

Diagnosis of *Staphylococcus aureus* intramammary infections by detecting antibodies in milk with an ELISA kit

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(Received 14 November 1991; accepted 30 January 1992)

Summary — An enzyme-linked immunosorbent assay (ELISA) for detecting *Staphylococcus aureus* antibodies in bovine milk samples and developed to differentiate infected from uninfected cows was evaluated by comparison with bacteriological examination. Thirty-two lactating cows from 3 dairy herds were selected on the basis of the pathogens isolated from their milk with confirmed culture at a second sampling. When compared to culture results considered as the reference, the accuracy of the ELISA test was 87.5% and showed an agreement of 89.5% and 84.6% for negative and positive samples respectively. For *S aureus* infected cows, there was no correlation between somatic cell counts and reactivity of composite milk samples to the exoantigens used in the ELISA. Neither was there any apparent relationship between numbers of infected quarters per cow and optical density values.

Staphylococcus aureus / mastitis / ELISA

Résumé — Diagnostic des infections mammaires à *Staphylococcus aureus* par détection des anticorps dans le lait à l'aide d'un kit ELISA. Un test ELISA, basé sur la détection d'anticorps dans le lait, et développé pour identifier les vaches ayant des infections mammaires à *Staphylococcus aureus*, a été évalué par comparaison au diagnostic bactériologique. En prenant en considération les résultats des analyses bactériologiques, 32 vaches provenant de 3 troupeaux laitiers ont été retenues pour cette étude. Estimée par rapport aux cultures bactériologiques, l'exactitude du test ELISA est de 87,5% avec une concordance de 89,5% et 84,6% respectivement pour les échantillons de lait négatifs ou positifs. Aucune corrélation n'a pu être établie entre les résultats du test ELISA et la numération des cellules somatiques des vaches infectées par *S aureus* ou le nombre de quartiers infectés par vache.

Staphylococcus aureus / mammite / ELISA

INTRODUCTION

Subclinical mastitis can affect quarters up to 50 times more than clinical mastitis (Bramley and Dodd, 1984). *Staphylococcus aureus* is the most common pathogen causing bovine chronic subclinical mastitis. The primary sources of this pathogen

are infected mammary glands, teat lesions and colonised teat ducts. Bacteria are readily transferred between teats and cows during milking. *S aureus* cannot be eradicated from the herd by anti-microbial therapy (Le Louedec, 1978; Dodd, 1983). Consequently, culling and segregation of infected cows are useful to control bacte-

rial transmission and to eradicate *S aureus* from a dairy herd. Specific detection methods are needed to optimize these control measures. The culture and bacterial examination of milk samples to identify the microbial pathogen is slow and costly. An additional problem of bacterial culture is the requirement of an aseptically collected milk sample. Recently, an enzyme-linked immunosorbent assay (ELISA) was developed to identify cows with *S aureus* intramammary infections by detecting specific antibody to staphylococcal exoproteins in milk (Adams *et al*, 1988). The ELISA kit can be used on unpreserved or chemically preserved milk and does not require an aseptic collection of milk. Results can be obtained in about 1 h by visual comparison with both negative and positive controls, or alternatively a spectrophotometric ELISA plate reader can be used.

The work presented here describes a preliminary evaluation of the ability of the commercial kit to differentiate *S aureus* infected cows from uninfected cows by comparing bacteriological results and ELISA.

MATERIALS AND METHODS

Herds and milk samples

Foremilk samples from all quarters were collected aseptically to determine existing intramammary infections in 3 herds consisting of 190 lactating cows. To avoid collecting samples containing blood-derived *S aureus* antibodies from cows which did not have active intramammary infections, only cows which had exceeded 30 days lactation after calving were included in this study (Adams *et al*, 1988). Additional foremilk samples were collected aseptically 1–3 weeks later and 32 cows were selected on the basis of the confirmation of the presence of previous isolated microorganisms.

