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From the National Veterinary Institute, Oslo, Norway and Department of Epidemiology and Preventive Medicine, School of Veterinary Medicine, Davis, California.

SERIOUSNESS AND STABILITY OF SUBCLINICAL MASTITIS ASSESSED BY QUARTER MILK SERUM ALBUMIN

By

G. Bakken and *M. Thorburn*

BAKKEN, G. and M. THORBURN: *Seriousness and stability of subclinical mastitis assessed by quarter milk serum albumin concentration.* Acta vet. scand. 1985, *26*, 273—285. — Bovine Serum Albumin (BSA) concentration in quarter milk samples from 51 cows examined twice, at 1½ months intervals, was related to subclinical mastitis diagnoses and to the change in diagnoses from the first to the second examination. The BSA-concentration increased with increasing scores of the California Mastitis Test (CMT). The concentration of BSA was higher if bacteria were isolated as compared to negative bacteriological findings, and it was higher if "major pathogens" (MaP) (Staphylococcus aureus or Streptococcus dysgalactiae) rather than "minor pathogens" (MiP) (S. epidermidis or α-streptococci) were isolated. There was no interaction in the "effects" of CMT-score and bacteria on BSA-concentration. Quarters which were healthy and pathogen-free at first examination and had a non-specific mastitis at the subsequent examination, had significantly higher BSA-concentration at first examination than those which remained healthy. Quarters with non-specific mastitis at the first examination and infectious mastitis at the next examination had higher BSA-concentration at the first examinition than those which turned out with other diagnoses. Quarters with infectious mastitis and MaP at first examination which had a latent infection at the next testing, had a lower BSA-concentration than those with other second examination diagnosis. In general, BSA seems to be a more sensitive parameter than CMT for showing the early establishment of an inflammatory reaction in the alveolar tissue.

CMT; bacteria; diagnostic methods; prognosis; laboratory methods; bovine serum albumin; udder health; major pathogens; minor pathogens.

The diagnosis of bovine subclinical mastitis is normally based on examination of quarter milk samples. Several suggestions are published for bacteriological and cytological procedures and for

interpretations of the results (*Anon.* 1967, *Klastrup & Schmidt Madsen* 1974, *Neave* 1975, *Anon.* 1981). The reliability of the diagnosis in routine examination, as part of a control program, has been emphasized (*Giesecke & Viljoen* 1974, *Anon.* 1975). The diagnosed severity of the disease and the estimated frequency of spontaneous recovery are of great importance when considering medical treatment or other control procedures. Serum albumin in milk is considered to be a sensitive indicator of the degree of inflammatory epithelial irritation in the mammary gland (*Schalm et al.* 1971). In the present study, the concentration of serum albumin (BSA) was measured in samples collected from different types of subclinical mastitis and from healthy quarters. Two samples were collected from each quarter with an interval of 1½ month. The objective was to investigate possible diagnosis-related variation in BSA-concentration and to look for possible relationships between the BSA-concentration in the first sample and the diagnosis found at the second examination.

MATERIAL AND METHODS

Animals and sampling

The quarter samples originated from 51 cows examined twice with an average interval of 46 (± 2.9) days. The total number of quarter samples was 408. Strict foremilk samples were drawn after disinfecting the teat ends with a cotton swab soaked in 70 % ethanol. The samples were collected at varying times of the day, although never the first 2 h after milking. California Mastitis Test (CMT) and bacteriological examinations (*Klastrup & Schmidt Madsen* 1974) were performed prior to the end of the following day. CMT-scores were recorded from 1 (normal) to 5. The samples were frozen within 48 h and stored at —20°C. The BSA-examinations were performed within 2 months of sample collection.

The samples originated from a large number of samples collected for a prospective study on the efficiency of a mastitis control program. The first sampling was made between 3 and 263 days post partum, the average distance from partus being 73 (± 73) days and the median 38 days. Cows ranged from first to ninth lactation; the average lactation number was 3.5. The average milk production per day was 22.6 (± 3.2) kg at first examination and 22.6 (± 4.8) kg at second examination. Based

Table 1. Mastitis diagnoses based on CMT-scores and bacteriological examinations.

