

## MULTI-LEVEL MODELLING OF QUARTER INFECTION DATA ESTIMATION OF VARIANCE COMPONENTS AND EFFECTS

Schukken Y.H.<sup>1</sup>, McDermott J.J.<sup>2</sup>, Lam T.J.G.M.<sup>1</sup>, Valkengoed P.<sup>1</sup>

*L'incidence de l'infection mammaire par S. aureus est suivie chez approximativement 500 vaches vivant dans 7 élevages. La complexité des données nécessite une approche de modélisation hiérarchique. Il s'agit de données binaires regroupées avec des mesures répétées. L'objectif de l'analyse était d'évaluer l'effet protecteur des agents pathogènes mineurs. Les résultats de l'analyse montrent qu'une prévalence élevée d'infection à C. bovis et la combinaison C. bovis avec Micrococcae coagulase négative sont associées à une faible incidence d'infection par S. aureus. La décomposition de la variance montre que la variation inter élevages est beaucoup plus faible que la variation intra vache. Le statut d'infection dépend essentiellement de l'état de la vache puis de celui du trayon. Les méthodes d'analyse de ce type de données demandent davantage de développement en épidémiologie vétérinaire.*

### INTRODUCTION

A possible reason for the sometimes high incidence of severe clinical mastitis in herds with a low bulk milk somatic cell count (BMSCC), is the decrease of the prevalence of minor pathogens. Several research workers have studied the effect of minor pathogens on infections with major pathogens in experimental situations resulting in sometimes contradictory results (Lam et al. 1997). Furthermore, the effects of experimental and natural infections with minor pathogens, may not necessarily be the same. Reports of studies on interactions between naturally occurring infections with minor and major pathogens are available. Again, these studies do not always reveal the same results. This may be due to differences in the definition of infection, or in the methods used to analyze the data. Different analytical methods sometimes produced different results, even when the same set of data was used (see Lam et al. 1997). The objective of the present study was to describe the effect of naturally occurring infections with minor pathogens on the susceptibility of the udder to natural infections with a major pathogen, specifically *S.aureus*. These data have previously been described using a matched case-control approach, where new infections with major pathogens were the cases, and the control was another quarter that did not experience an infection with a major pathogen from the same cow (Lam et al. 1997). This analysis controlled for confounding by farm, cow, parity, stage of lactation and other cow or environmental factors. However the general disadvantages of case-control studies also apply, and therefore no incidences or risk rates were available from this analysis. The purpose of this presentation is to evaluate an alternate option for analysis of longitudinal binary data with complex correlations within farm and cow.

### MATERIAL AND METHODS

During a 20 month study on the effect of discontinuation of post milking teat disinfection (PMTD) in low BMSCC herds, teats of left or right quarters of each cow in seven herds were disinfected after each milking. The seven herds in the study had a mean herd size of 56.2. Duplicate foremilk samples were collected from the quarters of all lactating cows before the study was started. Single quarter foremilk samples were collected every five to six weeks thereafter from all lactating cows. Samples were also collected from cows within one week after calving, from cows at drying off, and from quarters showing signs of clinical mastitis. Bacteriological procedures were according to National Mastitis Council standards (Harmon et al., 1990). A quarter was considered having an infection at the onset of the study when  $\geq 100$  cfu/ml of the same pathogen were cultured from both samples. A quarter was considered as having a new infection during the study, when  $\geq 500$  cfu/ml of the same pathogen were cultured from two of three consecutive milksamples, when  $\geq 100$  cfu/ml of the same pathogen were cultured from three consecutive milksamples, or when  $\geq 100$  cfu/ml of a bacterial species were cultured from a quarter showing signs of clinical mastitis. A quarter was considered to be eligible for a new infection after it had been found negative on that pathogen for at least two subsequent samplings. Only quarters eligible for a new *S.aureus* infection were included in the present analysis. Somatic cell counts were determined in each sample using an automatic cell counter. Data on cow level included parity and stage of lactation

### MODELLING

The data consisted of approximately 30,000 quarter observations from 470 cows on 7 farms. Hence clustering of information may be expected within farm, within cow, and within quarter (repeated measurements). To accommodate this, a multi-level model where farm and cow within farm were defined as random effects, and repeated measurements within quarter were correlated using an AR-1 structure (Diggle et al. 1994, Crowder and Hand 1995) was developed. With this correlation pattern corrected for, fixed effects at the cow and quarter level

<sup>1</sup> Department of Herd Health, Yalelaan 7, 3584 CL Utrecht, The Netherlands

<sup>2</sup> Department of Population Medicine, Ontario Veterinary College, Guelph, Ontario, Canada

were fitted. Variance components were estimated using REML procedures. The fixed part of the initial regression model was then:

$P(\text{S.aureus infection}) = \text{intercept} + \text{PMTD} + \text{previous minor pathogen (CNM, C.Bovis or both)} + \text{previous SCC} + \text{Front quarter} + \text{parity} + \text{lact. stage} + \text{error}$

Previous minor pathogens were coded as either absent or present (CNM = Coagulase Negative Micrococcae), PMTD was coded as either present or absent, previous SCC was classified as either below or above 500,000 cells per ml., parity was coded into first, second, and greater, and lactation stage was used as a linear effect of classes 0-25, 26-50, 51-100, 101-200, and > 200 days in milk.

