Epidémiol. santé anim., 1997, 31-32

THE INFLUENCE OF INTRAMAMMARY INFECTIONS WITH *STAPHYLOCOCCUS CHROMOGENES* AND *STAPHYLOCOCCUS WARNERI* OR *HAEMOLYTICUS* ON THE SOMATIC CELL COUNT IN DAIRY COWS*

Laevens H.[1], Deluyker H.[1], Devriese L.[2],de Kruif A.[1]

L'objectif de cette étude était d'estimer l'influence des infections intramammaires avec Staphylococcus (S.) chromogenes, S. warneri ou S. haemolyticus sur le taux cellulaire du lait chez les vaches laitières.
Chaque mois, les taux cellulaires transformés en $\log_e$ (lnSCC) des vaches suivies sur une lactation complète et ayant soit une bactériologie négative, soit une infection avec S. chromogenes, S. warneri et S. haemolyticus, ont été sélectionnés pour l'analyse statistique (ANOVA avec mesures répétées).
Les espèces de staphylocoques ainsi que le nombre de quartiers infectés ont eu une influence sur le lnSCC. Le lnSCC des vaches infectées par S. chromogenes dans un seul quartier (LSM = valeurs des moindres carrés moyens de lnSCC=4.50) et celui des vaches infectées par S. warneri ou S. haemolyticus dans plusieurs quartiers (LSM lnSCC = 4.93) diffèrent significativement des lnSCC chez les vaches à bactériologie négative (LSM lnSCC = 3.93) (p<0.001). Aucune différence statistique (p=0.11) n'a été observée entre les lnSCC des vaches infectées par S. warneri ou S. heamolyticus dans une seul quartier (LSM lnSCC = 4.08) et les lnSCC des vaches à bactériologie négative. Le stade de lactation et l'interaction entre le stade de lactation et le statut infectieux en fonction du nombre de quartiers infectés n'ont pas influencé les lnSCC (respectivement p=0.18 et 0.28).
En conclusion, à la fois S. chromogenes et S. warneri / heamolyticus ont affecté le lnSCC. Toutefois, l'augmentation du lnSCC a été plus importante pour des infections touchant plus d'un quartier mammaire et chez les vaches infectées par S. chromogenes.

INTRODUCTION

Since the mastitis control programs for major pathogens have been successfully implemented, the importance of the non-aureus or coagulase-negative staphylococci (CNS) increased. Nowadays the CNS, together with *Corynebacterium bovis* are the microorganisms most frequently isolated from herd surveys. The CNS have been reported as the leading cause of intramammary infections (IMI) in first lactation heifers, with the highest prevalence of infection present at calving and their influences on the bulk milk somatic cell count can not be ignored (3).
The objective of the study was to estimate the influence of IMI with *Staphylococcus* (*S.*) *chromogenes* and *S. warneri* or *S. haemolyticus* on the milk somatic cell count (SCC) in dairy cows.

MATERIALS AND METHODS

At monthly intervals, during a 20 month study period, milk samples of all cows on 25 Belgian dairy herds were collected for bacteriological examination and SCC measurement. Bacteriological examination was performed on aseptically collected quarter foremilk samples and isolation and initial identification of the microorganisms was done as described in the National Mastitis Council guidelines (2). The differentiation of non-aureus staphylococci was performed using the identification scheme described by Devriese et al. (1). SCC measurement was performed on mixed four-quarter milk samples with the Somascope (Delta Instruments, Drachten, The Netherlands).
Monthly SCC of bacteriologically negative cows and cows intramammarily infected with only *S. chromogenes* and only *S. warneri* or *S. haemolyticus* and observed over a whole lactation were selected for statistical analysis. Cows were considered bacteriologically negative when no microorganisms were isolated and no clinical mastitis was observed during the observed lactation. Cows were considered infected intramammarily with *S. chromogenes* or *S. warneri / haemolyticus* when the same microorganism could be isolated from the same quarter in 2 successive or 2 out of 3 successive milk samplings. The infected quarter became first negative with the first of 2 successive milk samples that were bacteriologically negative. A lactation was defined as the period from parturition to drying off or the period from parturition to the end of the observation period, when this period was longer than 250 days, whichever came first.
The data file, including all selected cows, was analysed with repeated measures analysis of variance (GLMM 1.0, Blouin DC and Saxton AM, Dep. Experimental Statistics, Louisiana State Univ. Baton Rouge) using the following models.