Diagnosis	CMT	Bacteria
Subclinical mastitis		
1. infectious mastitis, major pathogens (MaP)	3, 4, or 5	Staphylococcus aureus or Streptococcus dysgalactiae (MaP)
2. infections mastitis, minor pathogens (MiP)	4 or 5	S. epidermidis or alfa-streptococci (MiP)
3. non-specific mastitis	4 or 5	none
Latent infection	1 or 2	S. aureus or Str. dysgalactiae (= Map)
Healthy	1, 2, or 3	with or without growth of MiP

on the CMT-scores and the results of the bacteriological examinations, the quarter sample diagnoses are given in Table 1.

A total of 32 cows had subclinical mastitis (1 teat or more with infectious or non-specific mastitis) at the first examination. Different combinations of quarter diagnoses occurred within the same udder. The results from the first examination were not reported to the owners prior to the second sampling. The cows were not treated for mastitis between the first and second examination. A total of 11 cows had latent infections only (1 teat or more with latent infections, and no teats with subclinical mastitis) at the first examination.

Analytical methods

A single radial immunodiffusion test in 1 % agarose gel (Agarose immunodiffusion tablets, Bio-Rad Laboratories, cat.: 170—3002) was used for measuring the BSA-concentration. Anti Serum Albumin (Miles-Yeda Ltd, lot no R 722) was added to the agarose gel to a final concentration of 0.025 mg per ml agarose. Each plate was 10×10 cm and held 25 samples, 23 quarter samples and one "high" and one "low" control sample. The agar thicknes was 1.5 mm and the diameter of the wells 2.6 mm. Each well was inoculated with 8 microliters from thawed samples and incubated for 48 h at room temperature. The precipitation ring was measured using a graduated callipers. The recorded diameters were corrected according to the following formula:

$$BSA_C = BSA_O + (\bar{X}_L - BSA_L) \frac{(\bar{X}_H - \bar{X}_L) - (BSA_H - BSA_L)}{BSA_H - BSA_L} (BSA_O - BSA_L)$$

where

BSA_C = corrected BSA diameter
BSA_O = observed BSA diameter
BSA_L = observed diameter of the low BSA control
BSA_H = observed diameter of the high BSA control
$\bar{X}_L$ = mean of all observations of the low BSA control
$\bar{X}_H$ = mean of all observations of the high BSA control

It was important to establish whether the BSA levels in individual teats were influenced by the mastitis status of the other 3 teats in a given udder. For this reason, the sample correlation coefficient, r, was calculated between the BSA measurements of all quarters with CMT scores of 1 and the average CMT of the other three teats in individual udders.

Statistical mehods

A two-way analysis of variance (ANOVA) was performed on all quarter BSA measurements, regardless of examination number, to determine the effect of quarter CMT-score and bacterial diagnosis on BSA-concentration. The Bonferroni (*Neter & Wassermann* 1974) method of multiple comparisons was used to analyze specific factor effects.

Each first examination diagnosis was examined individually in order to determine if the BSA-concentration at first examination was related to the second examination diagnosis. This was done by performing separate one-way ANOVAs on each type of diagnoses from the first examination. When the analysis of variance indicated that at least 1 mean differed significantly, the Bonferroni method was used to find the specific differences.

RESULTS

The sample correlation coefficient between the BSA-measurements of quarters with CMT 1 and the average CMT-score of the other three quarters in the same udder was $r = -.0981$. This correlation is not significant ($.1 < P < .2$). It is thefore reasonable to assume that the BSA level in any given quarter is inde-

pendent of the mastitis status, as indicated by the CMT-scores, of the remaining quarters in the udder.

The distribution of the diagnoses at first and second examination is shown in Table 2. The per teat recovery rate, e.g. subclinical mastitis at first examination and healthy at second, varied from 71 % for α-streptococci to 0 % for Streptococcus dysgalactiae. Repetion of the diagnosis (stability) occurred in 75 % of the quarters with Staphylococcus aureus-mastitis and 80 % of the quarters with Str. dysgalactiae-mastitis. Corresponding figures for mastitis from which minor pathogens (MiP) (S. epidermidis or α-streptococci) were isolated, were zero.

