukocytes, acute BVD may enhance the pathogenesis of other infections (Bolin 1992).

I believe that the most important factor for the variation in the cows immune status in practice is BVD infection and maybe other viral infections.

It is my hypothesis that after a BVD infection, a herd of normal cows that can produce antibodies to the BVD infection, are immunosuppressed for 2 - 3 months. In that period the coliform mastitis cases are severe.

Selenium status has been shown to be of importance to the incidence of clinical mastitis and the severity of experimental E.coli mastitis. Smith et al. (1984) reported that cows supplemented during the dry period with vitamin E and given a single injection of selenium prior to calving, had a lower incidence of clinical mastitis (37%) and shorter duration of infection (62%) than unsupplemented controls. Studies with experimental E. coli intramammary infections found that cows supplemented

with 0.14 ppm selenium in the diet had less severe infections than unsupplemented controls with 0.04 ppm in the diet (Erskine et al. 1989).

The immune status of the herd can also be influenced by vaccination. Three vaccines is now being sold in the United States and widely used. In a field trial on of these vaccines has been shown to reduce the incidence of clinical coliform mastitis by 72%, which is statistically fewer cases (Cullor 1991).

Therapy.

Eberhart et al. (1979) suggested, stripping out, antibacterial therapy, fluid therapy, systemic glucocorticoids, antihistamines and calcium. This is still the way many practitioners treat coliform mastitis. I will emphasise some changes in this treatments with regard to antibiotics or not, concentrated fluids, new NSAID´s and pain relief, which I find important.

a. Stripping out.

The affected quarter should be milked out as thoroughly and as often as practical. Oxytocin given iv. may facilitate removal of bacteria, toxin, and inflammatory exudate. (Eberhart et al. 1979). I always use oxytocin and recommends stripping of the gland.

b. Fluids.

The mechanisms involved in endotoxic shock and, hence, coliform mastitis are more complex than simple dehydration. Cardiogenic, peripheral vascular capacitance, neurogenic, and other mechanisms contribute directly to decreased tissue perfusion and indirectly to the clinical manifestation of shock. (Erskine et al. 1993)

To reduce these problems, fluid therapy has been widely used.

Still clinical documentation lack in specific cases of coliform mastitis.

Blood et al. (1983) recommends balanced electrolyte solutions containing 5% glucose been given at a rate of 100-150 ml /kg body weight per 24 hours by continuous intravenous infusion.

The first 20-30 liters should bee given during the first 4-6 hours.

I personally has never used that large quantities.

Effective oral fluid therapy hinges on normal gastrointestinal absorption of administered fluid. At least gastrointestinal motility and probably other gut functions are impaired in the endotoxemic state (Erskine et al. 1993).

Green (1993) found no significant variation in severe clinical cases (recumbent or severely affected but no information's about CFU) treated by one of the three following regimes: first visit A: 30 l isotonic saline and 20 ml flunixin meglumine iv. B: 30 l isotonic saline iv. C: 20 ml flunixin meglumine iv. and at the second visit 18-24 hours later the to fluid groups were given 15 l isotonic saline iv. unless a

complete recovery had been made, 20 ml of flunixin meglumine were given to group A and C. The survival rate were around 50% in all groups indicating no great effect of the large quantities of fluids. Kidney damage indicated by high creatinin values can be expected after 1 day of disease (Katholm and Andersen 1992). These cows will die in spite of therapy so fluids should be used early in the treatment to avoid this.

As an alternative to great quantities of isotonic solutions Hines (1991) states to have good results by treating toxaemia cows with hypertonic salt solutions. He recommends 2 l of 7% NaCl solution to a Holstein cow.

Tyler at al. (1994a) infused endotoxin in one forequarter and treated the cows 4 hours later with either 5ml/kg body weight of isotonic saline solution or hypertonic saline solution. They found that the decreases in milk yield were greater in cows receiving isotonic saline solution at each of the 6 post treatment milkings.

Tyler et al. (1994b) found that mature lactating cows with endotoxin-induced chock had increases in plasmavolume after iv. administration of hypertonic saline solutions. Such increases in plasmavolume may be associated with improved tissue perfusion.

