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## Factors affecting the distribution of clinical mastitis among udder quarters in French dairy cows

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**Summary** — Factors related to the distribution of clinical bovine mastitis between rear and front quarters were studied using data from a 4 year survey of commercial dairy herds in western France. The study involved 844 mastitis cases affecting 597 lactations of 500 French Friesian cows from 44 herds. Risk factor hypotheses were related to certain aspects of lactation, udder conformation and management practices. Distribution was modelled using a hierarchical logistic regression. Rear quarters were affected in 61.9% of cases. The only significant risk factor was the cow's parity; rear quarter clinical mastitis was more frequent in primiparous than in multiparous cows. In this retrospective study, udder conformation did not seem to play a significant role in mastitis distribution. No overdispersion parameter was observed, indicating that each mastitis case could be considered as an independent event.

### **dairy cow / mastitis / distribution / risk factor**

**Résumé** — **Facteurs de variation de la distribution des cas de mammite clinique parmi les quartiers de la mamelle des vaches laitières en France.** Les facteurs de variation de la distribution des cas de mammite clinique entre les quartiers arrière et avant de la mamelle des vaches laitières ont été étudiés à l'aide des données d'une enquête longitudinale de 4 ans dans des exploitations laitières privées de l'Ouest de la France. L'étude a porté sur 844 cas de mammite ayant affecté 597 lactations chez 500 vaches de race Française Frisonne dans 44 troupeaux. Les facteurs de risque ont été recherchés parmi certaines caractéristiques de la lactation, de la conformation de la mamelle et des pratiques d'élevage. La distribution a été modélisée par une régression logistique hiérarchique. Les mammites étaient localisées sur les quartiers arrière dans 61,9 % des cas. Le seul facteur de risque statistiquement significatif était la parité de la vache : les mammites des quartiers arrière étaient plus fréquentes chez les primipares que chez les multipares. Dans cette étude rétrospective, la confor-

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mation de la mamelle n'a pas semblé influencer la distribution des mammites. Aucun paramètre de surdispersion n'a été observé, indiquant que les différents cas de mammite pouvaient être considérés comme des événements indépendants.

## **vache laitière / mammite / distribution / facteur de risque**

### **INTRODUCTION**

Mastitis is the most frequently occurring disease on French intensive dairy farms and in most cases of clinical mastitis, only a single quarter is affected (Faye et al, 1994). Farmers and veterinarians know that clinical mastitis occurs more often in rear quarters than in front ones, but few references are available supporting this point (Batra et al, 1976; Faull et al, 1983; Adkinson et al, 1993). In a recent paper, Adkinson et al (1993) analyzed the distribution of clinical mastitis among quarters. This paper presents an attempt to establish an explanatory model for this distribution, ie, to determine the risk factors for the asymmetry of udder infections.

### **MATERIALS AND METHODS**

#### **Survey**

This study was part of a prospective epidemiological survey, the main concern of which was to assess the herd-level and cow-level risk factors for clinical and subclinical peripartum diseases (Faye et al, 1989). It was carried out over 4.5 years (1986–1990) in 48 dairy herds in Brittany (France). Among these 48 herds, 44 had no missing data and were involved in this study. The farmers were members of the Milk Recording Scheme (MRS) and volunteered to participate in the survey. They were selected for their ability to detect and record diseases, as assessed by field veterinarians and confirmed over a pre-study period. In the selected herds, 98.5% of the cows were French Friesian.

A total of 8 945 lactations in 4 129 cows were surveyed. Average milk yield was 7 413 kg (range 1 653 to 12 471 kg) per lactation for cows

in milk for more than 300 days. Observers from the Veterinary Services visited the farms monthly to collect management data and to measure individual body conditions and dirtiness scores around calving (Faye and Barnouin, 1985). Technicians from MRS collected individual monthly milk samples from all lactating cows in order to determine production parameters.

Data were stored in a database managed by a relational database management system. The database was designed according to the MERISE method (Lescourret et al, 1993; Pérochon and Lescourret, 1994). Data were retrieved using a structured query language.

#### **Dependent variable**

The statistical unit was a clinical mastitis case. This was defined in terms of local symptoms (inflammation of the quarter, change in milk appearance), sometimes also associated with general signs (hyperthermia and prostration). The dichotomous dependent variable was the localization of the affected quarter (1 rear, 0 front). Cases of mastitis in the same quarter (and in the same cow) were assumed to be independent events, except those occurring within 3 months of an earlier case in the same lactation. These were not taken into account.

