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STAPHYLOCOCCAL NUCLEASE IN UDDER SECRETIONS OF COWS WITH ACUTE MASTITIS

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GUDDING, R.: *Staphylococcal nuclease in udder secretions of cows with acute mastitis.* Acta vet. scand. 1980, *21*, 79—95. — High concentrations of nuclease produced by Staphylococcus aureus were demonstrated within a few hours by direct examination of udder secretions from cows with a severe staphylococcal mastitis. A positive correlation between the nuclease concentration and the severity of the mastitis was found. The cows with high nuclease concentrations were generally young individuals and/or in the first 2 weeks of the lactation period. Most had a low titre of antibodies against staphylococcal nuclease. Two-thirds of the cows in which high nuclease concentrations were demonstrated were culled or died because of the mastitis attack.

The role which nuclease plays for staphylococcal virulence is discussed, and it is concluded that nuclease contributes to the pathogenicity of S. aureus.

mastitis; Staphylococcus aureus; nuclease; antinucleases.

Staphylococcus aureus produces several toxins and enzymes which are known to be of significance for virulence (*Abramson* 1972, *Jeljaszewicz* 1972). The role of these factors in the pathogenesis of bovine mastitis has recently been reviewed by *Anderson* (1976).

Most attention has been paid to the haemotoxins, in particular the α-toxin, and the coagulase as virulence and diagnostic criteria of S. aureus. Their significance with regard to pathogenicity has generally been based on in vitro tests, and on the demonstration of the biological effects of more or less purified toxins or enzymes administered to laboratory animals or applied to tissue cultures.

The enzyme deoxyribonuclease (nuclease, DNase) is produced by all or most strains of S. aureus and in recent years in-

terest in this enzyme in S. aureus diagnosis has increased. It has also been considered as being a virulence determinant.

In a previous short communication the presence of staphylococcal nuclease in quarter samples from cows with mastitis has been reported (*Gudding* 1976). The present paper gives a more detailed presentation of the bacteriological and epidemiological investigations which were carried out in order to study the development of acute staphylococcal mastitis with particular reference to the role of the staphylococcal nuclease.

MATERIALS AND METHODS

Samples and routine procedures

The material comprised selected quarter samples from mastitic cows sent to the National Veterinary Institute, Oslo, during the period April 1975 to April 1978. All samples were collected by veterinarians who also provided data regarding the identity and clinical status of the affected cows.

The quarter samples were routinely examined according to the methods described by *Klastrup & Schmidt Madsen* (1974). The mastitis diagnosis was also made according to their guidelines. The sensitivity of the isolated strains of S. aureus to antibacterial agents was determined as described by *Bakken & Gudding* (1978).

Nuclease activity and antinuclease titre

The concentration of staphylococcal nuclease in the quarter samples was determined in the Toluidine Blue DNA Agar (TDA) of *Lachica et al.* (1971). Aliquots of 0.1 ml milk were filled into wells with a diameter of 10 mm in the 2 mm thick agar layer. Both non pre-treated samples and samples pre-treated by being heated in a water-bath at 100°C for 10 min were examined. Nuclease demonstrated after heating is designated "heated nuclease" in order to distinguish it from the nuclease activity measured in non-heated samples which is designated "nuclease". The concentration of nuclease and heated nuclease is given in diffusion units (*Sandvik* 1962).

The titre of antibodies against the S. aureus nuclease in the milk samples was determined by the antinuclease test (*Gudding* 1977).

Group classification

Based on the nuclease concentrations of the affected quarter samples, the cows were divided into 3 groups, 1 of which was subdivided according to the anamnestic information given by the veterinary surgeons (Table 1). All cows in Group 1 and approx. 25 % of the cows in Groups 2, 3 and 4 originated from herds participating in a mastitis research programme from which data about the udder health of the cows and the herds were available. The 274 cows originated from 195 herds.

Staphylococcal α-toxin

The methods described by *Elek & Levy* (1950) and *Sandvik* (1955) were used for the demonstration of α-toxin production by the S. aureus strains. Paper strips soaked with antiserum against α-toxin and β-toxin were placed on blood agar prepared with 6 % washed erythrocytes from rabbit and cow, respectively. The strains were streaked at right angles to these paper strips and the plates incubated for 18 h at 37°C.