For the last two years I have used 1-2 l of hypertonic saline (7%) as a standard treatment to all coliform mastitis cases. The cows start to drink immediately and it seems to have a good clinical effect on the cows. I also use 500ml of 50% Invertose as a lot of the cases occur in the peripartum period with risk of ketosis and fatty liver. Depending of the clinical state (around 25% of the cases) I supply afterwards with 3-9 l of isotonic fluids preferably 5% glucosis.

c. Calcium

Significant decrease in serum calcium concentrations is observed at 4 hours postinjection of endotoxin iv., the decrease persisted until 24 hours. There was also a significant decrease in serum calcium 16 and 20 hours post intramammary infection with E. coli. (Griel et al. 1975). They found that intravenous administration of calcium borogluconate to two cows, that became recumbent in the endotoxin experiment, resulted in a reversal of the depression and rumen stasis.

Katholm and Andersen (1992) found in 9 clinical cases that calcium therapy were indicated in the first 24 hours of the disease as the cows in this period had a mild hypocalcæmia.

Oetzel (1985) also observed hypocalcaemia in field cases of E. coli mastitis.

Traditionally there has been a warning against intravenously calcium treatments to cows suffering from peracute mastitis e.g. Radostits (1961) that states that this treatment usually give a poor response or prove rapidly fatal.

I have never observed these adverse effects. On the contrary it seems to me that the cows benefit clinically from the calcium treatment, in the mild cases the cows start to eat and the recumbent cows may rise.

I always use 90g of calciumborogluconate in my treatment of coliform mastitis.

Calcium borogluconat in these doses has probably a potent and useful effect on the patients bloodpressure (Andersen 1996).

d. Antiinflammatorical drugs.

1. Steroidal antiinflammatory drugs

The major actions of glucocorticoids occur at protein transcription and translation resulting in blockage of TNF synthesis and lipocortin production. (Hardis and Kruse-Elliott 1990).

Glucocorticoids acts on the early mediators (first wave). This explains that the effect as a main rule is best early in the disease. Dose of dexamethason 1-3 mg/kg (Andersen 1996).

Lohuis et al. (1988) found that the milk losses fourteen days after experimentally induced coliform mastitis later were markedly reduced in 3 cows that were treated with 30 mg of dexamethason at the time of inoculation, compared to 3 control cows. Cows in the dexamethason group had less pronounced local clinical signs, the decrease in rumen frequency was less in the first period after treatment and the inhibition of rumen amplitude was significantly less during all observation periods.

b. Non-steroidal antiinflammatory drugs

Non-steroidal antiinflammatory drugs (NSAID´s) inhibit the synthesis of prostaglandins and thromboxanes by inhibition of the enzyme cyclooxygenase.

All NSAID´s do not necessarily act in the same manner. It has been demonstrated that several NSAID´s chelate iron. Other mechanisms, such as lipoxygenase inhibition and lysosomal membrane stabilisation, also may be important. (Erskine et al. 1993).

NSAID´s can in the treatment of acute coliform mastitis be expected to reduce the severity of systemic clinical signs, e.g. rectal temperature, heart rate, respiratory rate, and udder pain, but there is no direct evidence that they positively influence survival or milk production. (DeGraves and Anderson. 1990).

In acute mastitis induced by intramammary administration of endotoxin, flunixin meglumine significantly reduced rectal temperature and quarter signs of inflammation and improved clinically graded depression when compared with these signs in control cows. (Anderson et al. 1986). In experimental E.coli mastitis flunixin meglumine and flurbiprofen almost completely abolished the febrile response during the first 9 hours after infection, and the decrease in rumen motility was less pronounced compared to untreated controls (Lohuis et al. 1989).

The earlier mentioned clinical trail by Green (1993) showed no difference in the group with flunixin meglumine but without fluids, compared to a group treated intensively with fluids.

Shpigel et al. (1994) found that 2g of ketoprofen once daily together with sulfadiazine/trimethoprim in op to 5 days in a clinical trail with a control group showed that ketoprofen treated cows had a significant better result of the treatment of clinical mastitis where coliform mastitis cases were 64,2% of the cases.