#### **Covariates**

The risk factor hypotheses (table I) were selected among available data in the database. They involved factors likely to explain the unequal distribution of clinical mastitis between different quarters. Data had a hierarchical structure (mas-

**Table I.** Independent variables retained at the initial step.

<i>Independent variable</i>	<i>Code</i>	<i>Category number</i>	<i>Median</i>	<i>Mean or frequency</i>	<i>Standard deviation</i>
<i>Lactation level (n = 597)</i>					
Parity	PAR	–			
Milk production (kg at 305 days)	PROD	–	6 803	6 788	1 671
Dirty score at beginning of lactation (score 1–100)	DIRT	–	35	38.1	17.2
Frequency of diarrhoea during lactation (%)	DIAR	–	–	3.5	0.04
Frequency of lameness during lactation (%)	LAM	–	–	8.4	0.28
Frequency of udder trauma during lactation (%)	TRAM	–	–	2.0	0.14
<i>Cow level (n = 500) udder and teat conformation (score 1–9)</i>					
Front–rear udder balance	FRUB	–	5	4.88	0.92
Front teats gap	FTG	–	5	5.39	1.21
Lateral teats gap	LTG	–	5	4.97	0.89
Teat placement	TEU	–	5	4.66	1.40
<i>Farm level (n = 44)</i>					
Type of milking parlour (%)	TMP	0 Herringbone	–	95.4	–
		1 Tandem	–	2.3	–
		2 Other	–	2.3	–
Type of bedding (%)	BED	0 No straw	–	59.1	–
		1 Straw	–	40.9	–

titis within lactations, lactations within cows and cows within farms), and each covariate referred to a particular level in this hierarchy (table I).

Udder conformation changes along with successive lactations and milk production increases with the lactation number. Parity (PAR) was then retained as a possible risk factor.

Milking production (PROD) was defined as the standardized production at 305 days. Hind quarters produce more milk than fore ones. They might be more susceptible to clinical mastitis when the milk production increases. The covariate PROD was also considered as a possible confounder for parity.

Dirty score (DIRT) and diarrhoea (DIAR) were selected for hind teats, which are likely to be

more contaminated when cows are dirty. The dirtiness score was calculated using the method described by Faye and Barnouin (1985). The dirtiness score was attributed once at the beginning of the lactation.

Udder trauma (TRAM) can initiate clinical mastitis. Hind teats are more exposed to udder trauma than front ones; they are injured by rear legs when cows get up. Only lameness (LAM), diarrhoea and udder trauma cases occurring before clinical mastitis were selected.

Once a year (and once in the life of a given cow), a technician from the French Friesian Breed Improvement Association assigned cows an udder conformation scores. Four of these criteria were retained in this study: front–rear udder balance (FRUB), front teats gap (FTG), lateral

teats gap (LTG) and teat placement (TEU). These four scores were likely to be correlated. We assumed that mastitis localization was probably related to a set of conformation features rather than to a single one. A global conformation type was then constructed using ADDAD software (Lebeaux, 1989). Principal component analysis was run on the four scores, followed by an ascending hierarchical classification (second-order centered moment method, euclidian distance) of the subsequent factorial coordinates (Roux, 1985). The main classes of the hierarchy defined the categories for a global conformation covariate (CONF).

A given cow always goes to the same place in the milking parlour. According to the type of milking parlour (TMP), this might lead to udder trauma on a particular teat and subsequently to clinical mastitis.

The favourable effect of straw bedding versus raw soil on udder health is well known. Moreover, straw bedding might be more comfortable for the rear quarters. The type of bedding (BED) was retained as a possible risk factor.

### Statistical procedure

Conventional statistical modelling with dichotomous dependent variables involves logistic regression. The procedure recommended by Hosmer and Lemeshow (1989) was followed. Covariates were first described by univariate analysis (histograms, means, variance, quantiles of distribution, category frequencies) and screened for their association with the dependent variable (*t* test, ANOVA, Kruskal–Wallis test,  $\chi^2$  test). Irrelevant covariates were discarded (lack of variability or lack of association with the dependent variable). Graphs of the remaining covariates were plotted to check for linearity on the logit scale. Univariate logistic regressions were then performed with each covariate versus the dependent variable. Covariates with a significant parameter (Wald test,  $P \leq 0.30$ ) were included in a backward stepwise fixed effects logistic regression model. As a first step, param-

eters were calculated for all the covariates. The covariate for which the parameter had the lowest significant level, ie, the highest probability associated with the likelihood ratio statistic, was removed from the model. The process was iterated until the change in the model  $\chi^2$  reached a threshold ( $P = 0.25$ ).