Phage typing

The typing was carried out according to the method described by *Blair & Williams* (1961). The strains were tested with the phages of the international basic set for typing of bovine S. aureus, including the following phages and lytic groups: 29 and 52A (I), 3A and 116 (II), 6, 42E, 53, 75 and 84 (III), 42D, 102, 107 and 117 (IV) and 78, 118 and 119 (miscellaneous). Routine test dilution (RTD) of the phages and RTD×100 were used.

Health status of the cows and the herds

The anamnestic information about each cow was provided by local veterinarians. Data about the udder health of the affected cows, prior to and after the mastitis attack were available from the following sources: 1. Quarter milk samples from all cows in the herds; 2. Quarter milk samples of affected cows collected at the request of the laboratory; 3. Replies to a questionnaire sent to the farmers.

The udder health of the herds from which the mastitic cows originated, was evaluated in 2 different ways: 1. The prevalence rate of infectious mastitis and S. aureus mastitis in cows and quarters; 2. The cell count and antinuclease titre of bulk milk.

Data analyses

The material was coded and transferred to a computer for processing. The concentrations of nuclease, heated nuclease and the titre of antinuclease were transformed to square roots for the calculation of the average values. For the correlation analyses the clinical status of the cows was graded from 1 to 4, the latter figure applying for the most severe cases. The post mastitis status of the cows was converted into a quantitative variable as follows: Complete recovery = 1, subclinical mastitis = 2, dried or culled because of the mastitis = 3 and died as a result of the mastitis = 4.

RESULTS

Laboratory examinations

Nuclease, heated nuclease and antinucleases. The enzyme nuclease could sometimes be demonstrated in quarter samples from cows with staphylococcal mastitis by direct examination of the mastitic milk in TDA. High concentrations of staphylococcal nuclease (> 20,000 diffusion units per 0.1 ml) were found in samples from 65 out of approx. 1400 cows with mastitis caused by S. aureus. All the 65 animals suffered from an acute or peracute mastitis (Table 1). The number of cows with a nuclease concentration of 100—20,000 diffusion units per 0.1 ml in the same period exceeds 87 in Groups 2 and 3 (Table 1).

Table 1. Cases of mastitis grouped according to nuclease concentration in the udder secretions and clinical picture.

Group	Number of cows	Nuclease concentration (diffusion units per 0.1 ml)	Mastitis diagnosis
1	122	< 100	chronic, subacute, acute
2	42	100—20,000	subacute
3	45	100—20,000	acute, peracute
4	65	> 20,000	acute, peracute

Approx. 10 % of all the cows with staphylococcal mastitis belonged to this category. Average concentrations of nuclease, heated nuclease and antinucleases are shown in Table 2. In the heated samples, the nuclease was demonstrated in milk from all quarters with S. aureus mastitis. Both the concentrations of heated nuclease and the titres of antinucleases differed in the 4 groups ($P < 0.001$).

Table 2. Average concentrations of nuclease, heated nuclease and antinuclease in mammary secretions (diffusion units per 0.1 ml) in the 4 groups shown in Table 1.

Group	Nuclease*	Heated nuclease*	Antinucleases**
1	5 (n = 121)	4160 (n = 122)	6.5 (n = 72)
2	4324 (n = 42)	10120 (n = 36)	2.6 (n = 16)
3	4671 (n = 45)	15158 (n = 41)	1.4 (n = 16)
4	59467 (n = 65)	46573 (n = 48)	0.5 (n = 21)

* The value of the quarter sample with the highest figure.

** The value of the quarter sample with the lowest value.
Only cows in the period 10 to 250 days after parturition are included.

Bacteriological and cytological analyses. A diagnosis of mastitis could easily be made by visual assessment of the samples from the affected quarters in Groups 2, 3 and 4. The samples often looked like blood serum with a heavy sediment after centrifugation. However, the CMT*-score generally indicated a more moderate increase in the cell content. Samples from the infected glands in Group 4 were sometimes characterized by a subnormal pH when analysed in the laboratory and this observation was also occasionally reported by the practising veterinary surgeons who carried out the clinical examinations in the field. In general, a heavy growth of S. aureus was produced after primary inoculation on blood agar, primarily in Group 4 and to a lesser extent also in Groups 3 and 2. The number of colony forming units in Group 1 varied considerably.