1. $Y_{ijk} = \mu + INF_i + COW_{j(i)} + INFPER_k + INF_i * INFPER_k + \varepsilon_{ijk}$

where

Y_{ijk} = observed value (lnSCC of the monthly cow milk samples);
μ = overall mean;

* This study was made possible by a grant of the Belgian Ministry of Agriculture
[1] Department of Reproduction, Obstetrics and Herd Health and [2]Department of Bacteriology, School of Veterinary Medicine, Salisburylaan 133, B-9820 Merelbeke, Belgium

INF_i	= fixed effect of infection status (i = 0, 1, or 2 with 0 = bacteriologically negative, 1 = *S. chromogenes* infections, and 2 = *S. warneri* or *S. haemolyticus* infections);
$COW_{j(i)}$	= random effect of cow nested within INF_i (j = 1 to 78);
$INFPER_k$	= fixed effect of infected period (k = 0 or 1);
$INF_i * INFPER_k$	= interaction of infection status and infected period;
ε_{ijk}	= random error term.

The $INFPER_k$ variable was coded 1 during the infected period within a lactation and coded 0 in bacteriologically negative cows and during the uninfected period within a lactation of infected cows.

2. $Y_{ijk} = \mu + INFQUART_i + COW_{j(i)} + DIM_k + INFQUART_i * DIM_k + \varepsilon_{ijk}$

where

$INFQUART_i$	= fixed effect of infection status by number of infected quarters (i = 0 to 4 with 0 = bacteriologically negative cows, 1 = cows infected with *S. chromogenes* in a single quarter, 2 = cows infected with *S. chromogenes* in more than one quarter, 3 = cows infected with *S warneri* or *S. haemolyticus* in a single quarter and 4 = cows infected with *S. warneri* or *S. haemolyticus* in more than one quarter);
$COW_{j(i)}$	= random effect of cow nested within $INFQUART_i$ (j = 1 to 78);
DIM_k	= fixed effect of stage of lactation (k = 1 to 10);
$INFQUART_i * DIM_k$	= interaction of infection status by number of infected quarters and stage of lactation.

The stage of lactation variable was coded in 30-d periods, i.e. 1 = 1 to 30 DIM to 10 = 271 to 300 DIM.
The significance level of whole-plot and subplot fixed effects was estimated using the whole-plot ($COW_{j(i)}$) and the subplot (ε_{ijk}) random error term, respectively.

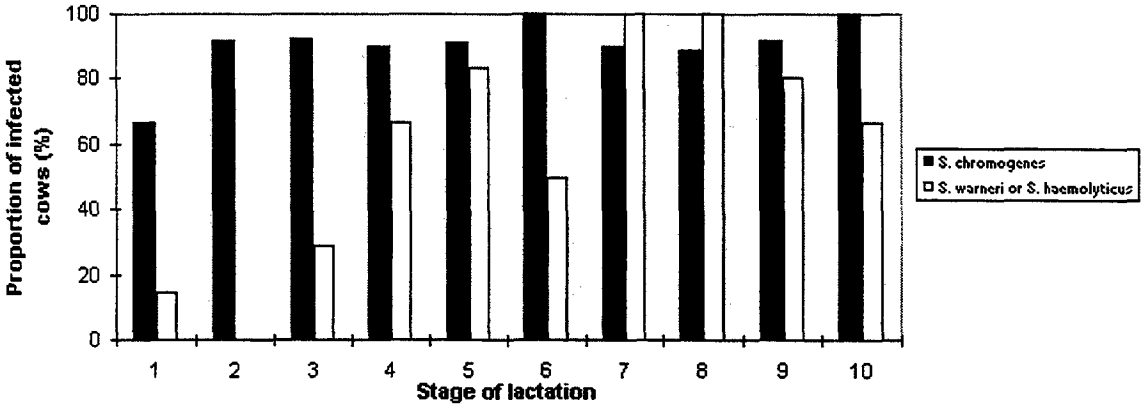
RESULTS AND DISCUSSION

The number of cows per parity and per number of infected quarters for each infection status class is given in Table I and the proportion of cows infected with *S. chomogenes* and *S. warneri* or *S. haemolyticus* in one or more quarters at a given time during lactation is presented in Figure 1.

Table I
Number of cows per infection status class

Infection status	Parity			Number of infected quarters		Total
	1	2	>2	1	>1	
Bacteriologically negative	28	15	13	-	-	56
S. chromogenes	9	2	2	12	1	13
S. warneri / haemolyticus	5	2	-	5	2	7

Figure 1
Proportion of *S. chromogenes* and *S. warneri* or *S. haemolyticus* infected cows in one or more quarters at a given time during lactation



In the first month of lactation, 66.6 % of the *S. chromogenes* infected cows were found positive whereas only 14.3 % of the *S. warneri* or *S. haemolyticus* infected cows were. The majority of the infections lasted to the end of

the lactation (84.6 % and 71.4 % for the *S. chromogenes* and the *S. warneri* or *S. haemolyticus* infected cows, respectively).

model 1

Within the *S. chromogenes* and the *S. warneri* or *S. haemolyticus* infected cows, the least squares means (LSM) lnSCC did not differ significantly (P = 0.49 and 0.24, respectively) between infected and uninfected periods (Table II). Therefore, in the subsequent analysis, infection status was entered as whole-plot effect rather than a subplot effect.

