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Acute Phase Response in Dairy Cows with Experimentally Induced *Escherichia coli* Mastitis

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[1]Faculty of Veterinary Medicine, Department of Clinical Veterinary Sciences, University of Helsinki, [2]Department of Biomedicine, Department of Medical Chemistry, University of Helsinki, [3]Department of Medicine, Division of Nephrology, University Central Hospital, Helsinki, Finland, and [4]Department of Obstetrics and Gynaecology, University of Veterinary Science, Budapest, Hungary.

Hirvonen J, Eklund K, Teppo AM, Huszenicza G, Kulcsar M, Saloniemi H, Pyörälä S: Acute phase response in dairy cows with experimentally induced *Escherichia coli* mastitis. Acta vet. scand. 1999, 40, 35-46. – Six Finnish Ayrshire cows were challenged intramammarily with 1500 CFU of *Escherichia coli (E. coli)* into single udder quarters, and the challenge was repeated into contralateral quarters 3 weeks later. All cows received flunixine meglumine once, and 3 of them were also treated with enrofloxacin. At the 2nd challenge, treatments were changed *vice versa*. The development of mastitis was followed by monitoring of systemic and local clinical signs, and with serial milk and serum samples. Intramammary challenge with *E. coli* produced clinical mastitis in all cows, the severity of the disease varying greatly between the animals. No significant changes between the 2 treatment regimens or sequent challenges were found for any of the clinical parameters. The response of each cow followed the same pattern after both challenges; three of the cows became mildly and the other 3 either moderately or severely affected. Two severely affected cows had to be euthanized because of severe mastitis.

Serum haptoglobin and amyloid-A concentrations peaked 2-3 days after bacterial challenge. Serum haptoglobin did not correlate with the severity of the disease. Serum amyloid-A rose gradually in the severely affected cows, and significant differences were found between severely versus moderately or mildly affected cows at day 4. Serum tumor necrosis factor alpha concentrations increased only in the severely affected cows. Serum cortisol response was prolonged in the severely diseased animals, and was significantly lower after the second challenge. Serum nitrite/nitrate concentration increased in the severely affected cows. This indicated excess nitric oxide production during acute *E. coli* mastitis. Strongly decreased milk production, and high bacterial growth in the infected quarters were best predictors for the outcome from acute *E coli* mastitis.

bovine; E. coli; acute phase proteins; haptoglobin; serum amyloid-A; nitric oxide.

Introduction

Bacterial lipopolysaccharide, often called endotoxin, is the key molecule in the development of coliform mastitis (*Carroll et al.* 1964). Endotoxin induces inflammatory response in the host animal, which sometimes can be deleterious for the animal itself (*Olson et al.* 1995). Due to the critical role of endotoxin in coliform mastitis, treatment of clinical coliform mastitis should be targeted to combat the effects of endotoxin. Instead, beneficial effect of antimicro-

bial agents in treatment of coliform mastits has been questioned (*Katholm et al.* 1992, *Pyörälä & Pyörälä* 1998).

The susceptibility of individual cows to coliform mastitis varies, and early-lactating cows are more susceptible (*Hill et al.* 1979, *Pyörälä & Pyörälä* 1998). For this reason, homogenous groups of cows are crucial in comparative trials on coliform mastitis. Another option is to decrease the between-cow variation in the challenge response by repeating the bacterial challenge with the same animals, and thus use each cow as its own control (*Pyörälä et al.* 1994).

The acute inflammatory response comprises numerous reactions in the host animal, collectively known as acute phase response (APR) (*Kushner* 1982, *Dinarello* 1984). APR limits the microbial invasion by several mechanisms, e.g. by infiltration of phagocytes to the inflammatory site and release of reactive oxygen intermediates from them, by synthesis of acute phase proteins (APP) in the liver, and by modulation of the secretion of hormones, including cortisol. The APR is initiated by production and release of pro-inflammatory cytokines, such as tumor necrosis factor α (TNFα) and interleukin-1 (*Baumann & Gauldie* 1994, *Pannen & Robotham* 1995). Bovine mammary macrophages are known to secrete TNFα in response to endotoxin (*Pighetti & Sordillo* 1994) and serum TNFα response has been reported to take place in acute *E. coli* mastitis (*Sordillo & Peel* 1992). Haptoglobin (Hp) is one of the most responsive APPs in the bovine (*Gruys et al.* 1994) and serum Hp concentration has been reported to be a sensitive inflammatory marker for acute *E. coli* mastitis (*Salonen et al.* 1996). Serum amyloid-A (SAA), a highly sensitive APP in the bovine, has been shown to increase in various inflammatory conditions (*Alsemgeest et al.* 1994).