Susceptibility pattern. The percentage of penicillin-resistant strains of S. aureus in Groups 1 to 4 was 11.5 (n = 122), 19.0 (n = 42), 4.7 (n = 43) and 4.8 (n = 63), respectively. All penicillin-resistant strains were susceptible to sulfonamides and all but 1 to tetracyclines. Resistance to streptomycin was recorded in strains from 8 of the 27 cows with penicillin-resistant S. aureus. This total was made up of 3 in each of Groups 1 and 2, and 1 in each of Groups 3 and 4.

Production of α-toxin and β-toxin. Out of 54 strains examined, 41 (76 %) were found to produce α-toxin. The percentage of strains producing α-toxin was lowest in Group 1 and highest in Group 4, the figures being: 67 % in Group 1, 71 % in

* California Mastitis Test.

Group 2, 80 % in Group 3 and 84 % in Group 4. All the strains examined produced β-toxin when cultivated on blood agar with washed bovine erythrocytes.

Phage typing. The phage pattern of the strains is presented in Table 3. Twenty-nine of 56 strains were lysed by phage 78. By combining the data from the toxin analyses and the phage typing the relative frequency of strains producing α-toxin and/or belonging to phage 78 was 72 % in Group 1, 86 % in Group 2, 90 % in Group 3 and 100 % in Group 4.

Table 3. Percentage distribution of cows in relation to the lytic groups of S. aureus (groups as in Table 1).

Group	Lytic group				Miscellaneous	Non-typable
	I	II	III	IV		
1 (n = 18)	0	0	22 (25)	11 (50)	44 (87)	22 (67)
2 (n = 8)	0	0	0	38 (50)	50 (67)	12 (67)
3 (n = 11)	0	0	0	0	73 (100)	27 (50)
4 (n = 19)	0	0	0	0	58 (73)	42 (100)

The figures in brackets give the percentage of strains in each lytic group producing α-toxin.

Epidemiological analyses

Clinical examinations. The type of mastitis (chronic, subacute, acute, peracute) shown by the cows in the various groups is presented in Table 1. Chronic, subacute and acute mastitis was diagnosed in respectively 52 %, 24 % and 24 % of the cows in Group 1. Average body temperature at the time of clinical examination was 39.4°C in Group 1, 39.4°C in Group 2, 40.7°C in Group 3 and 40.9°C in Group 4. (Twelve cows, 10 of which belonged to Group 4, suffering from acute or peracute mastitis with a temperature below 39.0°C were excluded).

Occurrence of mastitis in relation to age and time after parturition. The average age of the cows at the onset of the attack was found to be 6.0 years in Group 1, 4.9 years in Group 2, 4.8 years in Group 3 and 4.7 years in Group 4. It can be seen from Table 4 that most cases of clinical mastitis occurred during the first week after parturition. However, there was a difference between the groups in the time of onset as more than 55 % of the cows in Groups 3 and 4 were

Table 4. Percentage distribution of affected cows in relation to time after parturition (groups as in Table 1).

Group	Postparturition period (days)							
	0—7*	8—15	16—30	31—60	61—120	121—180	181—240	≥ 241
1 (n = 120)	25	3	5	12	11	15	14	16
2 (n = 34)	38	6	3	6	12	15	12	9
3 (n = 38)	47	11	5	8	5	8	8	8
4 (n = 58)	47	10	7	9	8	8	3	7

* The periods are numbered from 1 (0—7 days) to 8 in the correlation tests.

affected during the first 2 weeks after parturition, whereas the corresponding figure for Group 1 was 28 %.

Distribution of affected quarters and frequency of teat injuries. The rear quarters were affected or most severely affected in about 50 % of the cows of Group 1 and 60 % in Groups 2, 3 and 4. The frequency of injured teats was found to be 4.9 % in Group 1, 11.9 % in Group 2, 13.3 % in Group 3 and 15.4 % in Group 4. In addition to a higher frequency, comments from the veterinarians providing the samples indicated that teat lesions were more severe in Groups 3 and 4 than in Group 1. Teat lesions were equally distributed between front and rear quarters.

