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ANTIBODIES AGAINST STAPHYLOCOCCUS AUREUS NUCLEASE IN BULK MILK AS AN INDICATOR OF MASTITIS IN DAIRY HERDS

By

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GUDDING, R.: *Antibodies against Staphylococcus aureus nuclease in bulk milk as an indicator of mastitis in dairy herds.* Acta vet. scand. 1980, *21*, 96—107. — Titres of staphylococcal antinucleases and cell counts in bulk milk samples were compared as indicative criteria of the mastitis situation in dairy herds. The correlation coefficients between the prevalence rate of mastitis and the antinuclease titre and cell count, respectively, were of the same order of magnitude. The incidence of clinical mastitis showed a better correlation to the antinuclease titre than to the cell count. When cell counts and antinuclease titres were combined a more reliable selection of herds with a possible mastitis problem was achieved. The antinuclease test is consequently recommended as a supplement to cell counting when screening bulk milk.

antinucleases; cell count; bovine mastitis.

The selection of herds with a possible mastitis problem is generally based on an examination of components or properties of the milk which reflect differences between normal and pathological mammary secretions. The somatic cell count is recognized as the most accurate criterion for monitoring mastitis levels in dairy herds, and cell counting performed either by direct microscopy (*Prescott & Breed* 1910), electronic particle counting (*Tolle et al.* 1966) or fluoro-opto-electronic cell counting (*Schmidt Madsen* 1975) is applied world-wide in mastitis control programmes.

According to *Sandvik* (1975) antibodies against the nuclease of Staphylococcus aureus are present in milk from individual cows, probably as a result of an on-going or previous staphylococcal mastitis or infection/inflammation in other tissues. Antibodies were even demonstrated in samples of bulk milk.

Since S. aureus is the most frequent organism causing bovine mastitis in Norway, it was reasonable to assume that the titre of antibodies against this organism or its extracellular products in bulk milk might provide information as to the udder health of dairy herds in general and the level of staphylococcal infection in particular. A convenient method for the demonstration of antibodies against staphylococcal nuclease in series of samples has recently been developed (*Gudding* 1977). The aim of the present work was to compare the antinuclease titres and the cell counts in bulk milk as criteria for the selection of herds with a possible mastitis problem.

MATERIALS AND METHODS

Milk samples

The material comprised samples of bulk milk and quarter milk from 20 dairy districts subjected to routine examination as part of a mastitis control programme. The bulk milk samples originated from more than 6000 dairy herds during the period November 1975 to May 1978. Individual quarter milk samples were collected for bacteriological and cytological examinations from 1350 of these herds. The samples were generally collected on the farm and transported to the laboratory without refrigeration. Transport time was usually less than 24 h.

Examination of bulk milk

The routine for examination of bulk milk samples included somatic cell count and examination to demonstrate the possible presence of Streptococcus agalactiae and antibacterial substances.

Cell counts were carried out on formalin fixed samples in an electronic particle counter (Coulter Counter Fn and/or Coulter Milk Cell Counter) according to the manufacturer's instructions. About 20 % of the cell counts were performed at the laboratory of A/L Fellesmeieriet, Oslo, the remainder being carried out at the National Veterinary Institute, Oslo. Instruments at the 2 laboratories were intercalibrated at monthly intervals. Altogether, results of 49,872 bulk milk sample cell counts are included.

An agar diffusion method was used for the determination of antibodies against S. aureus nuclease (*Gudding* 1977). The antibody titre was determined in non-fixed and non-preserved samples. The results are expressed as the zone diameter minus the

well diameter as well as the number of diffusion units (*Gudding*). The antinuclease titre was recorded in 19,397 samples.

Examination of quarter milk

Routine examination of quarter samples from herds and the evaluation of results were performed as described by *Klastrup & Schmidt Madsen* (1974).

The udder health of the herds was classified according to the percentage of cows and quarters with infectious mastitis (CMT $\geqq 3$, and the presence of udder pathogenic microorganisms), infectious mastitis caused by S. aureus (hereafter called S. aureus mastitis; CMT $\geqq 3$, and the presence of S. aureus), non-specific mastitis (CMT $\geqq 3$, no demonstration of udder pathogenic bacteria) and latent infection with S. aureus (hereafter called S. aureus latent infection; CMT $\leqq 2$, and the presence of S. aureus).