The usual assumption of independence among observations was not met for the fixed effects model, because the data had a hierarchical structure. Furthermore, covariates referred to different levels (mastitis, lactation, cow and farm). Under these conditions, the data frame lay outside the field of fixed effects logistic regression. This could lead to biases in parameter estimates and their confidence intervals (Goldstein, 1987; McDermott et al, 1994). The hierarchical logistic model proposed by Goldstein (1987, 1991) was used to overcome these problems.

As far as mastitis and lactations were concerned, the hierarchical logistic model for the  $\pi_{ij}$  probability of the occurrence of mastitis *i* (level 1) within lactation *j* (level 2) on a rear quarter was:

$$\begin{aligned} \log[\pi_{ij}(1 - \pi_{ij})^{-1}] &= \text{logit}(\pi_{ij}) \\ &= \beta_0 + \beta_q X_{qij} + \beta_r X_{rj} + u_{0j} = \beta X + U_j \end{aligned}$$

where: Q level-1 covariates  $X_q$  described mastitis *i* within lactation *j*; R level-2 covariates  $X_r$  described lactation *j*;  $u_{0j}$  was a level-2 random variable  $\sim N(0, \sigma^2_0)$  associated with the constant  $\beta_0$ . ( $\beta X + U_j$ ) was the linear predictor:  $\beta X$  covered all fixed effects and  $U_j$  all random effects ( $u_{0j}$  in the model). With appropriate coding of the covariates,  $\beta_0$  can be interpreted as a baseline subject-specific log odds.

The level-2 random parameter  $u_0$  indicates the range of  $\beta_0$  fluctuations from one lactation to the next. Additional random effects can be obtained by allowing one  $\beta_q$  parameter (or more) to fluctuate from one lactation to the next, with a distribution of  $N(\beta_q, \sigma^2_q)$ , ie,  $\beta_{qj} = \beta_q + u_{qj}$  with  $u_{qj} \sim N(0, \sigma^2_q)$ . The full model for the observed response  $p_{ij}$  was:  $p_{ij} = \pi_{ij} + e_{ij}$ , where  $e_{ij}$  was a random variable with an extra-bino-

mial distribution, ie, its variance had both an overdispersion parameter and a binomial component:

$$\text{var}(e_{ij}) = \sigma^2 \pi_{ij} (1 - \pi_{ij})$$

Dependence among observations would lead to  $\sigma^2 \neq 1$ .

In the next step, the cow and farm were each considered as the second level in the analysis, ie, the level-2 random parameter  $u_{0j}$  indicated the range of  $\beta_0$  fluctuations from one cow (or one farm) to the next.

The full model described above is non-linear for both fixed and level-2 random parameters. Estimation procedures consider first a linearization of the function of the linear predictor, followed by an application of a standard procedure for the linear multilevel model using the iterative generalized least squares algorithm (Goldstein, 1986, 1989, 1991). Fitting the full model was achieved on a microcomputer, using the ML3E program (Prosser et al, 1991; Woodhouse et al, 1993) and macros written for multilevel binary response logistic models (Yang, 1993).

## RESULTS

The population under study consisted of 844 clinical mastitis cases occurring during 597 lactations (1.41 mastitis cases per lactation) among 500 cows belonging to 44 herds. Rear quarters were more affected than front ones (61.9 vs 38.1%). The difference between left and right quarters was low (48.6 vs 51.4%); this aspect of localization was not taken into consideration in the following steps.

### Covariates

Covariates describing type of milking parlour, occurrences of diarrhoea, lameness and udder trauma were deleted because of their lack of variability or their independence with the depen-

dent variable. Principal component analysis (figs 1a and b) revealed that udder conformation marks were correlated (fig 1b). Hierarchical ascending classification led to the choice of three classes corresponding to the two upper nodes of the hierarchy, ie, 34% of variance (fig 1c). These classes formed the categories for a synthetic covariate (CONF) describing udder conformation. The established classes were as follows. Class 1 ( $n = 161$ ) included cows with udder unbalanced to the rear. Teats were vertical but were established a long way from the median furrow of the udder. The front gap was normal for the French Friesian breed (15 cm). Class 2 ( $n = 248$ ) were cows with balanced udders. Teats were vertical and the front gap was normal but the teats were turned inwards. Class 3 ( $n = 91$ ), included cows with udders that were unbalanced to the front. The teats were established normally on the udder. The front gap between teats was over 15 cm and teats were turned outwards.

