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1 **Abstract**

2

3 Prevention of heifer mastitis is a key element in preventing mastitis. Since pathogenesis of  
4 heifer mastitis is not fully understood yet, combating it is arduous. The goal of this study was  
5 to examine whether the premature loss of the keratin plug of the teat canal represents an  
6 important risk factor for the development of heifer mastitis. 84 dairy heifers (German  
7 Holstein) from six high-yielding dairy herds in northern Germany were examined between  
8 March 2005 and June 2006. Each quarter was examined clinically at least three times before  
9 calving. If the teat canal of a quarter was open, secretion samples were collected for  
10 bacteriological analysis. After calving, four quarter samples were collected during the first  
11 lactation. A rapid increase of the proportion of open teat canals was detected in the pre-  
12 parturition period. No teat canals were open till 80 days before parturition, while 60% of the  
13 teat canals were open at 60 days before parturition. A time-scheduled infection pattern was  
14 observed: during the days 90 to 61, 60 to 31, and 30 to 0 before calving, skin inhabitants  
15 (coagulase-negative staphylococci, coryneform bacteria), cow-related, and environment-  
16 related microorganisms prevailed, respectively. A total of 77% of intramammary infections  
17 encountered at parturition had established previously. No significant association between the  
18 duration of open teat canals before calving and the udder health post partum was found.  
19 However, the incidence of clinical mastitis during first lactation was influenced largely by the  
20 duration of infection ante partum and the mastitis pathogen involved. This study shows that a  
21 high proportion of teat canals already opens several months before calving and that opening  
22 of the teat canal before calving is an important factor in the aetiology of heifer mastitis.

23

24 Keywords: Cattle, Bacteria, Heifer, Mastitis, Teat canal

25

26

## 1 1. Introduction

2

3 One of the most important economic issues in dairy farms is to reduce the occurrence of  
4 mastitis. Especially, mastitis in the first lactation reduces the life yield of milk cows with  
5 consequently high replacement costs in a herd. A recent Belgian study found that an increase  
6 of the somatic cell count (SCC) leads to, per natural logarithm step, milk losses of 0.13 kg per  
7 day of the lactation (De Vliegher et al., 2005). Furthermore, New Zealand authors reported  
8 that no compensatory effect in milk yield from uninfected quarters within udder was observed  
9 in first lactation heifers, in contrast to cows that had completed at least one lactation  
10 (Woolford et al., 1983).

11 It seems that heifer mastitis may develop in many different pathogenic ways. On one  
12 hand, calves and heifers suckling each others' teats can affect the development of the juvenile  
13 udder. This, in conjunction with the transmission of mastitis pathogens (e.g. *Streptococcus*  
14 *agalactiae*) is prone to lead to heifer mastitis (Schalm, 1942). On the other hand, one study  
15 found that stable flies can transmit pathogens such as *Staphylococcus aureus* from lactating or  
16 dry cows to non-lactating heifers (Nickerson et al., 1995). Intramammary infections (IMI) by  
17 coagulase-negative staphylococci (CNS) and environmental pathogens (i.e. environmental  
18 streptococci, coliforms) also occur, although the pathogenic mechanisms involved have not  
19 been fully unravelled yet.

20 However, some risk factors were found, among them seasonal and climate factors (Fox et  
21 al., 1995; Hallberg et al., 1995; de Vliegher et al., 2001). Nickerson et al. (1995) and Bassel et  
22 al. (2003) suggested that insufficient fly control and advanced age at first calving represented  
23 herd-specific risk factors. Poor hygiene in the calving area was associated with both increased  
24 prevalence of elevated SCC in heifers and increased incidence of clinical mastitis (Bareille et  
25 al., 2000; Reinecke et al., 2006). Contrary to expectations, group housing of pre-weaned  
26 calves, mastitic milk feeding and a high prevalence of coagulase-positive staphylococci in the

1 lactating herd do not necessarily translate into a high prevalence of coagulase-positive  
2 staphylococci IMI in heifers at calving (Roberson et al., 1994a). It appears that animal and  
3 teat-related risk factors are important in relation to heifer mastitis. IMI have been detected as  
4 early as 9 months pre-calving in heifers (Trinidad et al., 1990).