Table II
The least squares means lnSCC (x 10^3 cells/ml) of *S. chromogenes* and *S. warneri* or *S. haemolyticus* infected cows during the uninfected (0) and infected (1) period within a lactation (model 1).

Infection status	INFPER	
	0	1
S. chromogenes	4.45	4.59
S. warneri or *haemolyticus*	4.23	4.43

Table III
The least squares means (LSM) lnSCC and the geometric mean (geom. mean) SCC per infection status by number of infected quarters class (model 2).

INFQUART	LSM lnSCC (x 10^3 cells/ml)	geom. mean SCC (x 10^3 cells/ml)
0	3.94	51.3
1	4.50	90.3
2	5.54	256.1
3	4.08	59.4
4	4.93	138.3

model 2

The infection status by number of infected quarters variable was statistically significant (P < 0.001). The LSM lnSCC and the geometric mean SCC per infection status by number of infected quarters class is given in Table III. Stage of lactation and the interaction of stage of lactation and infection status by the number of infected quarters did not affect the lnSCC (P = 0.18 and 0.28, respectively).

Both the *Staphylococcus*-species and the number of infected quarters had an influence on the lnSCC. Comparison of the lnSCC between cows infected with *S chromogenes* ($INFQUART_1$ and $INFQUART_2$) and bacteriologically negative cows showed a significant difference (P < 0.001). The lnSCC differed between cows infected with *S. warneri* or *S. haemolyticus* ($INFQUART_3$ and $INFQUART_4$) and bacteriologically negative cows (P < 0.001) and also between *S. chromogenes* ($INFQUART_1$ and $INFQUART_2$) and *S. warneri* or *S. haemolyticus* ($INFQUART_3$ and $INFQUART_4$) infected cows (P < 0.001). Cows infected in a single quarter ($INFQUART_1$ and $INFQUART_3$) as well as the cows infected in more than one quarter ($INFQUART_2$ and $INFQUART_4$) had a higher lnSCC (P < 0.001) as compared with the lnSCC of the bacteriologically negative cows. Within the infected cows, the lnSCC differed significantly with the number of infected quarters (P < 0.001).

The lnSCC of cows infected with *S. chromogenes* in a single quarter, cows infected with *S. chromogenes* in more than one quarter and cows infected with *S. warneri* or *S. haemolyticus* in more than one quarter differed significantly with the lnSCC of bacteriologically negative cows (P < 0.001). No statistical difference (P = 0.11) was observed between the lnSCC of cows infected with *S. warneri* or *S. haemolyticus* in a single quarter and the lnSCC of the bacteriologically negative cows. The lnSCC of cows infected with *S. chromogenes* in a single quarter was higher (P < 0.001) as compared with the lnSCC of cows infected with *S. warneri* or *S. haemolyticus* in a single quarter. The lnSCC of cows infected with *S. chromogenes* in more than one quarter was also higher (P < 0.001) as compared with the lnSCC of cows infected with *S. warneri* or *S. haemolyticus* in more than one quarter. Within the *S. chromogenes* and the *S. warneri* or *S. haemolyticus* infected cows, the number of infected quarters also affected the lnSCC (P < 0.001 for both the *S. chromogenes* and *S. warneri* or *haemolyticus* infected cows).

CONCLUSION

It was concluded that both *S. chromogenes* and *S. warneri* or *S. haemolyticus* did affect the lnSCC. However, the increase of the lnSCC was more important when more than one quarter was involved and in cows infected with *S. chromogenes*.

ACKNOWLEDGEMENTS

The authors thank L. De Meulemeester, R. Vandermeersch, E. de Muêlenaere, N. Steendam, J. Hommez, B. Deheegher, T. Fevery, and the Flemish Cattle Breeding Association for their help in various ways.

REFERENCES

1. Devriese LA, Laevens H, Haesebrouck F, and Hommez J. A simple identification scheme for coagulase negative staphylococci from bovine mastitis. Res. Vet. Sci., 1994; 57: 240-244.
2. Harmon RJ, Eberhart RJ, Jasper DE, Langlois BE, and Wilson RA. Microbiological procedures for the diagnosis of bovine udder infections. In Proc. Natl. Mastitis Counc., 1990. Natl. Mastitis Council., Inc., Arlington, VA.
3. Smith KL, and Hogan JS. The importance of coagulase-negative staphylococci. Newsletter of the International Dairy Federation, 1995; n° 142: 26-29. Ed. Schällibaum M.