Nitric oxide (NO) is a multipotent molecule with numerous physiologic and pathologic functions. An inducible nitric oxid synthase is activated by endotoxin and pro-inflammatory cytokines in a variety of cell types, including macrophages and vascular endothelial cells (*Payen et al.* 1996). Bovine macrophages have recently been shown to express NO synthases upon stimulation with endotoxin (*Adler et al.* 1996). NO plays several roles in inflammatory reaction. For example NO participates in microbial killing by reactive nitrogen intermediates, which can interact with reactive oxygen products. In septic shock activation of inducible NO synthases and inhibition of constitutive ones can result in unresponsive vascular hypotension or multiple-organ failure. This could also contribute to the development of shock condition seen in severe coliform mastitis. NO is a very short-lived product in circulation. Nitrite (NO_2) and nitrate (NO_3), the 2 end products of NO metabolism, are rather stable and can be used for the evaluation of *in vivo* production of NO (*Moshage et al.* 1995).

It has been suggested that high blood urea nitrogen and creatinine concentrations indicate a poor prognosis in acute coliform mastitis (*Cebra et al.* 1996, *Katholm & Andersen* 1992). Endotoxin shock can decrease glomerular filtration rate and cause prerenal azotemia, thus increasing serum urea and creatinine levels (*Coles* 1986). Renal prostaglandins have a protective role by functioning as local vasodilators to maintain renal blood flow and glomerular filtration rate under adverse conditions, like hypovolemia and shock (*Davis* 1986)

The objective of this study was to examine the role of some parts of the APR in the pathogenesis of acute *E. coli* mastitis, including the possible role of inducible NO synthesis. We also studied the value of different parameters in predicting the outcome from acute coliform mastitis. Two different treatment regimens were used to test the role of antimicrobial therapy in *E. coli* mastitis.

Materials and methods

Animals, bacterial challenge and treatments

Six clinically healthy, early lactating (median lactation day 29; range 9-70) Finnish Ayrshire cows were used in the study*. The cows produced low somatic cell count (SCC) milk of <100,000 cells/ml with a mean of 17 l of milk/day (range 12-28 l). The cows were challenged with 1500 CFU of *E. coli* FT238 strain into a single udder quarter as described earlier (*Pyörälä et al.* 1994). The strain had been isolated from a cow suffering from clinical mastitis, and it was nonhemolytic, intermediately serum resistant, and *in vitro* sensitive to enrofloxacin (MIC<0.25 μg/ml). The cows were first challenged into one udder quarter, and 3 weeks later into the contralateral quarter.

The 6 cows were randomly allocated into 2 treatment regimens to study the effect of antimicrobial therapy on *E. coli* mastitis during early lactation phase. Twelve h after the bacterial challenge, when clinical signs of mastitis had developed, all 6 cows received a single flunixine meglumine treatment (Finadyne®, Schering-Plough, Kenilworth, USA; 2.2 mg/kg i.v.). Three of the animals also received enrofloxacin (Baytril®, Bayer AG, Leverkusen, Germany; 5 mg/kg first i.v., then s.c. once a day for 3 days). At the 2nd challenge 3 weeks later, treatments were changed so that cows treated with enrofloxacin at the first time did not receive it now and *vice versa*. The protocol to treat seriously affected cows included intensive fluid therapy from the 2nd post-challenge day before removal from the trial. Additional 10 healthy Ayrshire cows were used for a study of serum NO_2/NO_3 levels in normal dairy cattle.

* The experiment was approved by the Animal Experimentation Committee of the College of Veterinary Medicine, Helsinki, Finland.

Clinical examination and sampling

Systemic and local clinical signs were recorded throughout the experiment. The monitored systemic signs included attitude, heart rate, rumen motility, and appetite; local signs included swelling, colour, pain and milk appearance of the infected quarter. Systemic and local signs were scored on a 3-point scale (1 = no signs to 3 = severe signs) as described earlier (*Pyörälä* 1988). The daily milk yield was recorded for each cow during the experiment.