The average diameter of the corrected BSA-precipitation zone was 12.0 (± 2.9) mm, ranging from 6.5 to 29.7 mm. The average

Table 2. Distribution[1] of quarter mastitis diagnoses at 2 subsequent examinations.[2]

Diagnosis first examination (quarters)	Diagnosis at second examination (quarters)								
	Subclinical mastitis				Non-specific mastitis	Latent infections		Healthy	Total
	Sa	Sd	Se	α-Str		Sa	Sd		
Subclinical mastitis									
S. aureus	12	0	0	0	0	2	0	2	16
(Sa)	(75)	(0)	(0)	(0)	(0)	(12.5)	(0)	(12.5)	
Str. dysgalactiae	0	4	0	0	0	0	1	0	5
(Sd)	(0)	(80)	(0)	(0)	(0)	(0)	(20)	(0)	
S. epidermidis	0	0	0	0	1	1	0	3	5
(Se)	(0)	(0)	(0)	(0)	(20)	(20)	(0)	(60)	
alfa-streptococcus	0	0	0	0	1	0	1	5	7
(α-Str.)	(0)	(0)	(0)	(0)	(14.3)	(0)	(14.3)	(71.4)	
Non-specific	5	2	0	1	0	1	0	7	16
mastitis	(31.3)	(12.5)	(0)	(6.3)	(0)	(6.3)	(0)	(43.8)	
Latent infections[3]									
S. aureus	4	0	0	1	1	2	0	9	17
	(23.5)	(0)	(0)	(5.9)	(5.9)	(11.8)	(0)	(52.9)	
Healthy	3	1	1	1	6	1	3	122	138
	(2.2)	(0.7)	(0.7)	(0.7)	(4.3)	(0.7)	(2.2)	(88.4)	

[1] Frequencies are listed in each cell; percentages of second examination diagnosis for the individual first examination diagnoses are listed in parentheses in each cell.

[2] The second examination followed the first by approximately 1½ months.

[3] There were no latent Str. dysgalactiae quarters cultured in the 1st examination.

zone diameters for BSA are classified by CMT-scores and bacterial isolation in Table 3. This table include all quarter samples from both examinations. The two-way ANOVA performed on the data indicated that there was no significant interaction ($.4 < P < .5$) between the "effects" of quarter CMT-scores and bacterial type of quarter BSA-concentrations. Both factors, however, have significant main effects ($P < .001$). The lack of interaction, or presence of "additivity", means that the effect of CMT-score on BSA concentration was constant over all bacteria types. Ad-

Table 3. Average concentration (and standard deviation) of bovine serum albumin (BSA) in quarter milk samples grouped by California Mastitis Test (CMT) scores and bacteriological culture results.

CMT-scores	Bacteria*					Grand mean for
	Sa	Str. d	α-Str	Se	none	CMT-score
1	12.4	11.4	11.8	11.6	10.7	10.9[3]
	(2.0)	(0.7)	(1.6)	(1.0)	(1.6)	(1.7)
	n=17	n=2	n=9	n=9	n=181	n=218
2	12.2	12.6	13.9	12.1	10.7	11.4[2 3]
	(2.2)	(1.5)	(3.1)	(2.2)	(1.6)	(2.0)
	n=7	n=3	n=3	n=7	n=27	n=47
3	13.4	15.0	11.4	12.1	11.9	12.3[2]
	(3.1)	(3.0)	(1.8)	(2.6)	(2.9)	(2.8)
	n=13	n=3	n=8	n=9	n=33	n=66
4	14.5	15.8	12.7	13.3	15.2	14.6[1]
	(1.6)	(3.2)	(3.0)	—	(4.9)	(3.7)
	n=16	n=6	n=7	n=1	n=18	n=48
5	17.2	14.2	17.5	13.4	15.7	15.9[1]
	(3.5)	(0.7)	(10.6)	(3.0)	(3.7)	(4.4)
	n=11	n=3	n=3	n=5	n=7	n=28
Grand mean	14.0[1]	14.3[1]	12.7[2]	12.2[2]	11.3[3]	
for bacteria	(2.9)	(2.7)	(3.8)	(2.1)	(2.6)	
	n=64	n=17	n=30	n=31	n=266	

* Sa = S. aureus
Str. d = Str. dysgalacteiae
Se = S. epidermidis
α-str. = alfa-streptococci

[1 2 3] Means which have the same number superscript are not significantly different (for a family confidence coefficient of $\alpha = 0.20$). For example the mean BSA concentration of all S. aureus positive quarters is not significantly different from that of all S. dysgalactiae quarters, but is different from those of the remaining bacterial classifications.