Incidence of clinical mastitis

Cases of clinical mastitis are routinely recorded on the farm by veterinarians as part of the herd health card system currently in use in Norway. Data were retrieved from this system and used to calculate the herd incidence of mastitis (percentage of cows in the herd with clinical mastitis during a period of 1 year).

Data processing

Data analyses and all calculations were carried out using a computer. Average cell counts are presented as the geometric mean. Analyses comparing data from bulk milk and quarter milk examinations were performed on results obtained within a maximum interval of 180 days. Results are generally given from analyses of bulk milk samples collected 0 to 180 days before the corresponding quarter milk examinations. The health card data concerned the period September 1, 1975 to August 31, 1977, and were compared with laboratory results obtained in the same period.

RESULTS

The average cell count of the bulk milk samples, calculated as the arithmetic and geometric mean, was 350,000 cells per ml and 261,000 per ml, respectively. The antinuclease titre was on

Table 1. Correlation between cell count and antinuclease titre in 19,360 bulk milk samples.

Variables		Correlation coefficient	Level of significance
Zone diameter	Cell count	0.21	$P < 0.001$
Zone diameter	Logarithm of cell count	0.28	$P < 0.001$
Diffusion units	Cell count	0.21	$P < 0.001$
Diffusion units	Logarithm of cell count	0.25	$P < 0.001$

average 2.6 diffusion units per 0.1 ml, corresponding to a zone diameter in the antinuclease test of 5.5 mm.

As seen in Table 1, the correlation coefficients between the cell counts and the antinuclease titres were statistically significant. The zone diameter and the logarithm of the cell count showed the best correlation, and these 2 parameters were therefore used in subsequent calculations.

The correlation coefficients of zone diameter versus the logarithm of the cell count calculated for each dairy district varied from 0.16 to 0.46. For all but 1 district the level of significance was $P < 0.001$.

The frequency of samples with an antinuclease titre producing zone diameters of at least 6.0 mm and 7.0 mm was 30.1 and 12.4 %, respectively (Table 2).

Table 2. Average cell counts in relation to antinuclease titre in 19,360 bulk milk samples.

Antinuclease titre		Frequency %	Average cell count (geometric mean) (1000 per ml)
zone diameter (mm)	diffusion units (per 0.1 ml)		
0	0	42.7	219
4.0 and 4.5	1.0 and 1.4	9.4	244
5.0 and 5.5	1.9 and 2.6	17.6	290
6.0 and 6.5	3.6 and 5.0	17.7	336
7.0 and 7.5	7.0 and 9.6	8.8	422
8.0 and 8.5	13.8 and 18.3	2.5	458
9.0 and 9.5	25.4 and 35.0	1.1	497

The prevalence rate of infectious mastitis, S. aureus mastitis, non-specific mastitis and S. aureus latent infection is presented in Table 3. On average, 21.9 % and 9.3 % of the cows were as-

Table 3. Prevalence rate of cows and quarters with mastitis and udder infections based on the examination of quarter milk samples from 3670 herds.

Diagnosis	Percentage	
	cows	quarters
Infectious mastitis	21.9	7.3
Staphylococcus aureus mastitis	9.3	3.0
Non-specific mastitis	14.4	4.9
Staphylococcus aureus latent infection	5.2	1.7

signed a diagnosis of infectious mastitis and S. aureus mastitis, respectively. The overall average incidence of clinical mastitis based on the health card data was found to be 13.0 %.

Table 4 presents the correlation coefficients of the results from the examination of bulk milk and quarter milk, respectively. As well as the percentage of affected cows, the herd percentage of affected quarters was also used when calculating correlation coefficients. There were no statistically significant differences between the following correlation coefficients: a) corresponding coefficients for the same parameters examined at different periods (bulk milk analyses performed in the period 0 to 180 days before and after the quarter milk examinations, re-

Table 4. Correlation coefficients between the prevalence rate of mastitis/udder infections and the antinuclease titre (957 herds) and the cell content (3187 herds) of bulk milk, respectively*.