I try to use dexamethasoni isonicotinas 20-30 mg to cows at the first treatment and later ketoprofen 1,5g.

The clinical use of these drugs is based on the literature where the drugs often is used at the infection time or few hours later.

The effect in clinical cases where treatment often is started 12-24 hours after infection is difficult to evaluate from the experimental trails.

e. Pain relief

I think it is highly indicated to use some kind of pain relief in the cases with swelling and oedema in the quarter and skin. Petidine and methadone might be used as a systemic analgesic but it might also influence the cows ability to stand and drink and maybe eat.

At the present, I use an epidural injection of 1,5 ml xylazinhydrochlorid (20 mg base/ml), 1,5 ml morphine (20 mg/ml) and 7 ml of isotonic NaCl as pain relief therapy in clinical mastitis cases. This therapy can be expected to relief pain from the udder for 24 hours and does not induce systemic depression (Nielsen 1994).

f. Antibiotics

Coliform intramammary infections and particularly those caused by E. coli are of short duration and gives a false sense of antibiotic therapy effectiveness. Most coliform infections cause acute clinical cases and are eliminated spontaneously within seven days. Approximately 10% of coliform infections result in peracute clinical cases and even in these cases antibiotics are not of value (Smith and Hogan 1996). The effect of antibiotics in the therapy of coliform mastitis is therefor doubtful.

For the moment it has not been clarified if the outcome of disease by coliform mastitis can be positively influenced by the use of antibiotics. (Ekman et al. 1995a).

In the Swedish "antibioticapolicy" the use of antibiotic therapy in coliform mastitis is questioned (Ekman et al. 1995).

Norwegian strategy for reduction of the use of antibiotics points out that the oracial question in coliform mastitis is not what antibiotic to be used, but whether antibiotics should be used or not. The fagocytic activity is highly reduced after calving. In this period the cows defence against infection is

reduced and it may be more indicated to use antibiotics to coliform mastitis in this period rather than later in the lactation (Tørud et al. 1996).

In several studies with experimental E. coli challenge of the udder no statistical effect was observed with gentamycin i.m.m. (Erskine et al.1992), sulfadiazinum-trimethoprimum parenteral (Pyörälä et al. 1994) and colistin sulfate i.m.m (Pyörälä et al. 1994) compared to controls. In a clinical trial with mild clinical cases Amoxi-mast® i.m.m and Cefa-lak® i.m.m. had no significant effect compared to controls treated with oxytocin (Guterbock et al. 1993).

In an experimental study with cows in early lactation, Pyörälä et al. (1996) found that cows treated with flunixin meglumine and enrofloxacine eliminated the bacteria faster than controls treated with flunixin meglumine. The response to challenge varied greatly among cows. Two cows in the group not receiving antimicrobial therapy had to be euthanized 4 days after challenge. They concluded that in severe E. coli mastitis during early lactation it may be necessary to use a suitable antimicrobial therapy.

In an other experimental study Shpigel et al. (1997) found thatthe bacteriological cure rate of coliform cases treated with ampicillin and cloxacillin were 54% and much lower than cases treated with Cefquinome imm 83%, Cefquinome im 83 and cases treated with Cefquinome im and imm 95%. Shpigel et al. (1998) found in a clinical trail of coliform mastitis cases treated with sulphonamide/trimethoprim that the recovery rates were higher for the cases affected by coliforms that were sensitive to the combination 89,1 per cent than for cases affected by resistant coliforms 74,6 per cent

Table 14:Ellimination of coliform bacteria in 47 cows with severe clinical coliform mastitis devided in four groups according to colony density at direct culture and treatment without antibiotics or with danofloxacin

Colony density (CFU/cm^2) and treatment	day 0	day1	day 2	day 7	day14	day21
>300 - antibiotic	300	208	95	3	5	0
>300 + danofloxacn	300	169	68	4	1	0
<300 - antibiotic	116	68	25	1	0	0
<300+ danofloxacin	129	15*	2	1	1	0

* p<0,05

In 1996 I have treated all Limast® positive coliform mastitis cases solely with supportive therapy after the guidelines in this paper and in 1997-1998 a corresponding group were treated in ste same way but also with danofloxacin 1,25 mg/ kg. In table 14 the elimination curves for the coliform bacteria are shown There is a tendency that for the number of coliforms are lower in the treated group on day 1 and 2 and in the moderate group the reduction is significant on day 1 ($p<0,05$) and at day 2 the result has a probability of 0,07

The quicker elimination of bacteria can indicate a better cure in the group treated with danofloxacin this can also be seen in the clinical cure shown in table 15. Specially in the moderate group there is a tendency that danofloxacin group give a better cure with no reduction of yield in the affected quarter.