Health status before the attack. The percentage of cows with a previous history of mastitis, subclinical or clinical, was particularly low in Group 4 (Table 5). In Group 1, nearly 50 % of the cows had suffered an udder inflammation before the mastitis attack in question.

Table 5. Percentage of cows with and without a previous history of mastitis in the identical affected quarter* (groups as in Table 1).

Group	No previous mastitis	Mastitis
1 (n = 74)	51.4	48.6
2 (n = 16)	87.5	12.5
3 (n = 12)	66.7	33.3
4 (n = 20)	90.0	10.0

* Based on information from the dairymen and laboratory results obtained in the period 1 to 180 days before the attack.

Health status after the attack. Ten cows, 9 of which were in Group 4, died as a result of the mastitis (Table 6). In approx. half of the cows in the 3 groups with highest nuclease concentrations, lesions in the affected quarters were so severe that the quarters dried off, or the cows were culled either during or at the end of the lactation period. Even in Group 1 30 % of the cows were slaughtered because of the mastitis. Only 2 % of the cows in Group 4 and 4 % in Group 3 recovered completely.

Table 6. Udder health status after the mastitis attack given as the percentage of cows (groups as in Table 1).

Group	No mastitis	Subclinical mastitis of identical quarter		Dried/culled	Died
	laboratory diagnosis*	laboratory diagnosis*	information from the dairyman	because of the mastitis attack	
1 (n = 86)	34	27	9	30	0
2 (n = 31)	16	26	13	45	0
3 (n = 26)	4	31	8	54	4
4 (n = 48)	2	17	15	48	19

* Laboratory results obtained in the period 30 to 100 days after the attack.

Mastitis levels of the herds. The average prevalence rate of infectious mastitis and infectious mastitis caused by S. aureus in the herds from which the cows originated during the period 0 to 120 days before the attack was 29.1 % and 13.9 %, respectively, in all groups. The average antinuclease titre and the geometric mean of the cell count of the bulk milk in the period 0 to 180 days before the onset of the clinical mastitis were 3.6 diffusion units per 0.1 ml (zone diameter of 6.0 mm) and 296,000 per ml, respectively. There were no statistically significant differences between the 4 groups in this respect. Nor did the corresponding results for the period after the attack show any statistically significant differences when compared with those from the period preceding the onset of mastitis.

Correlation coefficients. As seen in Table 7, the concentration of nuclease and the titre of antinucleases in the quarter milk samples showed a negative correlation. However, the concentration of heated nuclease did not show any correlation to either that of nuclease or to the titre of antinucleases at

Table 7. Statistically significant correlation coefficients between parameters of udder health of the cow and the herd, postparturition period, antinuclease titre and concentration of nuclease.

Variables		Number of cows	Correlation coefficients	Significance level
Concentration of nuclease	Health status at clinical examination (1—4)	265	0.47	$P < 0.001$
Concentration of nuclease	Antinuclease titre (quarter milk)	125	—0.43	$P < 0.001$
Concentration of nuclease	Health status after the attack (1—4)	195	0.40	$P < 0.001$
Antinuclease titre (quarter milk)	Health status at clinical examination (1—4)	122	—0.31	$P < 0.001$
Concentration of nuclease	Postparturition period* (1—8)	250	—0.26	$P < 0.001$
Antinuclease titre (quarter milk)	Antinuclease titre (bulk milk)**	36	0.45	$P < 0.05$
Concentration of nuclease	Cell count (bulk milk)**	126	0.21	$P < 0.05$

* See Table 4.

** Including results of analyses in the period 90 days before to 90 days after the mastitis attack.

a significance level of $P < 0.05$. Furthermore, correlation tests were performed between the concentration of nuclease and heated nuclease and the antinuclease titre on one hand and, on the other hand, the following variables: The health status of the cow on clinical examination and after the mastitis attack, the cell count and the antinuclease titre of bulk milk, the post-parturition period (Table 4), the age of the cow and the herd percentage of cows with infectious mastitis and infectious mastitis caused by S. aureus. The coefficients which were found to be statistically significant are presented in Table 7.