Diagnosis	Correlation coefficients and level of significance			
	antinuclease titre (zone diameter)		cell content (log cell count)	
Infectious mastitis (% of cows)	$r = 0.23$	$P < 0.001$	$r = 0.24$	$P < 0.001$
Staphylococcus aureus mastitis (% of cows)	$r = 0.29$	$P < 0.001$	$r = 0.24$	$P < 0.001$
Non-specific mastitis (% of cows)	$r = 0.06$	$P < 0.05$	$r = 0.06$	$P < 0.05$
Staphylococcus aureus latent infection (% of cows)	$r = 0.17$	$P < 0.001$	$r = 0.20$	$P < 0.001$

* The bulk milk analyses were performed 0 to 180 days before the quarter milk examinations.

spectively, and bulk milk analyses performed in the period 90 days before to 90 days after the quarter milk examinations), b) coefficients for cell count versus a chosen parameter for udder health and coefficients for antinuclease versus the same parameter at corresponding periods and c) corresponding coefficients for prevalence rate of mastitis in cows and quarters, respectively.

Correlation between the incidence of clinical mastitis and antinuclease titre (zone diameter) was poor, and that between the former and the logarithm of the cell count even poorer, the coefficients being 0.15 and 0.04, respectively. However, due to the large number of observations, these coefficients and the difference between them were found to be statistically significant.

Table 5. Prevalence rate of infectious mastitis (IM) and Staphylococcus aureus mastitis (SM) and incidence rate of clinical mastitis (CM) in herds in relation to bulk milk cell count and antinuclease titre, respectively*.

Antinuclease titre (zone diameter in mm)	Cell count in 1000 per ml											
	0—200			201—400			401—700			≧ 701		
	IM	SM	CM	IM	SM	CM	IM	SM	CM	IM	SM	CM
0	13.8**	4.9**	10.8**	17.5	5.6	10.7	22.3	8.1	10.2	17.6	4.9	11.1
	n=16***		n=1619	n=128		n=1122	n=39		n=354	n=14		n=102
4.0—5.5	17.5	9.4	13.4	21.7	11.3	13.5	26.4	13.6	13.3	29.7	8.0	14.5
	n=94		n=794	n=120		n=938	n=50		n=375	n=33		n=113
6.0—7.5	21.8	11.4	15.1	23.4	13.0	14.8	27.4	14.2	15.6	34.1	24.0	16.0
	n=64		n=559	n=105		n=932	n=73		n=554	n=47		n=275
8.0—9.5	30.6	13.8	13.9	25.0	13.6	17.4	30.8	21.3	14.6	35.4	24.1	16.9
	n=5		n=50	n=17		n=112	n=10		n=104	n=12		n=50

* The bulk milk analyses were performed 0 to 180 days before the quarter milk examinations.
** Percentage of cows.
*** Number of herds.

When the correlation coefficients were calculated for each dairy district a great variety in the results was observed. The highest coefficients with a significance level $P < 0.05$ for the antinuclease titre versus the prevalence rate of infectious mastitis and S. aureus mastitis and the incidence rate of clinical mastitis were 0.73 ($n = 11$), 0.49 ($n = 36$) and 0.33 ($n = 135$),

respectively. The corresponding coefficients for the cell count versus the same parameters were 0.49 (n = 39), 0.75 (n = 8) and 0.17 (n = 233), respectively. The lowest coefficients for all parameters were close to zero.

The prevalence of mastitis/udder infections and the incidence of clinical mastitis in relation to different bulk milk cell counts and antinuclease titres are presented in Tables 5 and 6. The prevalence rate of infectious mastitis and S. aureus mastitis was generally highest in herds with high cell counts and high antinuclease titres in the bulk milk. The incidence of clinical masti-

Table 6. Prevalence rate of mastitis/udder infections and incidence rate of clinical mastitis in herds grouped according to high/low cell count and high/low antinuclease titre in the bulk milk.

	Cell count (1000 per ml) and antinuclease titre (zone diameter in mm)							
Diagnosis	< 500	< 7.0	< 500	≧7.0	≧ 500	< 7.0	≧ 500	≧7.0
Infectious mastitis	19.0*	(n=675)**	23.2	(n=76)	28.0	(n=147)	33.2	(n=59)
Staphylococcus aureus mastitis	8.7*	(n=675)	12.3	(n=76)	13.3	(n=147)	21.3	(n=59)
Non-specific mastitis	11.1*	(n=675)	14.1	(n=76)	11.4	(n=147)	16.8	(n=59)
Staphylococcus aureus latent infection	7.2*	(n=675)	9.5	(n=76)	13.4	(n=147)	13.6	(n=59)
Clinical mastitis	12.4*	(n=5851)	15.7	(n=679)	13.1	(n=1023)	16.0	(n=401)

* Percentage of cows.
** Number of herds.

tis increased more in relation to higher antinuclease titres than to increasing cell counts.