In severe cases with carpet growth, less than half of cases cure. That corresponds with the findings of Katholm et al. (1989) for cases treated with tetracycline iv. and cefoperazone i.m.m.

Of the 5 cows that cured clinically in the severe group without danafloxacin, 3 were bacteriological infected at control day 21. One of them with persisting coliform infection at day 1,2,7,14, and 21. In the moderate group where 13 of the 14 cows were milked, 2 were bacteriological infected with a new bacteria in the earlier infected quarter.

Table 15:Clinical status on day 21 in 47 cows with severe clinical coliform mastitis devided in four groups according to colony density at direct culture and treatment without antibiotics or with danofloxacin

Colony density (CFU/cm) and treatment	N	Clinically Restored	Reduced Yield	Dry teat	Dead
>300 - antibiotic	14	5	1	7	1
>300 + danofloxacin	8	2	0	6	0
<300 - antibiotic	14	10	3	1	0
<300+ danofloxacin	11	11	0	0	0

For cows treated without antibiotic days from calving and the SCC at control day prior to infection day in the severe and moderate group were compared. Cows in the severe group were not significantly closer to calving. The mean value is more than 2 months from calving, indicating that not all severe cases occur around calving. The cow that died were 3 days post partum.

The cow SCC at control day prior to infection were low in booth groups. This was expected as the percentage of coliform mastitis is high in herds with low SCC (Smith and Hogan 1996).

The tendency although not statistically significant that mild cases had the lowest cow SCC was unexpected.

Conclusion of treatment of a cow with coliform mastitis..

Milk is sampled to verify diagnosis and predict prognosis.

Intensive treatment at first visit.

Treatment is based on supportive treatment. Stripping out, calcium, 7% sodiumcloride, isotonic fluids, antiinflammatory drugs and pain relief.

Value of antibiotics is still to be investigated.

REFERENCES.

Andersen P.H.: Endotoxic shock, pathofysiology, diagnosis and treatment. 1996, In Kruuse 100 års Jubilæumssymposiun, 2. October, Hotel Munkebjerg, Denmark.

Anderson K.L., A.R. Smith, R.D. Shanks, L.E. Davis and B.K Gustafsson: Efficacy of flunixin meglumine for the treatment of endotoxin-induced bovine mastitis. American Journal of Veterinary Research 1986, 47, 6, 1366-1372

Barkema.H.W., Y.H.Schukken, T.J.G.M. Lam, M.L. Beiboer, H. Wilmink, G. Benedictus and A. Brand.: Incidence of clinical Mastitis in Dairy Herds grouped in Three Categories by Bulk Milk Somatic Cell Count. J. Dairy Science, 1998, 81, 411-419

Blood D.C., M.O.Radostits and J.A. Henderson: Veterinary Medicine, Sixth edition, Baillière, Tindall 1983, 474

Bolin S.R.: BVD: What's the latest. XVII World Buiatrics Congress, St. Paul, Minnesota, USA 1992, Vol 2. 155-157

Bramley A.J.: Sources of Streptococcus uberis for the dairy herd. 1. Isolation from bovine feces and cattle bedding. Journal of Dairy Research 1982, 49, 369-373.

Bramley A.J. and F. H. Dodd. Reviews of the progress of Dairy Science: Mastitis control - progress and prospects. Journal of Dairy Research, 1984, 51, 481 - 512.

Bramley A.J.: Mastitis Physiology or Pathology? In, New insights into the pathogenesis of mastitis. Flemish Veterinary Journal, 62, 1991 Suppl. 1, 3-11

Cullor J.S.: The Escherichia coli J5 vaccine: Investigating a new tool to combat coliform mastitis. Veterinary Medicine, 1991, August 836-844.