Milk samples were collected from the challenged quarter of each cow prior to challenge, and from 12 h post challenge (PC) twice a day (before milking, at 8 a.m. and 5 p.m.) for 2 days, and thereafter before every morning milking for 1 week. Milk bacterial counts were determined by plate count method. Indicators for mastitis, milk SCC and N-acetyl-β-D-glucosaminidase (NAGase) activity, were also measured. Milk SCC was measured with a Fossomatic instrument, and NAGase activity with a commercial Milk NAGase Test kit (*Pyörälä et al.* 1994).

Blood for serum samples was drawn from jugular vein prior to bacterial challenge, at 12 h PC, thereafter twice daily (at 8 p.m. and a.m.) for 3 days and once daily (at 8 a.m.) for the rest of the experimental period. The samples were analyzed for serum haptoglobin (Hp), serum amyloid-A (SAA), tumor necrosis factor alpha (TNFα), cortisol, nitrite/nitrate (NO_2/NO_3), urea, and creatinine concentration.

Analytical methods

Serum Hp concentration was analyzed chromatographically as described by *Salonen et al.* (1996). Human Hp standards were used. The results were expressed as g/l of Hp. SAA concentration was measured by radial immunodiffusion using antiserum to human amyloid-A as described earlier (*Maury & Teppo* 1984). Purified human amyloid-A protein was used as stan-

dard. The detection limit of the assay is 5 mg/l for human SAA. Serum TNFα concentration was analyzed using human TNFα radioimmunoassay kit (Necroton®, F. J. C. National Research Institute for Radiobiology and Radiation Hygiene, Budapest) with a human standard. The detection limit of the assay is 20 pg/ml. Serum cortisol concentration was analyzed using a direct radioimmunoassay method developed in Budapest University of Veterinary Science (*Korody et al.* 1998). The results were expressed as nmol/l.

Serum NO_2/NO_3 concentration was analyzed essentially as described by *Verdon et al.* (1995). Before analysis, serum samples were first deproteinized by ultrafiltration with regenerated cellulose membrane filters (Ultrafree®-MC 10000 NMWL Filter Unit, Millipore, Bedford, MA). Serum urea and creatinine concentrations were determined with an automated analyzer using routine methods (*Fabiny & Ertigshausen* 1971, *Gutmann & Bergmeyer* 1977).

Differences in examined parameters between mildly and moderately or severely affected animals were analyzed by repeated measures analyses of variance with clinical response as between factor, and treatment and time after challenge as within factors. Significances of F-ratios for within factors were evaluated using Greenhouse-Geisser adjusted p-values. Differences in profiles between the 2 clinical response groups were tested also by comparing changes between successive time points after bacterial challenge. Time points with any missing data were excluded from the analyses. Period and carry-over effects were tested using t-test procedures as described earlier (*Jones & Kenward* 1990). Differences between survived and euthanized animals were studied using t-test at selected time points.

Results

Clinical findings

Clinical mastitis with mild, moderate or severe signs was developed within 12 h PC in all of the cows. The clinical response varied greatly among the cows, but the response of each cow followed the same pattern after both challenges. Three cows showed mild, and the other 3 either moderate or severe clinical signs of mastitis. No significant differences between the enrofloxacin-treated vs non-treated cows or sequent challenge times were found for any of the clinical parameters. In case of mild and moderate mastitis, systemic clinical signs disappeared within 2-3 days, and local signs approximately within one week (Fig. 1 b,c). Two cows developed severe systemic and local clinical signs of mastitis. The poor clinical condition of these 2 animals became more apparent at 2 days after challenge, while the others recovered quickly. After day 2 PC, the clinical condition of these two cows deteriorated rapidly, and the cows became recumbent. These cows were excluded from the trial 4 days PC and had to be euthanized. For one of these cows the challenge was the second. The 2 euthanized cows belonged to the group treated with flunixin only. The detailed results from treatment effects will be presented in a separate paper later.

Bacteriological results

The first bacteriological milk samples were taken 12 h PC when there were already differences in bacterial numbers between the challenged quarters. The 3 cows with mild infection reduced bacterial growth more rapidly than the other 3 animals with moderate or severe mastitis (Fig. 2a). The 2 severely affected cows showed the highest *E. coli* counts, the numbers remaining high for 3 days (Fig. 2 a); this difference was not statistically significant. The bacteria were eliminated from the mildly and moderately affected animals within 2-4 and 3-8 days, respectively.