### Screening steps

The covariates CONF, FRUB, TEU and DIRT were discarded because of their lack of association with the dependent variable. The remaining covariates describing udder conformation (LTG and FTG) were discretized in three equalized ordinal categories. Milk production (PROD) showed a quadratic variation of the logit. This covariate was then squared (PROD2) for use in subsequent analysis. Categories 2, 3 and 4 of the covariate PAR were combined and the resulting covariate had two categories: 1 (primiparous) and 2 (multiparous). The covariates FTG, PROD2 and PAR were retained after univariate logistic regression, together with the bedding covariate (BED), though the latter's parameter was slightly beyond the cut-off point (0.31 vs 0.30).

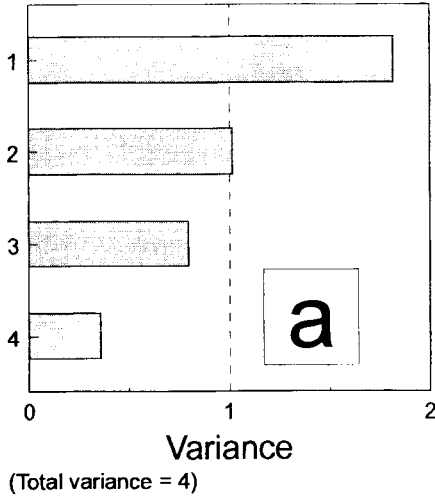
### Fixed effects logistic model

Parity (PAR) had an odds ratio significantly lower than 1 (0.64 with 95% confidence interval

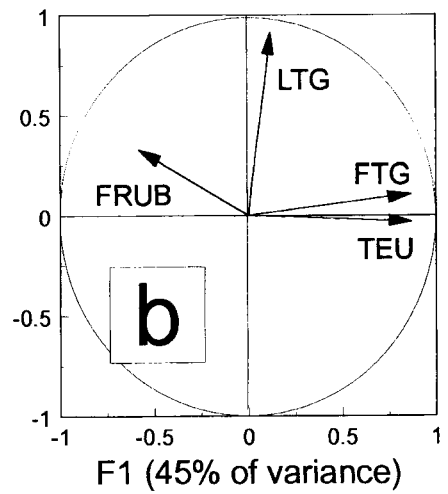
of 0.48–0.85, table II); the probability of mastitis occurring in the rear quarters was in reverse proportion to the parity number. The other effects were not significant, in particular the squared

milk production. This means that milk production was a confounder for parity in this study. All the possible second-order interaction terms were tested but none of them was significant.

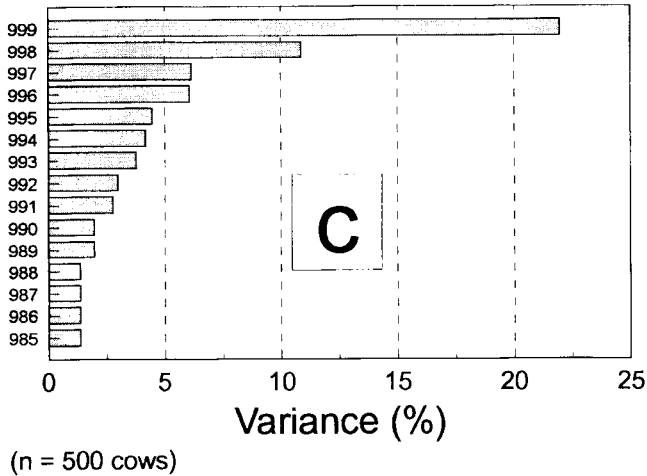
**Eigen value number**



**F2 (26% of variance)**



**Upper nodes of the hierarchy**



**Fig 1.** Constructing the synthetic conformation covariate using principal component analysis: eigen values of the correlations matrix (a), correlations circle (b) for udder conformation marks and hierarchical ascending classification (c). F1 first principal component; F2 second principal component; FRUB front-rear udder balance score; FTG front teats gap score; LTG lateral teats gap score; TEU teat placement score.