DeGraves F.J. and K.L. Anderson: Ibuprofen treatment of endotoxininduced coliform mastitis. International Conference Mastitis: Physiology or Pathology ?, Gent, Belgium, 1990, 140

DeGraves F.J. og K.L. Anderson: Anti-Inflammatory Therapy for Acute Coliform Mastitis. International Symposium on Bovine Mastitis. Indianapolis U.S.A. September 13-16, 1990 p 261-265

Eberhart R.J., R.P. Natzke, F.H.S. Newbould, B. Nonnecke, and P. Thompson: Coliform mastitis - A Review. J Dairy Sci. 1979, 62, p 1-22

Eberhart R.J.: Coliform Mastitis, Veterinary clinics of North America: Large Animal Practice 1984, 6, 287-300.

Ekman T., A.Franklin, C.H.Sandgren og P.Jonsson: Förslag till behandling av klinisk mastit hos ko. Svensk Veterinärtidning, 1995a, vol 47, 16, p 713-718.

Ekman T., A.Franklin, C.H.Sandgren og P.Jonsson: Antibiotikapolicy vid behandling av mastit hos ko - "revisited". Svensk Veterinärtidning, 1995b, vol 47, 15, p. 665-669.

Erskine R.J., R.J. Eberhart, L.J. Hutchinson, S.B. Spencer and M.A. Campbell: Incidence and types of clinical mastitis in dairy herds with high and low somatic cell counts. J. Am. Vet. Med. Assoc. 1988, 192, 6, 761 - 765.

Erskine R.J., R.J. Eberhart, P.J. Grasso et al: Induction of Escherichia coli mastitis in cows fed selenium-deficient or selenium-supplemented diets. Am. J. Vet. Res. 1989, 50, 2093 - 1300.

Erskine R.J., R.C. Wilsom and M.G. Riddell.: The pharmacokinetics and efficacy of intramammary gentamicin for the treatment of coliform mastitis. Int. Symp. on Bovine Mastitis, Indianapolis, USA, 1990, 256-260.

Erskine R.J., R.C. Wilson, G.M. Riddell et al.: Intramammary gentamycin as a therapy for experimental Escherichia coli mastitis. J. Am. Vet. Med. Assoc. 1992, 53, p 375 - 381.

Erskine J.R., J.H. Kirk, J.W. Tyler and F.J. DeGraves: Advances in th therapy for mastitis. In Update on Bovine Mastitis, The Vet. Clinics of North America, Food animal practice,1993, Vol 9, 3, 499-517.

Funke H.: Kliniska mastiter. Infektionspanorama, kliniska symptom m m. Svensk Veterinärtidning 1983, 35, 14, 707-711.

Funke H. and Strandberg P.: Intramuskulær behandling af klinisk mastitis med benzylpenicillin - ester (Leocillin®) i henholdsvis 3 og 5 dage. Intern rapport Løvens Kemiske Fabrik. 1991.

Griel L.C., A. Zarkower and R.J. Eberhart: Clinical and clino-pathological Effects of Escherichia coli Endotoxin in Mature Cattle. Can. J. comp. Med. 1975, 39, January, p 1-6

Green M.J., L.E.Green and P.J.Cripps: Low bulk milk somatic cell counts and endotoxin-associated (toxic) mastitis. The Vet. Rec. 1996. March 30, s 305-306

Grommers F.J., Y.H. Schukken, D. van de Geer and A. Brad: Bulk milk cell count (BMCC) shift and clinical mastitis in low BMCC herds in the Netherlands. Int conf. on mastitis. St. Georgen, Österreich, 1989, 70 - 74

Grønborg-Pedersen H.: Kviemastitis. Med særlig henblik på peripartum-perioden. Dansk Veterinær Tidsskrtft 1991, 74, 369-375.

Guterbock W.M.,A.L. Van Eenennaam, R.J. Anderson, I.A. Gardner, J.S. Cullor and C.A. Holmberg: Efficcacy of intramammary antibiotics for treatment of mild clinical mastitis caused by environmental pathogens. J. Dairy Sci. 1993, 76, p 3437-

Hanssen H.P. and Ø.R. Jepsen: Mannitol-agar til dyrkning af yverbe tændelsesbakterier. Dansk Veterinær Tidsskrift 1984, 67, 119 - 122.