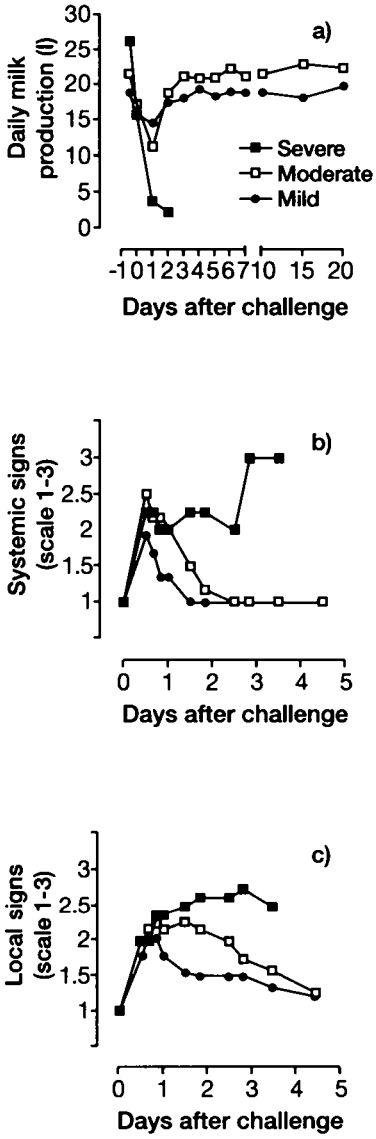

Figure 1. Mean a) daily milk production, score for b) systemic clinical signs, and c) local clinical signs in the mildly, moderately, and severely affected dairy cows after an intramammary administration of 1500 CFU of *E. coli* into single udder quarter. The data is combined from 2 successive challenges carried out at 3 weeks intervals with 6 cows.

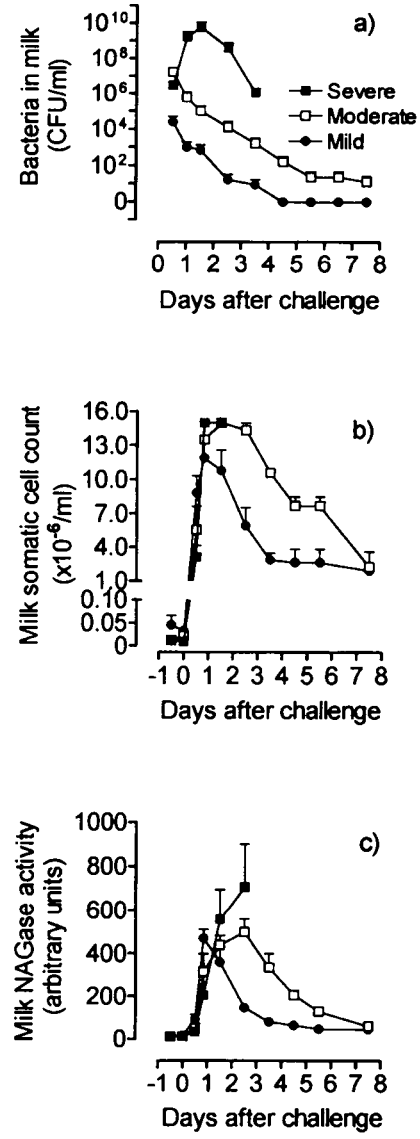

Figure 2. Mean (± SEM) a) milk bacteriological count, b) milk somatic cell count, and c) milk NA-Gase activity of the administered quarter in the mildly, moderately, and severely affected dairy cows after an intramammary administration of 1500 CFU of *E. coli* into single udder quarter. The data is combined from 2 successive challenges carried out at 3 weeks intervals with 6 cows.