Somatic cell counts (SCC) and ELISA were performed on composite foremilk samples ob-

tained by mixing 0.8 ml of each quarter sample from each cow.

Bacteriological examination and somatic cell counts

A 0.025-ml sample of each quarter milk sample was plated on aesculin sheep blood agar medium. All the cultures were incubated aerobically for 24–48 h at 37 °C before being examined for the identification of bacterial colonies. SCC were determined by use of a Coulter counter as recommended by the International Dairy Federation (1984).

Enzyme-linked immunosorbent assay

The ELISA was performed as indicated in the manufacturer's instructions (ProStaph I, ProScience Corp, Sterling VA, USA).

Each composite sample and positive and negative controls were assayed in duplicate. The optical density (OD) at 405 nm was read with an automated microplate reader. A positive ELISA result was defined as any OD equal to or greater than the positive control. A sample with an OD value less than 85% of the positive control was classified negative, and suspect when the OD values was 85–100% of the positive control.

RESULTS

According to bacterial culture results, samples were divided into groups lettered from A to E (table I).

Composite samples from cows with either non infected quarters (group A) or quarters infected by coagulase-negative staphylococci (group B) had the lowest values for SCC and ELISA tests. For cows with one or more quarters infected by *S aureus* (groups D and E), there was no correlation ($r = 0.16$) between SCC and ELISA values.

Table I. Relationship between either infection status of the mammary gland or somatic cell counts and ELISA values.*Relations entre le statut infectieux des mamelles ou le nombre de cellules somatiques du lait avec les valeurs ELISA.*

Groups	No of cows	Pathogens isolated ^a	Somatic cell counts ^b (x 10 ³ /ml)		ELISA ^b (OD)	
			Mean	Range	Mean	Range
A	6	None	191	160 – 263	0.103	0.030 – 0.314
B	7	Staph coagulase negative	272	180 – 313	0.115	0.047 – 0.404
C	6	Major pathogens ^c except <i>S aureus</i>	1 362	316 – 2 883	0.308	0.062 – 0.550
D	8	<i>S aureus</i> + <i>C bovis</i> ^d	2 640	519 – 5 146	0.537	0.107 – 1.102
E	5	<i>S aureus</i> + <i>Str uberis</i>	3 764	3 095 – 5 554	0.883	0.490 – 1.934

^a Pathogens isolated from the different milk quarter samples of each cow. ^b Values recorded from composite samples from each cow. Value for positive control in ELISA was 0.397. ^c Major pathogens isolated were *E coli*, *Str uberis*, *Enterococcus faecalis*, *Erterococcus faecium*, *Str bovis*. ^d Only one cow without *C bovis* infection.

Comparison between culture and ELISA results is shown in table II. None of the samples was classified as suspect by ELISA test. An agreement of 89.5% and

Table II. Comparison of ELISA reactivity and bacteriological results.*Comparaison entre les résultats obtenus en ELISA et les cultures bactériologiques.*

<i>S aureus</i> culture ^a	ELISA ^b classification (No of samples)		
	Positive	Negative	Total
Positive	11	2	13
Negative	2	17	19

^a Culture of quarter milk samples. ^b ELISA for milk antibody to *S aureus* was performed on composite milk samples.

84.6% was recorded for culture negative samples and culture positive samples respectively. Two quarter milk samples were found positive by culture for *S aureus* and the corresponding composite cow milk samples negative by ELISA for antibody. The SCC values for these samples were 519 000 and 1 906 000/ml. Of the 2 samples from cows infected with an organism other than *S aureus* classified positive on the ELISA, SCC's were 393 000 and 2 883 000/ml. Culture results on these samples were respectively 2 coagulase-negative staphylococci (2 quarters infected) and 1 *Escherichia coli* with 3 *Corynebacterium bovis* (4 quarters infected). As indicated in table III, there was no apparent relationship between the number of quarters infected by *S aureus* for each cow and the OD values recorded for composite samples in the ELISA test.

Table III. Relationship between the number of quarters infected by *S aureus* and the ELISA reactivity of composite milk samples.