In this model, quarter effects were tested against the residual error, and cow effects were tested against the cow\*effect interaction. All analysis were done using SAS Proc Mixed, macros using this Proc Mixed, and other descriptive procedures in SAS (SAS v6.11, 1995).

## RESULTS

A total of 26,289 quartersamples from 462 cows in 7 farms were available for analysis. In these quartersamples the total number of incident *S.aureus* infections was 146(0.5%), the prevalence of Coagulase Negative Micrococcae was 13.8%, and C. Bovis prevalence was 45.8%. Incident *S.aureus* infections occurred most frequently in older parities, in hind quarters, and in early lactation.

Variance component estimation of herd and cow within herd effects resulted in a non-significant herd effect, and a significant cow effect and quarter effect, however residual error was by far the largest component. Relative sizes were .00143, .0233, .0101 and 1 for herd, cow within herd, quarter within cow and residual respectively.

Results of regression analysis are presented in Table I. Parity appeared to be significantly associated with new *S.aureus* infections. Young animals had a significantly lower incidence of infection.

**Table I**  
**Results of regression analysis: fixed effect estimates and variance components**

Parameter	Estimate	Std. Error	Significance
<b>Fixed effects:</b>			
Intercept	.007579	.00193	.0112
Parity 1	-.00529	.00159	.0009
Parity 2	-.00318	.00155	.0398
Parity 3	ref		
Quarter front	-.00163	.00110	.138
Quarter hind	ref		
PMTD	-.00547	.00143	.0001
Previous CNM	.00707	.00266	.0078
Previous C.Bovis	-.0326	.00160	.042
Previous C.Bovis*previous CNM	-.00601	.00303	.047
<b>Random effects:</b>			
herd	.0000083		
cow	.0001334		
quarter	.0000583		
residual	.005734		

## DISCUSSION

Analysis of variance components showed that herd level variance in these data was limited, quarter level variance was small, and variance within cow was the largest component. The relative low herd effect appears to be caused by herd selection. Only seven herds, with excellent udder health management as indicated by low BMSCC were included in the study. A random selection of sufficient herds would be more relevant to determine herd variance components. Variability due to cow was important higher than variability due to quarter. Apparently, in these data, cow variance is more important, and *S.aureus* infection is more related to cow level variables than to quarter variables. It should be considered that in this analysis some important quarter level variables such as quarter location and infection status of minor pathogens were already corrected for in the fixed effects. Further studies on the dynamics of infection should focus more on cow level variability, and factors explaining susceptibility of cows to infection with *S.aureus*.

The analysis of the fixed effects indicated that at the quarter level infection status, and post milking teat disinfection were significant, a the cow level only parity was a significant variable. Especially a previous infection with *C.bovis*, and the combination of *C.bovis* and Coagulase Negative Micrococcae resulted in a lower incidence of *S.aureus* infections. A prevalent infection of Coagulase Negative Micrococcae was associated with a higher incidence of infection with *S.aureus*. These results coincide with the previous observed results from the case-control analysis of the same data (Lam et al. 1997). Protective effect of *C.bovis* was also observed in this analysis, however it was not statistically significant in contrast to the current results. Hence, longitudinal analysis

of all these data appears to be more informative compared to the previous case-control analysis that omits considerable data, particularly with respect to the variability of the responses. Thus we gained both incidence information and also increased the power of the analysis.

Coagulase Negative Micrococcae were apparently not associated with a decreased incidence of *S.aureus*. Only when CNM infection was combined with *C.bovis* infection, a decrease in incidence was observed. Although CNM is not associated with a decreased incidence in infection, it was reported that quarters infected with both *S.aureus* and CNM showed a lower CFU of *S.aureus*, and that duration of infection was also shorter. Hence an interaction of CNM and *S.aureus* may still lead to a lower prevalence of *S.aureus* in a herd. Prevalence of infection of CNM was also much lower than prevalence of *C.bovis*. Hence, the impact of CNM on population dynamics of *S.aureus* appears to be smaller compared to the impact of *C.bovis*.

Analysis of binary clustered data with repeated measurements currently requires advanced analytical programs. Further development of these programs, and a further application of this in veterinary epidemiology appears possible. The framework of generalized linear mixed models allows complexities as described in this paper.

## REFERENCES

- Crowder M.J., Hand D.J., 1995. Analysis of repeated measures. Chapman & Hall, London.
- Diggle P.J., Liang K.Y., Zeger S.L., 1994. Analysis of longitudinal data. Clarendon Press, Oxford.
- Harmon R.J., Eberhart R.J., Jasper D.E., 1990. Microbiological procedures for the diagnosis of bovine udder infection. National Mastitis Council, Arlington, Va.
- Lam T.J.G.M., Schukken Y.H., van Vliet J.H., Grommers F.J., Tielen M.J.M., Brand A., 1997. Effect of natural infection with minor pathogens on susceptibility to natural infection with major pathogens in the bovine mammary gland. American Journal of Veterinary Research 58(1), 17-22.
- SAS, Statistical Analysis System version 6.11, 1995. SAS Institute Inc., Cary NC.