Changes in milk parameters

Milk SCCs of the challenged quarters were already high 12 h PC (Fig. 2b) and the numbers had increased slightly more in the 3 mildly affected cows. The moderately and severely affected animals responded with a short delay, achieving the peak within 1 day PC. After that, the SCCs started to decrease in all animals, approaching the preinfection levels within 3 weeks PC. The milk SCC response was affected by the challenge time. After the 2nd challenge, the mean SCC was significantly higher ($p<0.05$) at 12 h PC, whereafter the counts declined more rapidly than after the 1st challenge. Milk NAGase activity of the challenged quarters was also increased within 12 h (Fig. 2c). Again, the increase was more rapid in the mildly affected cows, which reached the peak NAGase activity within 1 days PC, followed by a rapid decrease during the following days. The NAGase response of the moderately and severely affected animals peaked at day 2 PC, and decreased more slowly than in the former group of cows. The decrease in NAGase activity was more rapid than in SCCs, and after 7 days only minor activity was seen. The repeated challenge affected the NAGase response in the same manner as with SCC, but the effect of challenge time was not statistically significant. The mean decrease in daily milk production one day PC for mildly, moderately, and severely affected cows was 8, 35 and 77 per cent, respectively. The strong decrease in severely affected animals was significantly different from that of the other cows on day 1 PC ($p<0.05$). In the mildly and moderately affected animals, milk production normalized rapidly (Fig. 1a).

Changes in serum parameters

Bacterial challenge induced an increase in serum Hp and SAA concentrations in all 6 cows (Fig. 3a,b). Hp levels were normalized within 7 days, and SAA levels by the 6th day PC. Hp response of the 2 cows with severe mastitis did not differ significantly from the others, one of them having only moderate Hp response during the acute phase of infection. In SAA concentrations, significant differences between the mild or moderate vs. serious responders were seen on day 3 ($p<0.001$).

TNFα concentrations of individual cows varied remarkably before challenge, having a range from <20 (undetectable) to 293 pg/ml. The mildly and moderately affected cows did not show any serum TNFα response during the experiment (Fig. 4a). The 2 cows with severe mastitis showed a 2-fold response at 1-2 days PC. Despite the severe clinical condition of these animals, their serum TNFα concentration returned to background levels within 3 days.

Serum cortisol concentration was increased in all 6 cows at 12 h PC; the increase was approximately 10-fold (Fig. 4b). The 2 cows with severe mastitis showed elevated cortisol levels until they were euthanized (Fig. 4b). The cortisol peak was significantly less pronounced after the 2nd challenge ($p<0.001$).

Before challenge, serum NO_2/NO_3 concentration of the experimental cows varied between 12 and 51 μmol/l. After the challenge, serum NO_2/NO_3 concentration first decreased in all animals, followed by an increase in the severely affected cows at 1-2 days PC (Fig. 5a). The NO_2/NO_3 patterns of other 10 healthy cows were also examined. In these animals the serum NO_2/NO_3 levels varied between 1-30 μmol/l, the levels being higher in dry cows, and after feeding (data not shown). The serum urea and creatinine concentration were slightly increased in the moderately and severely affected cows at day 1-2 PC (Fig. 5b,c). The increased urea and creatinine concentrations returned to background levels within 3 days PC.

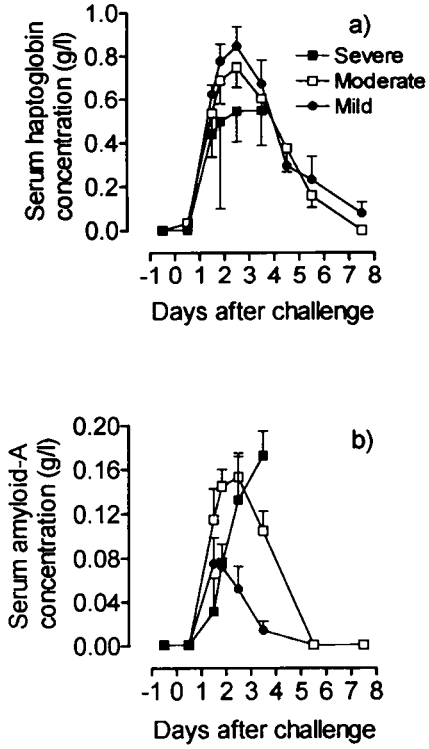

Figure 3. Mean (± SEM) a) serum haptoglobin, and b) serum amyloid-A concentration in the mildly, moderately, and severely affected dairy cows after an intramammary administration of 1500 CFU of *E. coli* into single udder quarter. The data is combined from 2 successive challenges carried out at 3 weeks intervals with 6 cows.