DISCUSSION

In the present study, staphylococcal nuclease was demonstrated in the Toluidine Blue DNA Agar (TDA) by a direct examination of quarter milk samples from cows with mastitis caused by S. aureus. The presence of high concentrations of nuclease in quarter samples from cows with mastitis is a result of a very heavy growth of an organism which excretes the enzyme into the environment. S. aureus is an abundant producer of nuclease in vitro, and the present results show that the enzyme is also excreted in vivo in infected tissues. The concentration in the quarter milk samples of cows in Group 4 is of the same order of magnitude as that recorded in broth cultures of S. aureus.

The activity of "free" nuclease as measured by direct examination of samples from mastitic quarters will be determined not only by the original amount of enzyme excreted, but also by the amount of neutralizing antibodies present. In Group 4, the antibody titre was very low, being on average 0.5 diffusion units per 0.1 ml in quarters with the lowest titre.

The concentration of heated nuclease more accurately reflects the total excretion of nuclease by the microorganism, as the neutralizing antibodies are inactivated by the heat treatment. As shown in Table 2, concentrations of heated nuclease in Groups 1, 2 and 3 were significantly higher than those of nuclease, particularly in Group 1. The increase in the difference between the concentrations of heated nuclease and nuclease seemed to coincide with the increase of the antinuclease titre. The average concentration of the heated nuclease was less than that of the nuclease in Group 4. In addition to the insignificant amount of antibodies, this was most probably due to a slight inactivation of the nuclease during the heat treatment.

The levels found in Group 1 (Table 2) are representative for those recorded in samples from cows with a subclinical mastitis prior to the clinical case. Samples from this category of cows are characterized by high antibody titres, and concentrations of heated nuclease significantly higher than those of the nuclease (*Gudding* 1980 c).

All the strains examined produced β-toxin and most of them α-toxin. There was an increasing percentage of α-toxin producing strains coincident with an increase of group number and consequently the severity of the disease. The role of the α-toxin in the pathogenesis of peracute mastitis seems undisputable. Numerous reports, reviewed by *Anderson* (1976) conclude that the α-toxin, producing damage to membranes, vasoconstriction and a subsequent ischaemic necrosis, is essential for the development of peracute staphylococcal mastitis.

Korbecki & Jeljaszewicz (1965) demonstrated that α-toxin and nuclease caused damage to KB-cells (*Eagle* 1955) as a result of a synergistic effect. Even in combination with subtoxic dosis of α-toxin they found that nuclease produced complete lysis of all cellular structures. Most of the strains examined in the present work produced α-toxin, and this property most frequently characterized the groups with the highest nuclease concentrations. The theory of synergism between α-toxin and nuclease provides an explanation for these observations. The fact that α-toxin production was not demonstrated in all strains may have been due to insignificant in vitro production of the toxin. However, this observation may also indicate that this haemotoxin is not a prerequisite for the development of a severe S. aureus mastitis.

The role of the β-toxin is probably more interesting when considering the pathogenesis of bovine mastitis, as all strains produced this haemotoxin. Sphingomyelin is a major constituent of membranes of the mammary gland, being concentrated in the outer exposed side of the membrane (*Keenan* 1974, *Patton & Keenan* 1975). It seems very likely that the β-toxin, which is a sphingomyelinase C, interferes with the surface membrane. Although contradictory results have been reported, membrane damage due to β-toxin activity has been demonstrated experimentally (*Wadström & Möllby* 1971).

The staphylococcal nuclease can hydrolyse both DNA and RNA, and it may thus cause lysis of both the nucleus and cyto-

plasmatic organelles such as the ribosomes if the DNA or RNA are exposed to the enzyme. The toxic effect of staphylococci on the cell is the combined result of the activity of a number of different metabolites. The nuclease exerts its toxic effect after prior action by membrane-damaging agents.

The observation of *McKee & Braun* (1962) that DNA digest, produced as a result of DNase activity, stimulated growth of coagulase-positive staphylococci fits another piece of knowledge into place in the puzzle of staphylococcal virulence. An infection with a nuclease producing staphylococcus would seem to be an auto-catalytic process, metabolites of the nuclease activity stimulating the growth of the organism and increasing the elaboration of staphylococcal extracellular products. The results of *McKee & Braun* also provide some explanation of the observation made in the present study that cows with a low antinuclease titre are more likely to get a severe attack of staphylococcal mastitis than cows with a high titre of nuclease neutralizing antibodies.