The prevalence rate of latent infection with S. aureus showed a similar pattern of distribution to that of infectious mastitis and S. aureus mastitis. The same tendency could also be observed for non-specific mastitis, but the differences in prevalence rates between high and low cell counts and high and low antinuclease titres, were generally smaller than for infectious mastitis and S. aureus mastitis.

According to Table 7, 63 % of the herds with a high antinuclease titre (zone diameter ≧ 7.0 mm) and 36 % of the herds with a low antinuclease titre (zone diameter < 7.0 mm) had a

Table 7. Percentage of herds with a high mastitis prevalence rate at low and high bulk milk cell counts and/or antinuclease titre, respectively.

Bulk milk parameter	Percentage of herds	
	infectious mastitis prevalence rate $\geqq$ 20	Staphylococcus aureus mastitis prevalence rate $\geqq$ 10
Cell count (1000 per ml) $<$ 500	48 (n=2155)*	37 (n=1637)
Cell count (1000 per ml) $\geqq$ 500	68 (n= 570)	52 (n= 440)
Antinuclease titre (zone diameter) $<$ 7.0 mm	46 (n= 588)	36 (n= 463)
Antinuclease titre (zone diameter) $\geqq$ 7.0 mm	65 (n= 135)	63 (n= 131)
Cell count (1000 per ml) $<$ 500 and Antinuclease titre (zone diameter) $<$ 7.0 mm	43 (n= 470)	35 (n= 234)
Cell count (1000 per ml) $\geqq$ 500 and Antinuclease titre (zone diameter) $\geqq$ 7.0 mm	78 (n= 65)	75 (n= 44)

* Number of herds.

prevalence rate of S. aureus mastitis of $\geqq$ 10 %. The difference between these 2 figures (27 %) was the largest found when 1 single parameter of bulk milk analyses was applied for screening udder health. When the cell count results were combined with the results of the immunological analyses, the corresponding difference increased to 40 %.

It took a technician about 30 min to analyse 100 bulk milk samples, both with regard to cell count and the antinuclease test. The cell counting in this study was performed using an automatic milk cell counter connected to a printer, but without technical equipment for direct transfer of results to a computer.

DISCUSSION

The antinuclease test is a simple agar diffusion test which can be used for the determination of the titre of antibodies against staphylococcal nuclease in biological fluids, including milk. The present study has shown that the titre of staphylococcal antinucleases in samples of bulk milk can provide information regarding the udder health of dairy herds. The reliability of this information as a true indicator of udder health in a herd is about the same as that of cell counting.

In studies of the relationship between cell counts and udder health, correlation coefficients in the range $r = 0.50$ to 0.87 have been documented (*Tolle et al.* 1969, *Postle et al.* 1971 and *Pearson & Greer* 1974). The cell counts found in the present study

did not, however, correlate so well to the herd infection level. This discrepancy may be explained by several factors. In contrast to the above-mentioned investigations, the present study was based entirely on milk samples taken for routine examination. Consequently, samples of bulk milk and quarter milk were not collected at the same time. Sample collection by different individuals and transport of non-refrigerated samples may also partly explain the deviation in results obtained. The fact that approx. 15 % of the farmers still deliver milk in churns (not bulk tank) also contributes to the heterogeneity of the material. The fact that there was a relatively large difference (39 %) between the arithmetic and geometric mean and also that the logarithm of the cell count was the best estimate for the cell content of bulk milk, indicates a substantial scatter of the results in individual herds. However, it should be emphasized that the large number of observations gave a high statistical significance to the present results. Most previous studies are based on data from comparatively few herds, and significance levels are not reported.

The correlation coefficients between the antinuclease titre and the prevalence rate of mastitis are also low. However, a comparison with the coefficients for cell count reveals the antinuclease titre of bulk milk to be as equally reliable an indication of the mastitis status in dairy herds as the cell count.

Samples for cell counting and the antinuclease test were always collected simultaneously, and so the reservations taken with regard to sample collection and transport should apply in both cases.