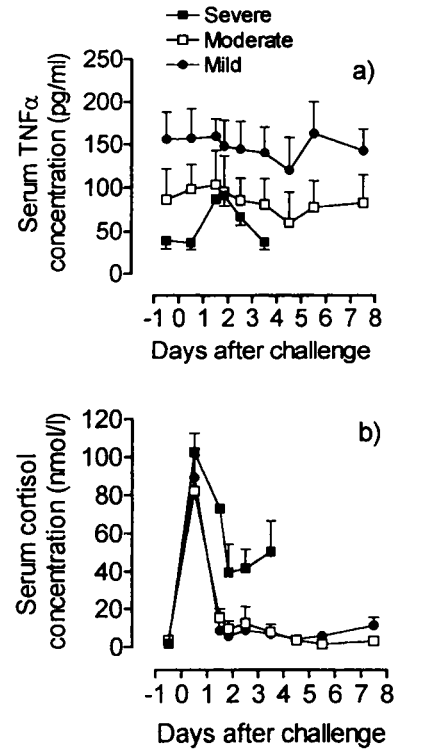

Figure 4. Mean (± SEM) a) serum tumor necrosis factor alpha, and b) serum cortisol concentration in the mildly, moderately, and severely affected dairy cows after an intramammary administration of 1500 CFU of *E. coli* into single udder quarter. The data is combined from 2 successive challenges carried out at 3 weeks intervals with 6 cows.

Discussion

The intramammary infection with 1500 CFU of *E. coli* bacteria produced a clinical mastitis in all cows, but the clinical response varied greatly between animals. This indicates that the severity of response to coliform infection is dependent on the cow, as also has been stated before (*Eberhart et al.* 1979). In our previous study using the same model, the infection induced no severe coliform mastitis cases during late lactation period (*Pyörälä et al.* 1994). The higher susceptibility of early-lactating cows to severe coliform mastitis is well acknowledged (*Hill et al.* 1979, *Hill* 1981). In this study the cow responses in the first and second challenge 3 weeks further into lactation were principally similar, but no animals were challenged immediately after calving when they probably had been even more prone to serious disease.

The clinical condition of the 2 severely affected cows started to deteriorate at 1-2 days PC, which is much later than the onset of clinical signs. This makes it difficult for the practicing veterinarian to find the animals with poor prog-

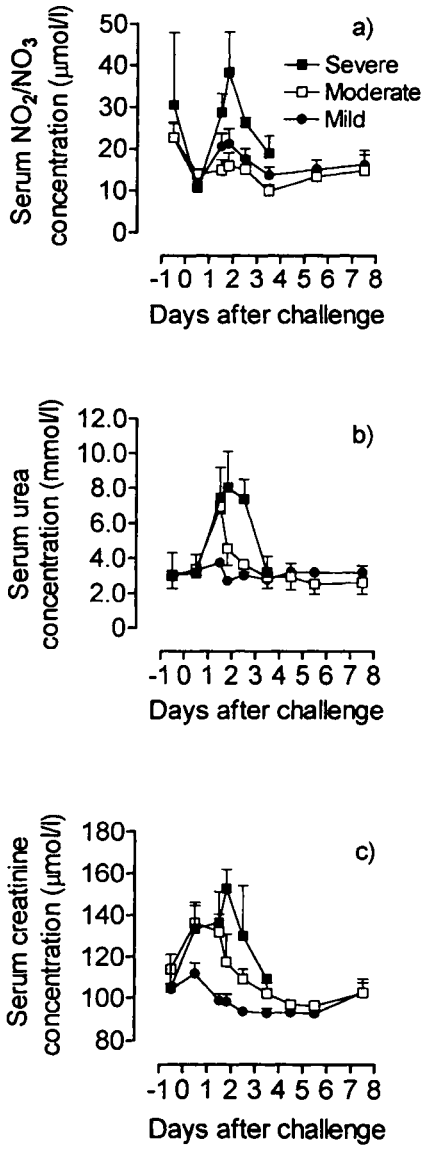

Figure 5. Mean (± SEM) a) serum nitrite and nitrate, b) serum urea, and c) serum creatinine concentration in the mildly, moderately, and severely affected dairy cows after an intramammary administration of 1500 CFU of *E. coli* into single udder quarter. The data is combined from 2 successive challenges carried out at 3 weeks intervals with 6 cows.

nosis for more intensive treatment early enough. In our study, the severity of mastitis was significantly related to bacterial counts in milk. This finding is supported by several authors (*Lohuis et al.* 1990, *Vandeputte-van Messom et al.* 1993, *Shuster et al.* 1996).