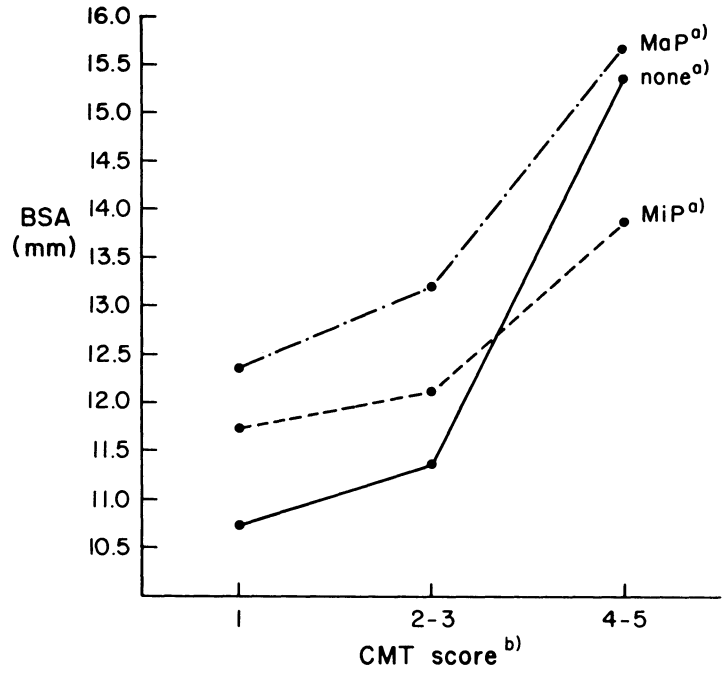
ditionally, the differential effect of bacteria type on BSA concentration was constant over all CMT-scores.

Table 3 shows the specific factor level differences, with a family confidence coefficient, $\alpha = .20$. The results can be summarized as follows:

1 The mean BSA-concentration of quarters with "none" bacteria was significantly lower than that of other quarters (bacteria isolated).
2 The mean BSA concentration for quarters with mastitis from which S. aureus or Str. dysgalactiae were isolated, were similar.
3 The mean BSA-concentration for quarters with mastitis from which S. epidermidis or α-streptococci were isolated, were similar.
4 The mean BSA-concentration of quarters infected by major pathogens (MaP) (S. aureus or Str. dysgalactiae) were significantly higher than of quarters infected by minor pathogens (MiP) (S. epidermidis) or α-streptococci).
5 The mean BSA-concentration of quarters with CMT-score of 4 and 5 were similar, but significantly higher than those of quarters with CMT-scores of 3 or less.

The findings from Table 3 are also presented in Fig. 1. In this figure the mean BSA-diameter for the three bacterial groups: none, MiP, and MaP are plotted for the three CMT-score groups: 1, 2 and 3, 4 and 5. This figure shows the increasing trend in the BSA-diameter as CMT-score increased. It also shows the consistently higher average BSA-diameter associated with quarters in which MaP were isolated. The relationship between CMT and quarters with MiP and between CMT and quarters with no bacteria ("none") appear, however, to contradict the lack-of-interaction results described earlier. At low CMT-scores (≤ 3) "none" quarters have the lowest average BSA-concentration, while a high CMT-scores (≥ 4) "none" quarters have higher average BSA-diameters than do MiP quarters.

Table 4 groups the mean BSA-concentrations for the different first examination diagnoses by the second examination diagnoses. It was necessary to group some diagnoses together in order to have an adequate sample size for the analysis. A one-way ANOVA was performed on the quarter samples in each of the following 6 first examination diagnoses: infectious mastitis with MaP;



a) none (——) = no bacteria
MiP (– – –) = minor pathogen (*Staphylococcus epidermidis* or alfa-streptococcus)
MaP (—·—) = major pathogen (*Staphylococcus aureus* or *Streptococcus dysgalactiae*)

b) The mean BSA for each of the 3 bacterial groups is compared at 3 levels of CMT: (i) CMT = 1; (ii) CMT = 2 or 3; (iii) CMT = 4 or 5.

Figure 1. Mean bovine serum albumin (BSA) concentrations (quarter) for CMT score-bacterial isolation combinations from first and second examinations.

infectious mastitis with MiP; non-specific mastitis; latent infections; healthy with MiP; and healthy with no pathogens.

In each analysis, the first examination BSA-concentration was the dependent variable, and the second examination diagnosis was the grouping or the independent variable. Four specific second examination diagnoses were considered: infectious mastitis with MaP or MiP; latent infection with MaP; non-specific mastitis; and healthy quarters. The purpose of this analysis was to determine if, for a given first examination quarter diagnosis, the BSA-concentration was related to a later (second examination) quarter condition. Such information provides an indication of the predictive, or prognostic value of BSA-measurements.