It should be emphasized that the nuclease was demonstrated by direct examination of excretions from inflammatory processes, and as seen in Table 7 the concentrations of the nuclease were positively correlated with the severity of the inflammation. Consequently, the results of the present study strongly indicate that this enzyme is one of the factors which contribute to the udder pathogenicity of S. aureus.

Nucleases (DNases) are produced by other organisms causing mastitis, especially certain streptococci. DNases are also present in normal bovine colostrum (*Gudding* 1979 a, b). Consequently possibilities for misinterpretations exist, at least theoretically. However, with a few exceptions, the excretion of DNase by streptococci into mastitic milk is slight compared with that of S. aureus (*Gudding* 1980 b). Moreover both streptococcal and normally occurring DNases lack certain properties essential for demonstration of nuclease by the original TDA method (*Gudding* 1979 a, b). High concentrations of nuclease may rarely be present in mastitic milk from which S. epidermidis is isolated, but this enzyme can be distinguished from the S. aureus nuclease by enzymo-serology (*Gudding* 1980 e).

The demonstration of the nuclease is preferably performed in a laboratory where temperature, time and other conditions can be standardized. However, even 1 drop of the mastitic milk on the TDA incubated at room temperature can produce pink

coloured zones in the agar. This observation and the fact that nuclease activity is seen after only 1 h, if the enzyme concentration is high and experimental conditions optimal, make the method suitable for rapid diagnostic use in the field. At lower enzyme concentrations and at incubation temperatures below 37 °C, the time required for a distinct colour change to occur in the agar will be prolonged. In the present study, the zone diameters in the TDA, after incubation at 37 °C for 18 h, were in the range 10 to 27 mm, corresponding to between 100 and > 150,000 diffusion units per 0.1 ml.

The other laboratory and epidemiological examinations also revealed some interesting findings. The subnormal pH occasionally observed in the mastitic milk from the most severe cases was most probably a result of lactose fermentation. This observation has generally been associated with mastitis caused by Gram negative lactose-fermenting organisms such as E. coli and other organisms of the coliform group. The observations in the present work indicate that a subnormal pH might also be caused by growth of S. aureus in the mammary gland.

The frequency of penicillin resistant strains in Groups 1 and 2 is similar to that reported by *Bakken & Gudding* (1978). In the groups with the most severe mastitis (Groups 3 and 4), less than 5 % of the strains were penicillin-resistant. This low percentage indicates that the cows comprising these 2 groups originated from herds in which the use of antibiotics was limited. Penicillin is the predominant antibiotic in mastitis therapy in Norway, generally in combination with streptomycin, and these antibiotics are administered as initial therapy to most cows. The results of the susceptibility tests together with the recovery rates in the 4 groups show that the low response to therapy in cases with severe mastitis is not due to bacterial resistance to the therapeutic agent, but more probably to damage of the udder tissue by the microorganism before therapeutical concentrations are established.

The phage type pattern of S. aureus strains isolated from cases of mastitis in Norway is somewhat unique, as 78 seems to be the predominating phage type (*Niskanen & Koiranen* 1977). This was also the case in the present investigation. Although the material was small, it is noticeable that none of the strains in Groups 3 and 4 was lysed by phages in the lytic groups 1 to 4, and all but 1 of the strains belonging to these lytic groups were

isolated from cases of chronic or subacute mastitis. The α-toxin producing strains seem to be equally distributed among "non-typable" strains and strains belonging to the group "miscellaneous".

Most cows suffering from acute and peracute S. aureus mastitis had a high fever. The 10 in Group 4 with a subnormal body temperature were all severely affected and 5 of them died or were killed in a moribund state. Information from local veterinarians did not suggest that delayed discovery could explain the rapid and dramatic course of the disease. It is more reasonable to attribute it to the rapid growth of an enzyme and toxin producing organism overcoming the normal and acquired defence mechanisms of the host.