Hakogi E., H. Tamura, S. Tanaka, A. Kohata, Y. Shimada and K. Tabuchi: Endotoxin Levels in Milk and Plasma of Mastitis-Affected cows Measured with a Chromogenic Limulus Test. Veterinary Microbiology 1989, 267-274.

Hardie E.M. and K. Kruse-Elliott: Endotoxic shock, Part 2: A review of treatment. J.Vet. Int. Med., 4, 1990, p 306-314

Hill A.W.: Factors influencing the outcome of Escherichia coli mastitis in the dairy cow. Res. Vet. Sci. 1981, 31, 107-112.

Hill A.W.: Somatic cells - Friends or foes? In, New insights into the pathogenesis of mastitis. Flemish Veterinary Journal, 62, 1991 Suppl. 1, 217-232.

Hines J.A.:7% Saline IV, A New Approach to an Old Problem. 24th annual convention, American Association of Bovine Practitioners, September 18-21, 1991, Orlando, Florida. Proceedings, s. 142

IDF: Bulletin of the International Dairy Federation, no. 211 3-9, 1897.

Jarp J., Bugge H.P. and Larsen S.: Clinical trial of three therapeutic regimens for bovine mastitis. Veterinary Record ,1989, 124, 630-634.

Jarløv N., P.H.Andersen and Michael Hesselholt: Pathophysiology of Experimental Bovine Endotoxicosis III: Prevention of endotoxin-induced synthesis of thromboxane A2, prostacycline (PGI2) and prostaglandin E2 by some non-sterioidal anti-inflammatory drugs. XV World Buiatrics Congress, Spain, Palma de Mallorca 1988, 1422-1427

Jensen N.E.: Mastitis. Definition, aetiology, pathogenesis and diagnostics. Cattle specialist education, Copenhagen, 1987, 1-19.

Jonsson P. og T. Ekman: Bovin Mastit - Dyrkning - diagnostik -Terapi. Svensk Veterinärtidning 1988, 40,12, Supplement 17 s. 1 - 55

Johnsson P. and Holmberg O.: Interaktion mellam mikroorganismer och värdsdjur vid bovin mastit. Bovin Mastit - Dyrkning - diagnostik -Terapi. Svensk Veterinärtidning 1988, 40,12, Supplement 17, 11-20

Kaartinen L. and Jensen N.E: Use of N-acetyl -B-D-glucosaminidase to detect teat canal inflammations. Journal of Dairy Research, 1988, 55, 603-607.

Katholm J.: Pyogenes - mastitis. Dansk Veterinær Tidsskrift 1988, 71, 5, s. 257 - 258.

Katholm J., O.R. Jespersen and N. Agger: Peracute coliform mastitis. Experiences from 70 cases in 2 general practices. International Conferences on mastitis 1989, 29 May - 2 June, St Georgen/Längsee, Kärnten, Austria, p 8-13.

Katholm J og P. Haubro Andersen: Acute coliform mastitis in dairy cows: endotoxin and biochemical changes in plasma and colony-forming units in milk. Veterinary Record November 28, 1992, 513- 515

Katholm J. and Andersen P.H.: Severe coliform mastitis in Practice, Efficacy of a therapy regimen not including antibiotics. XX World Buiatrics Congress, Sydney Australia 1998, p. 261-264

Katholm J: Colimastitis. Hjemmedyrkning og terapi. Dansk Veterinærtidsskrift. 1989, 72, 4, 171-177.

Lohuis J.A.C.M., W. Van Leeuwen, J.H.M Verheijden, A.S.J.P.A.M. Van Miert and A. Brand: Effect of Dexamethasone on Experimental Escherichia coli Mastitis in the Cow. J. Dairy Sci. 1988, 71, p. 2782-2789

Lohuis J.A.C.M., W Van Leeuwen, J.H.M. Verheijden, A. Brand and A.S.J.P.A.M. Van Miert: Effect of Sterioidal Anti-Inflammatory Drugs on Escherichia coli Endotoxin-Induced Mastitis in the Cow. Journal of Dairy Science 1989a, 72, 241-249.