Milk NAGase activity reflected the SCC closely and may demonstrate the difference in leucocytic function between the mildly and moderately or severely affected animals. NAGase activity of the mildly affected quarters increased, peaked, and declined more rapidly than in the moderately and severely affected ones. The SCC and consequent NAGase patterns of this study support the important role of phagocytic activity in bovine coliform mastitis. The mobilization and function of leucocytes are important factors affecting the pathogenesis of coliform mastitis, and these factors may be suppressed during the puerperal period (*Hill et al.* 1979, *Hill* 1981, *Kehrli et al.* 1989, *Cai et al.* 1994, *Shuster et al.* 1996). Delay in leucocyte diapedesis into the infected gland can result in an exponential growth of *E. coli* bacteria, and in development of severe mastitis. This is in agreement with the results of our study.

There was a prominent decrease in milk production in the 2 cows with fatal mastitis. Milk production one day PC was an accurate indicator for subsequent development of severe coliform mastitis in this study. This finding is supported by previous reports (*Vandeputte-van Messom et al.* 1993).

Experimental *E. coli* mastitis was able to trigger a strong serum APP response, as also has been reported before (*Salonen et al.* 1996). In one study (*Werling et al.* 1996), no Hp response was detected in heifers after intravenous low-dose-infusion of endotoxin. This can be due to the different administration route and/or endotoxin dose. In our study, the Hp response seemed not to be related with the severity of the disease, and Hp failed to discriminate the fatal *E. coli*

infections from others. There are only few reports on SAA in bovine mastitis or endotoxin-induced experimental mastitis (*Rossevatn et al.* 1992, *Werling et al.* 1996). Here SAA concentration was related to the severity of the disease: in the cows with fatal mastitis SAA levels progressively rose. Serum SAA thus appeared to be a promising indicator for the course of *E. coli* mastitis.

The serum TNFα response of the severely affected cows was rather low when compared with other studies where bovine TNFα has been measured radioimmunologically (*Kenison et al.* 1990, *Peel et al.* 1990, *Sordillo et al.* 1991). In our study, TNFα response could only be seen in the severely diseased cows; the initial and final concentrations were lower in them as compared with the mild or moderate responders. One weakness in our analysis was that human TNFα standard was used. In one recent study on naturally occurring coliform mastitis (*Nakajima et al.* 1997), serum TNFα was found to be high in surviving animals, but low in animals that died and in healthy controls. The transient increase of serum TNFα concentration of the severely affected cows at day 1-2 PC together with maximum bacterial growth in milk may indicate the role of TNFα in pathogenesis of *E. coli* mastitis in the bovine; this has also been shown by *Sordillo et al.* (1992). In our study, the serum TNFα concentration returned to background levels within 72 h PC. This is likely due to down-regulation of TNFα production. The local TNFα production can also continue without reflecting in circulation (*Myers & Murtaugh* 1995). It must be noted that the twice a day sampling was presumably not frequent enough to study the TNFα response during the first 3 days.

All cows responded to bacterial challenge with increased serum cortisol concentration. In the mildly and moderately affected cows the increase was temporary, being similar to the increase after intramammary infusion of endotoxin (*Paape et al.* 1974, *Jackson et al.* 1990). Instead, the 2 severely affected cows had increased cortisol levels until being euthanized. This reflects the continuation of endotoxin release and consequent APR in these animals, and the need of cortisol for the down-regulation of APR (*Baumann & Gauldie* 1994).