The distribution of mastitis in relation to age in Group 1 was similar to that presented in data from the Norwegian Health Card System (*Anon.* 1978). The mastitis attacks in Groups 2, 3 and 4 affected young animals to a greater extent. One of the reasons for this age distribution may be a lower titre of antibodies against S. aureus in heifers and young cows (*Gudding* 1980 d).

The first week post partum is generally critical with respect to the development of clinical mastitis. The fact that nearly half of all mastitis cases in Groups 3 and 4 appeared in the first week after parturition is more evidence that parturition leads to debilitation of the cow which should be compensated by optimalization of the physical and microbiological environment.

The significance of the cow's environment for the pathogenesis of acute and peracute mastitis is illustrated by the fact that 15 % of the cows in the group with the highest nuclease concentration had a clinical teat injury.

As can be seen in Table 5, nearly half of the cows in Group 1 had a previous history of mastitis as compared with only 10 % in the group with the most severe attacks. The findings of the present work indicate that cows which have previously suffered from mastitis have the ability to limit and terminate a clinical mastitis more effectively than cows with no previous history of mastitis. It is reasonable to deduce that a clinical or more especially a subclinical mastitis, caused by microorganisms, stimulates the defence mechanisms of the animal, first of all the immunological ones, better enabling the animal to destroy the microorganism and reestablish milk production. These findings

thus provide a basis for future work on artificial stimulation of humoral or cellular immunity as part of a mastitis control programme.

In addition to the high percentage of cows which left production after the mastitis attack, it is discouraging to ascertain that only one third of the cows in Group 1 recovered completely. These results again confirm the poor prognosis in staphylococcal mastitis.

Based on experience from some of the herds, it was postulated before analysing the data that the cows with the highest nuclease concentrations in the mastitic milk were likely to have originated from herds with a better udder health than those with a low nuclease concentration. However, this hypothesis was not confirmed as there was no difference between the groups either in mastitis prevalence rate, bulk milk cell count or antinuclease titre. The values for all these parameters were generally somewhat higher than corresponding average values for all herds examined in the laboratory district concerned (*Gudding* 1980 a).

In conclusion, it should be pointed out that an inflammation of the udder is the combined result of a complex of positive and negative factors related to the host, the microorganism and the environment. The nuclease of S. aureus is one of the bacterial factors. In addition to the diagnostic significance, the demonstration of nuclease in udder secretions from mastitic cows in concentrations which are positively correlated to the severity of the disease, is a strong indication that this enzyme is a virulence determinant of S. aureus.

REFERENCES

Abramson, C.: Staphylococcal enzymes. *In* Cohen, J. O., ed.: The Staphylococci. Wiley-Interscience, New York 1972, 187—248.

Anderson, J. C.: Mechanisms of staphylococcal virulence in relation to bovine mastitis. Brit. vet. J. 1976, *132*, 229—245.

Anonymous: Rapport fra vurderingskomitéen for helsekort. Helsekortordningen 1976—77. (Report of the Animal Health Card Assessment Committee, 1976—77). Norsk Vet.-T. 1978, *90*, 451—458.

Bakken, G. & R. Gudding: In vitro antibiotic sensitivity test of Staphylococcus aureus isolated from mastitic milk. Nord. Vet.-Med. 1978, *30*, 15—17.

Blair, J. E. & R. E. O. Williams: Phage typing of staphylococci. Bull. Wld Hlth Org. 1961, *24*, 771—784.

Eagle, H.: Propagation in a fluid medium of a human epidermoid carcinoma, strain KB. Proc. Soc. exp. Biol. (N. Y.) 1955, *89*, 362—364.

Elek, S. D. & E. Levy: Distribution of hæmolysins in pathogenic and non-pathogenic staphylococci. J. Path. Bact. 1950, *62*, 541—553.

Gudding, R.: Heat stable nuclease in mastitic milk. Acta vet. scand. 1976, *17*, 501—502.

Gudding, R.: An agar diffusion method for the determination of antibodies against Staphylococcus aureus deoxyribonuclease. Acta vet. scand. 1977, *18*, 480—493.

Gudding, R.: The demonstration and characterization of deoxyribonuclease of streptococci group A, B, C, G and L. Acta vet. scand. 1979 a, *20*, 102—121.