5 Several authors demonstrated that the prevalence of IMI increases as the calving date  
6 approaches and was highest during the last trimester of pregnancy. This suggests that heifers  
7 may be most susceptible during that period of gestation, this being most likely associated with  
8 the rapid mammary gland development during that time (Oliver and Mitchell, 1983; Fox et  
9 al., 1995; Aarestrup and Jensen, 1997). An increased age at calving is a significant risk factor  
10 for *S. aureus* and environmental pathogen IMI (Bassel et al., 2003).

11 Reported quarter-level IMI prevalence ranges from 29 to 86% before calving and 18 to  
12 55% at calving (Meaney, 1981; Oliver and Mitchell, 1983; Trinidad et al., 1990; Pankey et  
13 al., 1991; Roberson et al., 1994a; Fox et al., 1995; Myllys, 1995; Aarestrup and Jensen, 1997).  
14 These papers found IMIs with particularly CNS, *S. aureus* and environmental pathogens in  
15 heifers close to parturition.

16 Regarding the importance of teat apex colonisation by microorganisms as a risk factor,  
17 there is a number of different opinions. De Vlieghe et al. (2003) found that teat apex  
18 colonisation before calving with *Staphylococcus chromogenes* prevented IMI with major  
19 pathogens in heifers after calving. By contrast, heifers with teat skin colonized by *S. aureus*  
20 were 3.3 times more likely to develop IMI with the same pathogen at calving than were non-  
21 colonized heifers (Roberson et al., 1994b). Zadoks et al. (2002), on the other hand, claimed  
22 that this association was only suggestive and not significant. Nevertheless, local quarter  
23 immune factors do have an important influence on the risk of clinical mastitis (Green et al.,  
24 2004).

25 The premature loss of the keratin plug of the teat canal was suggested to be a major risk  
26 for heifer mastitis (Williamson, 2002). In older cows, closure of the teat canal at an early

1 stage of the dry period reduced the risk of a new IMI significantly (Dingwell et al., 2004).  
2 Applying an internal teat sealer after infusion of antibiotics at dry-off also yielded a more  
3 effective protection against new IMI than applying an antibiotic for dry-off alone (Sanford et  
4 al., 2006). In a pilot study, infusion of a teat sealant approximately 4 to 6 wk before calving  
5 was found to be effective at decreasing the prevalence of postcalving (0 to 4 d) IMI of  
6 *Streptococcus uberis* in heifers by 84% (Parker et al., 2007). In a following study it was  
7 shown, that the use of an internal teat-canal sealant in heifers (median = 39 d before calving)  
8 reduced the postcalving IMI prevalence and the incidence of pathogen-associated clinical  
9 mastitis postcalving by decreasing the incidence of new IMI (Parker et al., 2008).

10 The objective of this study was to evaluate whether losing the keratin plug in non-  
11 lactating heifers' teat canals would influence udder health during the first lactation. For this,  
12 84 non-lactating heifers from dairy farms in Lower Saxony were sampled under field  
13 conditions, and the relations among the opening of the teat canal, IMI and the development of  
14 clinical mastitis were described.

## 16 **2. Materials and methods**

### 18 *2.1. Herds and Animals*

19  
20 Six dairy herds in Northern Germany were selected to participate in the study. In all herds  
21  $\geq 50\%$  of the heifers had a first test-day cow-level SCC  $> 100,000$  cells/ml. Mean herd size  
22 was  $170 \pm 87$  (ranging from 100 to 328) cows. Mean lactational yield at the beginning of the  
23 survey amounted to  $9855 \text{ kg} \pm 650 \text{ kg}$ , mean geometric bulk milk SCC to  $184,000$  cells/ml  
24 (ranging from  $97,000$  to  $356,000$  cells/ml). Mean age at first calving was 25 months (SD = 2.5  
25 months). In every farm surveyed, heifers and cows were housed in a free-stall with cubicles in  
26 the rest area. Heifers were incorporated into the lactating herd at five to two weeks before

1 calving. All herds were fed a total mixed ration (lactating cows also received transponder-  
2 mediated concentrate in relation to their individual milk yield in three herds). Heifers were  
3 not milked before calving. Farms were equipped with herringbone (n = 4) or rotary (n = 2)  
4 milking parlours. During March and April 2005 100 German Holstein non-lactating  
5 heifers were selected that were reported to be between 195 and 210 days of gestation. Study  
6 animals had to be clinically healthy and pregnant at the start of the study. A total of 16  
7 animals were excluded due to absent gestation (n = 7) and several other culling reasons not  
8 associated with the udder (stroke of lightning, abortion, leg fracture; n = 9).