Mammary macrophages form the first-line alarming and defense system in case of bacterial colonization of the udder. According to the authors' knowledge, there are no reports on the role of NO in bovine mastitis. Theoretically, acute coliform infection could activate inducible NO synthases and be a potent source for excessive release of NO. In circulation, NO is rapidly metabolized to NO_2 and further to NO_3 by interaction with oxyhaemoglobin (*Moshage et al.* 1995, *Payen et al.* 1996). Both NO_2 and NO_3 are rather stable in serum samples. The measurement of serum NO_2/NO_3 concentration from blood to study NO metabolism is not without difficulties: actual production of NO_2/NO_3 is underestimated because of their distribution to extracellular fluid, and it can also be affected by alterations in extracellular fluid volume and renal function in diseased animals (*Zeballos et al.* 1995). Furthermore, ruminant nitrogen metabolism is a complicated network. Serum NO_2/NO_3 levels are strongly affected by the stage of lactation, the amount and quality of feed and water, the metabolic activity of the forestomachs, and the convertion of these salts to other compounds and their excretion in the urine. However, the increase in serum NO_2/NO_3 concentration in the severely affected cows may indicate excess production of NO, because the feed intake and the consequent intake of exogenous NO_2/NO_3 were reduced due to the disease. Nevertheless, it is concluded that serum NO_2/NO_3 concentration does not seem to be a reliable marker for indigenous NO production during acute coliform

mastitis in the bovine. The possible role of NO in bovine mastitis requires more detailed *in vitro* and *in vivo* studies.

The temporary increase of serum urea and creatinine concentration in the moderately and severely affected cows can be explained by decreased renal function as a consequence of APR. Simultaneous catabolic breakdown of proteins may also result in an increase of serum urea concentration. The normalization of serum urea and creatinine levels even in severely affected cows may at least partly have been affected by the intensive fluid therapy administered.

The experiment was carried out by repeating the bacterial challenge with the same animals after an interval of 3 weeks. The benefit of this model is to decrease the between-cow variation in the challenge response as each cow is used as its own control. It has been demonstrated that previous exposure of mammary gland to endotoxin may change the effects of subsequent exposure (*Rainard & Paape* 1997). In that study, mammary gland was shown to become sensitizated to endotoxin. However, the challenge interval was 24 h only. In other studies, after repeated exposure to endotoxin, tolerance to endotoxin has been shown to develop (*Olson et al.* 1995). In our previous study with the same experimental model as here, we found a decrease in serum Hp response after the 2nd challenge (*Salonen et al.* 1996). Here we were not able to repeat this result, possibly because of the limited number of animals. In the present study, the serum cortisol response was significantly lower after the repeated challenge; this may indicate suppressed production of pro-inflammatory cytokines. On the other hand, the rapid increase in milk SCC after the repeated challenge indicates that the milk inflammatory cells were primed by the previous challenge with *E. coli*. However, this did not seem to trigger stronger systemic APR.

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Sammanfattning

Akut fas respons hos kor med experimentellt inducerad Escherichia coli mastit.

För att åstadkomma experimentell mastit, inokulerades en juverfjärdedel hos sex finska ayrshire kor med 1500 CFU av *E. coli*. Tre veckor senare inokulerades kornas kontralaterala juverfjärdedel. Utvecklingen av mastit följdes upp genom att iakttaga allmänna och lokala kliniska symptom, och genom analys av serum och mjölkprover. Intramammär inokulation med *E. coli* ledde till klinisk mastit hos alla kor, men svårighetsgraden varierade mycket mellan olika kor. Tre kor insjuknade milt, en måttligt och två allvarligt. De två allvarligt insjuknade korna måste avlivas p.g.a. grav mastit.

Serumkoncentrationerna av haptoglobin och amyloid-A nådde sina maximala nivåer 2-3 dagar efter inokulationen. Serummängden av haptoglobin korrelerade inte med sjukdomens svårighetsgrad. Serumkoncentrationen av amyloid-A steg gradvis hos de allvarligt insjuknade korna, och ledde till signifikanta skillnader mellan grupperna på den fjärde dagen. Serumkoncentrationen av tumor nekros faktorn α steg endast hos de allvarligt sjuka korna. Kortisol-responsen i serum var förlängd hos de allvarligt sjuka korna och signifikant lägre efter den andra inokulationen. Serumhalten av nitrit/nitrat steg hos kor med grav mastit; eventuellt ett tecken på ökad kväveoxidproduktion i samband med akut *E. coli* mastit. Å andra sidan är nitrit/nitrat halten i serum inte en pålitlig markör för ökad kväveoxid produktion. Kraftigt minskad mjölkproduktion och snabb bakterietillväxt i den infekterade juverfjärdedelen visade sig vara de viktigaste symptomen som tyder på dålig prognos vid akut *E. coli* mastit.

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