Lohuis J.A.C.M., W Van Leeuwen, J.H.M. Verheijden, A. Brand and A.S.J.P.A.M. Van Miert: Flunixin Meglumine and Flurbiprofen in cows with experimental Escherichia coli mastitis. The Veterinary Record 1989b, 124, 305-308.

Lohuis J.A.C.M.: Some aspects of treatment of coliform mastitis. Cornell Vet. 1990, 80, 223-227.

Lohuis J.A.C.M., Y.H. Schukken, C. Burvenich and A. Brand: Preinfection migration of blood polymorphonuclear leukocytes and the outcome of experimental Escherichia coli mastitis in the cow. Int. Symp. on Bovine Mastitis, Indianapolis, USA, 1990, 20-25.

Lohuis J.A.C.M., Y. H. Suhukken, J.H.M Verheijden, A. Brand, and A.S.J.P.A.M. Van Miert: Losses in milk production following experimental Escherichia coli mastitis in lactating cows related to the severity of systemic signs during the acute phase. J. of Dairy Sci., 1990, 73, p 333-341

Morin D.E. and P.D. Constable: Characteristics of cows with clinical mastitis and no bacteria isolated from milk.. XX World Buiatrics Congress, Sydney Australia 1998, p. 237-241

Nielsen C. Grøndahl: Personal communication. 1994

Oetzel G.R.: Compendium on continuing Education (1985), 7, p. 237

Pyörälä S.: Recovery from acute mastitis. Symposium on Mastitis Control, Espoo, Finland, 1986, 163-171.

Pyörälä S. and E. O. Pyörälä: Efficacy of parenteral administration of three antimicrobial agents in treatment of clinical mastitis in lactating cows: 487 cases (1989-1995). J Am Vet Med Assoc, 1998 , 212, 3, 407-412

Pyörälä S., J. Hirvonen, L. Kaartinen and P. Harmoinen: The effect of antimicrobial therapy on experimental induced <u>Escherichia coli</u> mastitis during early lactation. XIX World Buiatrics Congress, 1996, 8 - 12 july, Edinburgh, United Kingdom, p 306-309

Pyörälä S., L. Kaartinen and H Käck: Efficacy of two therapy regimens for treatment of experimentally induced <u>Escherichia coli</u> mastitis in cows. J. Dairy Sci. 1994, 77, p 453-461

Radostits O.M.: Coliform mastitis in cattle. Can. Vet. Jour., 1961, vol 2, no 11, p 401-405

Sandholm M. and T. Mattila: Mechanisms of infection and inflammation of the mammary gland - an overview. Symposium on mastitis control and hygienic production of milk. Espoo, Findland, 1986, 7-13.

Sandholm M., Ali-Vehmas T., Nyholm K., Honkanen-Buzalski T. and Louhi M.: Failure mechanisms in lactational therapy of staphylococcal mastitis. In, New insights into the pathogenesis of mastitis. Flemish Veterinary Journal, 62, 1991 Suppl. 1, 171-186.

Sandholm M. and Pyörälä S.: Coliform Mastitis, 1995, in The bovine udder and mastitis, University of Helsinki, Faculty of Veterinary Medicine, p 149-160

Schukken Y.H., H.W. Barkema and T.J.G.M. Lam: Udder health programs: Present state and future perspectives. XX World Buiatrics Congress, Sydney 6-10 July 1998, 225-229

Shpigel N.Y., F. Longo, A. Saran og G. Ziv:Ketoprofen efficacy as adjunctive therapy in the treatment of field cases of clinical mastitis in dairy cows. XVIII World Buiatrics Congress august 29-september 2, 1994, Bologna, Italy, s. 595-597.

15. Shpigel N.Y.,D. Levin, M. Winkler, A. Saran, G.Ziv and A. Böttner: Efficacy of cefquinome for treatment of cows with mastitis experimentally induced using Escherichia coli. J. Dairy Sci, 1997, 80, 318-323

16. Shpigel N.Y., M. Winkler, G.Ziv and A. Saran:Relationship between in vitro sensitivity of coliform pathogens in the udder and the outcome of treatment for clinical mastitis. The Veterinary Record 1998, february 7, 135-137

Smith K.L., K. Henry and D.A. Todhunter: Diagnosis of coliform intramammary infection, Journal of Dairy Science 1985, 68, supp 1., 170.