Table 4. Mean first examination quarter BSA concentrations classified by first and second examination quarter diagnosis: Means (and standard deviations).

1st examination diagnosis (quarters)	2nd examination diagnosis (quarters)			
	Infectious subclinical mastitis (MaP or MiP)	Non-specific mastitis	Latent infections	Healthy
Subclinical mastitis				
Major pathogens (MaP)**, a	14.5[1]	none	11.0[2]	18.5[1]
(Staphylococcus aureus or Streptococcus dysgalactiae)	(2.1)		(2.0)	(5.4)
Minor pathogens (MiP)	none	14.0	12.3	15.5
(S. epidermidis or α-streptococci)		(6.2)	(1.4)	(6.3)
Non specific mastitis**	16.5[1]	none	10.7	11.6[2]
	(2.7)		(0)	(2.8)
Latent infections	12.6	9.0[n]	11.8	12.2
	(1.4)	(0)	(3.2)	(2.2)
Healthy				
Minor pathogens	15.1[n]	12.8	none	12.1
	(0)	(3.6)		(2.4)
No pathogens*, a	11.1[1 2]	15.2[1]	11.1[1 2]	10.9[2]
	(1.5)	(3.3)	(0.6)	(1.6)

*, ** For the given 1st examination diagnosis, at least one mean BSA, grouped by 2nd examination diagnosis; differs (* = $P < .05$; ** = $P < .01$).

a Transformations were performed on the dependent variable (BSA) to achieve equal variances for analysis.

1, 2 Means which share the same numerical superscript do not differ significantly ($P > .1$). Means with superscript 1 are significantly larger than means with superscript 2 ($P < .05$). (Superscripts are only shown for 1st examination means where preliminary analysis indicated that at least one mean differed).

n These groups were excluded from any analysis due to a sample size, n=1.

For infectious mastitis with MiP, latent infection (MaP) or healthy quarters with MiP, the BSA-concentration at first examination did not differ significantly according to the diagnoses found at second examination. The ANOVA indicated, however, that the BSA-concentration for at least one of the diagnoses at second examination differed significantly for the following three first examination diagnoses: mastitis with MaP ($P < .01$); non-specific mastitis ($P < .01$); and healthy quarters with no patho-

gen ($.01 < P < .05$). Bonferroni tests performed on each of these three diagnoses gave the following results:

1 For infectious mastitis with MaP, the first examination BSA-concentration for quarters which subsequently were found to have a latent infection, was significantly lower than that of those quarters which still had mastitis ($.01 < P < .05$) or became healthy ($P < .01$). There was no significant difference ($P < .1$) in the mean BSA-concentration of the latter 2 groups.
2 For non-specific mastitis, the mean BSA-concentration of quarters which developed infectious mastitis (MaP or MiP) by the second examination was significantly higher ($P < .01$) than that of quarters which became healthy.
3 For healthy pathogen-free quarters, the mean BSA-concentration of quarters which had non-specific mastitis at second examination was significantly higher ($.01 < P < .05$) than that of those which remained healthy. However, the mean BSA-concentration of healthy quarters which had infectious mastitis or latent infection at second examination, did not differ significantly ($P > .1$) from either that of subsequent non-specific mastitis quarters or that of quarters which remained healthy. No difference was detected in the BSA-concentration of healthy MiP quarters which subsequently developed non-specific mastitis or remained healthy.

DISCUSSION

The results of Table 3 indicate that BSA-concentrations were related independently to CMT-scores and bacteria. This is also illustrated in Fig. 1. Specifically, for any given bacterial type, the mean BSA-concentration increased as the CMT-score increased. This effect was constant, statistically, over all bacterial types. The observed specific bacterial means in Table 3 do not follow this pattern, but such fluctuations are expected due to sampling variations.

Conversely, for any given CMT-score, the mean BSA-score was highest for quarters infected with MaP, next highest for those infected with MiP and lowest for quarters with "none" bacteria. Again, this effect was constant, statistically, over all CMT-scores. If, for instance, the CMT is 1, then the expected BSA-concentra-

tion on the basis of the CMT effect alone should be approximately 10.7 to 11.1 ($X \pm 1.96$) (standard error). However, the additive bacterial effect must also be considered. If the BSA-concentration is substantially greater than 10.9, it is quite likely that the quarter is infected by some pathogen. The higher the BSA-concentration, the more likely a MaP is infecting the udder.