Gudding, R.: DNases in milk and blood sera from different species. Acta vet. scand. 1979 b, *20*, 404—416.

Gudding, R.: Antibodies against Staphylococcus aureus nuclease in bulk milk as an indicator of mastitis in dairy herds. Acta vet. scand. 1980 a, *21*, 96—107.

Gudding, R.: Nucleases of some udder pathogenic organisms. In vivo and in vitro production. Acta vet. scand. 1980 b, *21*. In press.

Gudding, R.: Measurement of Staphylococcus aureus nuclease and antinucleases. Applicability for the assessment of mastitic milk. Acta vet scand. 1980 c, *21*. In press.

Gudding, R.: Antibodies against staphylococcal and streptococcal nucleases in bovine blood serum and milk. Acta vet. scand. 1980 d, *21*. In press.

Gudding, R.: Nuclease of Staphylococcus epidermidis isolated from mastitic milk. Production and some properties. Acta vet. scand. 1980 e, *21*. In press.

Jeljaszewicz, J.: Toxins (Hemolysins). *In* Cohen, J. O., ed.: The Staphylococci. Wiley-Interscience, New York 1972, 249—280.

Keenan, T. W.: Composition and synthesis of gangliosides in mammary gland and milk of the bovine. Biochim. biophys. Acta (Amst.) 1974, *337*, 255—270.

Klastrup, O. & P. Schmidt Madsen: Nordiske rekommendationer vedrørende mastitisundersøgelser af kirtelprøver. (Nordic recommendations concerning mastitis examinations of quarter samples). Nord. Vet.-Med. 1974, *26*, 197—204.

Korbecki, M. & J. Jeljaszewicz: Action of staphylococcal toxins in cell cultures. J. infect. Dis. 1965, *115*, 205—213.

Lachica, R. V. F., C. Genigeorgis & P. D. Hoeprich: Metachromatic agar-diffusion methods for detecting staphylococcal nuclease activity. Appl. Microbiol. 1971, *21*, 585—587.

McKee, J. J. & W. Braun: Effects of enzymatic digests of DNA on staphylococci. Proc. Soc. exp. Biol. (N. Y.) 1962, *109*, 166—170.

Niskanen, A. & L. Koiranen: Correlation of enterotoxin and thermonuclease production with some physiological and biochemical properties of staphylococcal strains isolated from different sources. J. Food Prot. 1977, *40*, 543—548.

Patton, S. & T. W. Keenan: The milk fat globule membrane. Biochim. biophys. Acta (Amst.) 1975, *415*, 273—309.

Sandvik, O.: En undersøkelse over karakteristiske hemolyseforhold innen den bovine mikrokokkflora. (Investigations on typical types of hemolysis within the bovine micrococcal flora). Nord. Vet.-Med. 1955, 7, 378—396.

Sandvik, O.: Studies on casein precipitating enzymes of aerobic and facultatively anaerobic bacteria. Thesis, Veterinary College of Norway, Oslo 1962, 116 pp.

Wadström, T. & R. Möllby: Studies on extracellular proteins from Staphylococcus aureus. VII. Studies on β-haemolysin. Biochim. biophys. Acta (Amst.) 1971, *242*, 308—320.

SAMMENDRAG

Stafylokokknuklease i jursekret fra kyr med akutt mastitt.

Høye konsentrasjoner av nuklease produsert av Staphylococcus aureus kan påvises i løpet av få timer ved en direkte undersøkelse av jursekretet fra kyr med alvorlig stafylokokkmastitt. Det er funnet en positiv korrelasjon mellom nukleasekonsentrasjonen og graden av sykdom. Kyr med store mengder nuklease i sekretet var vanligvis yngre dyr og/eller de befant seg i de to første ukene av laktasjonsperioden. De fleste av dem hadde et lavt titer av antistoffer mot stafylokokknuklease. Omtrent to tredjedeler av kyrne med høye nukleasekonsentrasjoner ble slaktet eller de døde på grunn av mastitt-tilfellet.

Betydningen av enzymet nuklease for virulensen av stafylokokker blir diskutert. En av konklusjonene er at enzymet nuklease bidrar til patogeniteten hos S. aureus.

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