Smith K.L., J.H. Harrison, DD Harrison et al.: Effect of Vitamine E and Selenium Supplementation on Incidence of Clinical Mastitis and Duration of Clinical Symptoms. Journal of Dairy Science. 1984, 67, 1293-1300.

Smith K.L. and J.S. Hogan: Future prospects for mastitis control. XIX World Buiatrics Congress, Edinburgh 8-12 july 1996, 263-269

Thomas L.H., J.A. Leigh and A.W. Hill:Do Neutrophils perform any useful function in defence against mastitis caused by Streptococcus uberis? International Conference Mastitis:Physiology or Pathology? University of Ghent, 1990, 84.

Tyler J.W., F. J. DeGraves, R. J. Erskine, M.G. Riddell, H.C. Lin and J.H. Kirk: Milk production in cows with endotoxin-induced mastitis treated with isotonic or hypertonic sodium chloride solution. J.A.V.M.A. 1994a, 204,12, p 1949-1952

Tyler J.W., E.G. Welles, R. J. Erskine, H.C. Lin, M.A. Williams, J.S. Spano, J.T. Gaslin and K.A. McClure: Clinical and clinicopathological changes in cows with endotoxin-induces mastitis treated with small volumes of intravenously administered isotonic or hypertonic sodium chloride. Am. J. Vet. Res. 1994b, 55, p 278-287

Tørud E., R. Lang-Ree, A. Raage, S. Støverud, T. Sivertsen, S.A. Ødegaard, H. Sørum, K. Grave, O. Austbø, T. Grøndalen, R.A. Aass, and O. Østerås: Strategier for å redusere forbruk av antibakterielle midler i storfeproduksjonen - med særlig vekt på behandling av mastitt. 1996 Helsetjenesten for storfe.

Vandeputte-van Messom G., C. Burvenich, E. Roets, A.M. Massart-Leën, R. Heyneman, W.D.J. Kremer and A. Brand: Classification of newly calved cows into moderate and severe responders to experimental induced Escherichia coli mastitis. J. Dairy Research. 1993, 60, p 19-29.

Veterinærdirektoratet: Om mastitisbekæmpelse og laboratoriemæssige undersøgelser i forbindelse hermed. Veterinærdirektoratets vejledning af 15 februar 1992

Waage S.: Akutt mastitt assosiert med speneskade - mikrobiologiske funn. Nordisk Mastitt- og Reproduksjonsmøte, Trondheim, Norway, 1991a, 148-149.

Waage S.: Bovin mastitt forårsaket av Staphylococcus aureus -sammenheng mellom klinisk manifestasjon og bakteriestammenes penicillinfølsomhet in vitro. Nordisk Mastitt- og Reproduksjonsmøte, Trondheim, Norway, 1991b, 150-151.

Waage S., P. Jonsson og A. Franklin: Evaluation of a Cow- Side Test for Detection of Gram-Negative Bacteria in Milk from Cows with Mastitis. Acta vet. scand. 1994, 35 s. 207-212.

Waage S.: Comparison of two regimes for the treatment of clinical bovine mastitis caused by bacteria sensitive to penicillin. Vet. Rec. 1997, December 13, 616-620

Wadström T.: Molecular Aspects on Pathogenesis of Wound and Foreign Body Infections due to Staphylococci. Zbl. Bakt. Hyg. 1987, A 266, 191-211.

Zorah K.T. R.C.W. Daniel and A.J. Frost: Detection of bacterial antigens in milk samples from clinical cases of bovine mastitis in which culture is negative. The Veterinary Record, February 27, 1993 208-210.

Østerås O.: Mastittbehandling på besetningsnivå. Nordisk Mastitt- og Reproduksjonsmøte, Trondheim, Norway, 1991, 91-96.

Åstrøm G.: Juverinflamation-behandling, Svensk Veterinärtidning, 1988, 40, supplement 17, 32-34.