Fig. 1, however, revealed a possible interaction effect between bacteria and CMT-scores, which, although not significant statistically, may be of important biological significance. The average BSA-diameter associated with non-specific mastitis was much higher than expected from the ANOVA results. Although this might be attributed to sampling variability, other influences may be important. These quarters may, for instance, have an infectious subclinical mastitis without shedding bacteria.

The results of Table 4 indicate that there were additional factors influencing the quarter BSA-concentration. For certain quarter diagnoses, the future condition (1—2 months later) of the quarter affected the present BSA-concentration. Thus, in the example above, a quarter with CMT 1 and a BSA-concentration substantially higher than 10.9 need not be infected by pathogens. It could, instead, be a pathogen free quarter which, as in Table 4, develops a non-specific mastitis in the subsequent month. It appears, in fact, that the elevation in the BSA-concentration of low CMT-score quarters is even more marked in pathogen-free quarters which later develop non-specific mastitis than it is in latent infected quarters.

From the results discussed above, it can be concluded that healthy quarters with high BSA-concentration have a higher tendency to develop a non-specific mastitis than those having a low BSA-content. Furthermore, animals with non-specific mastitis with high BSA-concentration end up with infectious mastitis more frequently than those with the other diagnoses.

Non-specific mastitis comprises a high proportion of all subclinical mastitis diagnoses (*Bakken* 1981). The stability of this diagnosis has been found to be low. The pathological significance of this condition has repeatedly been discussed in, for instance, the International Dairy Federation (*Klastrup* 1983), which establishes procedures for diagnosing subclinical mastitis. According to the prevailing results, the BSA-concentration will add valuable information for assessing the prospective condition of quarters having non-specific mastitis at a given time.

According to Table 4, BSA-concentrations in quarters with persistent infectious mastitis and in those becoming healthy did not differ. The quarters which turned from subclinical to latent infections had, on the other hand, a significantly lower BSA-concentration at the first examination. This finding supports that of *Giesecke & Viljoen* (1974) who claimed that a high percentage of subclinical mastitis judged by CMT and bacteria are "relevant teat canal infections". As far as theraphy is concerned, it is important to differentiate between persistent conditions and those recovering spontaneously.

The sample size in this study is too small to realistically attempt to define useful cut-off points for BSA-based diagnoses. Because of the relatively large number of CMT 1 (pathogen-free quarters) and the need to distribute the remaining quarter sample diagnoses over many categories, several of the standard errors in Table 3 are quite large. Therefore, it was not possible to statistically distinguish between specific CMT/bacteria combinations on the basis of BSA-concentration.

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SAMMENDRAG

Subklinisk mastitt hos ku. Varighet og alvorlighetsgrad vurdert ut fra melkeprøvens innhold av bovint serum-albumin.

Speneprøver fra 51 kyr uttatt med 1½ måneds mellomrom ble undersøkt med henblikk på mulig subklinisk mastitt og for innhold av bovint serumalbumin (BSA). Det var et økende innhold av BSA med økende celletall vurdert med California Mastitis Test (CMT). BSA-innholdet i bakteriologisk positive prøver var høyere enn tilsvarende i bakteriologisk negative prøver. I prøver hvorfra Staphylococcus aureus eller Streptococcus dysgalactiae ble isolert, fantes høyere innhold av BSA sammenlignet med prøver hvorfra S. epidermidis eller α-streptococcer ble isolert. Det var ingen samspilleffekt mellom CMT-resultatet og bakterietype på BSA-konsentrasjonen.

BSA ved første undersøkelse for kjertler som var friske både ved første og andre gangs undersøkelse, var lavere sammenlignet med tilsvarende kjertler som var friske første gang og som andre gang hadde mastitt uten funn av bakterier (mastitt). BSA ved første undersøkelse for kjertler som første gang hadde mastitt og andre gang hadde infeksiøs mastitt, var høyere enn tilsvarende for kjertler som første gang hadde mastitt og som andre gang hadde andre diagnoser enn infeksiøs mastitt.

Generelt synes BSA å være et parameter som avdekker en betennelsestilstand i juret på et tidlig tidspunkt.

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Reprints may be requested from: G. Bakken, the National Veterinary Institute, P. O. Box 8156, Dep., N-0033 Oslo 1, Norway.