**Table II.** Parameters for the fixed effects and hierarchical (upper level = farm) models.

Variable	B	$S_B$	$\chi^2$	Wald	df	P
<i>Fixed effects model</i>						
Constant	0.8	0.35	–	6.43	1	0.01
BED	0.19	0.16	–	1.44	1	0.23
PAR	–0.45	0.15	–	9.09	1	0.003
<i>Hierarchical model</i>						
<i>Fixed effects</i>						
Constant	0.64	0.17	14.88	–	1	10 <sup>–4</sup>
BED	0.18	0.18	1.03	–	1	0.31
PAR	–0.45	0.15	9.61	–	1	0.002
<i>Random effects</i>						
$e_{ij}$	0.99	0.05	0.09	–	1	0.76
$u_{oj}$	0.06	0.06	1.10	–	1	0.57

$B$  parameter estimate;  $S_B$  standard deviation of parameter estimate;  $\chi^2$  value of the  $\chi^2$  statistic;  $df$  degrees of freedom; Wald: value of the Wald statistic;  $P$ : probability of null hypothesis; BED type of bedding (0 no straw; 1 straw); PAR categorized parity (0 primiparous; 1 multiparous);  $e_{ij}$  level-1 random variable;  $u_{oj}$  level-2 random variable.

### Hierarchical model

The overdispersion parameter was not significant when mastitis was considered as the lower

**Table III.** Goodness of fit for the hierarchical logistic model.

Variable	Predicted cases	Observed cases
BED = 0		
PAR = 0	72.7	73
PAR = 1	76.3	76
BED = 1		
PAR = 0	155.3	155
PAR = 1	218.7	219

$\chi^2 = 0.0$ ,  $df = 3$ ,  $P = 1.0$

Predicted cases = number of predicted values > 0.5 for each covariate pattern; number of observed cases for each covariate pattern; BED type of bedding (0 no straw; 1 straw); PAR categorized parity (0 primiparous; 1 multiparous);  $\chi^2$  value of the  $\chi^2$  statistic;  $df$  degrees of freedom;  $P$  probability of null hypothesis

level and lactation as the upper level. The same result was found when cow or farm was taken as the upper level. Tables II and III show the estimates and statistics with farm as the upper level. They were close to those calculated by the fixed effects logistic regression. Absence of any overdispersion parameter indicates that rear–front mastitis localizations could be considered as independent events in this study. The level-2 random parameter associated with the constant was not significant. No other random effect was observed; the parity effect was the same for all farms.

### DISCUSSION

This study confirmed that rear quarters are more affected than front ones. The difference decreased with parity which was found to be the only significant factor associated with the distribution of mastitis.

The same rear–front trends were observed in a 30-year retrospective study of the Louisiana State University Dairy Research Herd (US) by



Adkinson et al (1993). Rear quarters were affected more than front ones (28.4 vs 24.9%,  $n = 2407$ ,  $P < 0.05$ ), but their difference was lower. This result was also inconsistent with ours in that the proportion increased with parity. In similar conditions to those of Adkinson et al (1993), Batra et al (1976) observed more mastitis in the rear quarters (29.5%) than in the front ones (26.5%) but the difference was not significant. On the other hand, our results were similar to those of Faull et al (1983) who, in a prospective study of 400 Friesian cows on an English experimental farm, observed 31% of new cases of clinical mastitis in the front quarters. Variation factors for this distribution were not discussed however. In addition, the other three studies were performed on experimental farms, ie, with probably quite different management practices than in commercial Breton farms.

Several authors have pointed out that teat–floor distance is a risk factor for udder trauma and mastitis (Kubicek and Meinecke, 1978; Janicki and Balukiewicz, 1980; Poutrel, 1983). As the distance decreases with parity (Kubicek and Meinecke, 1978), one might consider that unbalanced udders would promote mastitis in the rear quarters. However, rear–front udder balance was not related to mastitis distribution in this study.

It seems difficult to explain how parity itself would promote a higher susceptibility to mastitis in the front quarters than in the rear ones. Parity effect is likely to be an indicator of hidden features such as tissues ageing or udder immune status, that change between first and subsequent lactations and that would induce an overall udder weakness. The absence of any overdispersion parameter indicates that the covariates included in the model took data clustering into account. The hidden features therefore ought to be closely related to parity.

In conclusion, this study confirmed the asymmetry of mastitis distribution between front and rear quarters in dairy cows, but parity was the only factor which could be identified to explain the difference.

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