Moreover, samples subjected to immunological testing did not contain any preservative, whereas formalin was added to the samples for cell counting.

The great variation in correlation coefficients found with regard to the various dairy districts confirms the heterogeneity of the material. However, the high degree of correlation found between parameters of udder health from bulk milk and quarter milk in some districts is another indication that the antinuclease titre is as reliable as cell count as a measure of the mastitis status.

The prevalence rate of non-specific mastitis was generally poorly correlated to the cell count and the antinuclease titre. Bulk milk and quarter milk samples were not taken at the same

time, and the low degree of correlation would therefore seem to indicate that non-specific mastitis is of a more fluctuating or intermittent nature than infectious mastitis.

The low average correlation coefficient between the cell count and the incidence rate, even though statistically significant, does not reflect the real relationship between these 2 parameters. When grouped into dairy districts, the highest coefficient was found to be 0.17. This figure is a strong indication that bulk milk cell counts do not reflect the level of clinical mastitis as well as they do the level of subclinical mastitis. The antinuclease titre seems to be better to reflect the herd situation as regards clinical mastitis, as both the average and the highest correlation coefficients were significantly higher than the corresponding coefficients for cell count.

The results of bulk milk analyses primarily give a picture of the level of infection in the dairy herd. However, as the farmers are usually more interested in the incidence rate of the disease, a screening method which also takes clinical mastitis into account would be advantageous.

The figures in Table 5 show that, by combining the results of cell counting and the antinuclease test, it is possible to select herds with a considerably higher prevalence/incidence or more herds with a certain prevalence/incidence than when applying 1 bulk milk test alone.

As an example, a limit of 0.7 mill. cells per ml selects 11.1 % of the herds with an average prevalence rate of infectious mastitis of 30.7 %. If the group of herds with the highest antinuclease titre (zone diameter $\geqq 8.0$) or even herds with a cell count > 0.4 mill. per ml and zone diameter $\geqq 6.0$ were included, the prevalence rate of infectious mastitis would be 30.0 % and 29.1 %, and the percentage of herds selected 14.4 % and 22.0 %, respectively.

According to Table 7, 48 % of the herds with a cell count below 0.5 mill. per ml had a prevalence rate of infectious mastitis of 20 or more ("false negatives"). The percentage of "false positive" results (cell count $\geqq 0.5$ mill. per ml and prevalence rate < 20) was found to be 32. As can be seen from the table a combination of the cell count results and the results of the antinuclease test give a reduction in the number of "false positives" and "false negatives" when selecting herds with a possible mastitis problem.

A comparison of practical, technical and especially the economical aspects of the 2 methods comes out in favour of the antinuclease test. This method is inexpensive both with regard to equipment and reagents, and is reasonably quick and simple to perform. The critical points and sources of error are the addition of enzyme to the agar and the storage of the plates. Consequently, these procedures should be highly standardized (*Gudding* 1977). Another drawback is the lack of suitable standard antinucleases.

Though the results of the present work seem promising, the antinuclease test is not considered to be an alternative to cell counting in laboratories which have already developed automatic or semi-automatic routines. However, the test can be used as a supplement to cell counting to give a more reliable selection of herds with a possible mastitis problem. The test may also be a useful tool in mastitis control in regions where S. aureus is frequently isolated from mastitis samples and where the screening of mastitis herds is either based on laborious methods such as direct microscopy or on inaccurate, indirect cell counting methods, or where no system for mastitis screening of bulk milk has been established at all.

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SAMMENDRAG

Antistoffer mot Staphylococcus aureus-nuklease i leverandørmelk som indikator på mastitt i storfebesetninger.

Titeret av antistoffer mot Staphylococcus aureus-nuklease og innholdet av celler i leverandørmelkprøver er sammenlignet som kriterier for mastitt i storfebesetninger. Korrelasjonskoeffisienten mellom forekomsten av mastitt og henholdsvis antinukleasetiteret og celletallet var av samme størrelsesorden. Frekvensen av klinisk mastitt var bedre korrelert til antinukleasetiteret enn til celleinnholdet.

Ved å kombinere celletallet og antinukleasetiteret oppnås en mer pålitelig utvelgelse av besetninger med et mulig mastittproblem, og antinukleasetesten er derfor anbefalt som et tillegg til celletelling av leverandørmelk.

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