Relation entre le nombre de quartiers infectés par S aureus et les valeurs obtenues en ELISA sur le lait de mélange de chaque vache.

No of quarters per cow	No of infected cows	Optical density (mean)
1	10	0.609
2	1	1.934
3	2	0.615

DISCUSSION

The accuracy of the ELISA test used herein, defined as the ability of the assay to give correct results relative to bacterial culture, was 87.5%. The specificity and the sensitivity which are the ability of the ELISA test to identify true negative and true positive samples was 89.5% and 84.6% respectively. These percentages were slightly less than those previously reported (Matsushita *et al*, 1990).

The 2 ELISA false negative reactions found in the 13 *S aureus* culture positive samples may indicate either infections not involving the gland itself but restricted to the teat canal or existence of infections which had not been present long enough to promote detectable antibody. As these samples remained culture positive upon resampling and had SCC of over 500 000/ml, the first hypothesis can reasonably be set aside.

Although most intramammary infections are persistent and spontaneous cure is not frequent (Rainard and Poutrel, 1982), the 2 samples which were found to be negative for culture of *S aureus* but positive by ELISA could be explained by intermittent shedding of *S aureus* or presence of anti-

bodies resulting from a previous spontaneously-cured staphylococcal infection.

Also false positive results in ELISA may have resulted from either cross-reactivity with antigens of other microorganisms or leakage of antibodies into the milk compartment promoted by inflammation. Indeed, these samples were cultured positive for coagulase-negative staphylococci and *Escherichia coli* associated with *Corynebacterium bovis*. Lack of reactivity to the *S aureus* antigens of milk samples from most cows infected by coagulase-negative staphylococci (group B) and other major pathogens (group C) is in favour of the specificity to exoantigens selected in the ELISA test (Adams *et al*, 1988). The exudation of immunoglobulins from the blood into the milk compartment which occurs during acute infections, for example *E coli* and *Streptococcus uberis* infections, could explain the presence in the milk of naturally acquired antibodies to *S aureus* antigens. The limited number of samples with this type of infection does not allow us to draw a conclusion on this point. However, the lack of correlation between SCC and OD values previously reported for infections caused by microorganisms other than *S aureus* (Adams *et al*, 1988) and confirmed in this study for staphylococcal infections suggests that inflammation alone is not likely to be a major cause of false positive reactions in the ELISA test and that antibodies to *S aureus* exoantigens may be mainly produced in the mammary gland.

Most of the staphylococcal infections were correctly diagnosed from composite milk samples although the majority of *S aureus* infections were established solely in one quarter of each cow, as shown in table III. This and the lack of correlation between the OD values in ELISA recorded on composite milk samples and the number of quarters from each cow infected by

S aureus indicate that the limit of detection of the ELISA test used was good enough to be apparently unaffected by dilution of antibodies.

The diagnosis of subclinical mastitis is usually based on identifying pathogens isolated from aseptically taken milk samples, and by measuring the effects of the inflammation on the milk. Errors occur because pathogens are not always isolated from infected quarters, milk samples can be contaminated, and the extent of the inflammation is variable. Concerning *S aureus* infections, our results confirm that the disagreement between bacterial culture and ELISA antibody test is minimal, as previously reported (Matsushita *et al*, 1990). Taking into account the different causes of false negative and false positive results, it is likely that the number of discordant results should be lowered by additional testing.

In conclusion, the ELISA test used in this study is accurate in differentiating *S aureus*-infected from uninfected cows. The test is fast and can be performed either on chemically preserved or fresh milk and does not require aseptic collection. It is therefore suitable for the screening of large numbers of samples and offers a useful diagnostic tool to the dairy farmer and the veterinarian for implementing a mastitis control strategy.

ACKNOWLEDGMENTS

This work was supported by Laboratoire Sogeval (Laval, France). The authors wish to thank M Ducelliez for technical assistance and P Rainard for his critical review of the manuscript.

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