## 9 10 2.2. Sampling and laboratory procedures

11  
12 The clinical investigation of each heifer started 10 weeks before the anticipated calving  
13 date. Numbered collars and ear tags were used to identify the heifers in each herd. The heifers  
14 were immobilized and secretion samples for microbiological tests were taken from open  
15 udder quarters. The assessment of open or closed udder quarters was done using the method  
16 described by Williamson (2002). It involves applying light pressure to the teat sinus by using  
17 a gentle milking action with the thumb and index finger, allowing the contents to slip upward  
18 within the teat. If the pressure applied by this action results in a drop of secretion at the  
19 orifice, the teat is classified as being 'open', otherwise as 'closed'. Only open quarters were  
20 sampled. Due to the small volume of secretion present in most glands, foremilk secretion was  
21 not discarded strictly before collection of the sample *ante partum*. Sampling was repeated  
22 until parturition at 4-week intervals. After calving, duplicate samples of quarter foremilk were  
23 collected for bacteriological culturing in the first week and in intervals of 10 weeks up to the  
24 end of the first lactation. Additional samples were collected when clinical mastitis occurred.  
25 Teat ends were cleaned and disinfected with ethanol (70%) before sampling. Strict foremilk  
26 (first jets) was discharged, and then two times 10 ml of milk were collected aseptically from



1 each udder quarter into sterile vials. Samples were kept at 4°C until cyto-bacteriological  
2 examination took place. Milk samples from clinical mastitis cases were frozen at -20°C for up  
3 to four weeks before processing.

4 For culturing, 10 µl of each milk sample were spread on blood agar plates (5%  
5 defibrinated sheep blood, Oxoid, Wesel, Germany). The plates were incubated aerobically at  
6 37°C and examined after 24 h and 48 h. The colonies were provisionally identified on the  
7 basis of Gram stain, morphology, and haemolysis patterns and the numbers of each colony  
8 type were recorded. The representative colonies were then subcultured on blood agar plates  
9 and incubated aerobically at 37°C for 24 h to obtain pure cultures. Catalase and coagulase  
10 production was tested for Gram-positive cocci. Specific identification of staphylococci was  
11 made using the coagulase test. Gram-positive, catalase-negative isolates were tested by  
12 CAMP, aesculin-reaction, growth at 45°C and commercial micromethods (Oxoid DR0575M  
13 Strep Plus Kit, Germany). Some Gram-positive rods were identified with simple procedures,  
14 e.g. *Corynebacterium bovis*. Gram-negative rods were subcultured on Violet Red Bile Agar,  
15 tested afterwards for oxidase and indole-reaction and additionally cultured in triple sugar iron  
16 agar and Simmons citrate agar. This method allowed bacteria identification of genus or  
17 species level in most cases; otherwise, unidentified organisms were recorded as either Gram-  
18 negative or Gram-positive.

19 Culture status of milk samples was defined according to the procedures recommended by  
20 the German Veterinary Association (GVA, 2002). Precalving, a quarter was considered to  
21 have an IMI when > 500 CFU/ml of a bacterial species was cultured, and 1 to 3 bacterial  
22 species were isolated. In postcalving samples, IMI was considered if in duplicate samples >  
23 500 CFU/ml of the same bacterial species was cultured, and 1 to 3 bacterial species were  
24 isolated. A milk sample was defined as being contaminated if > 3 bacterial species were  
25 isolated. The SCC of every milk sample was determined by fluorescence (Fossomatic 360,  
26 Foss Electric, Denmark).

1

2 *2.3. Statistical analyses*

3 The association between teat canal closure before calving, bacteriological findings and  
4 udder health in the first lactation was analyzed using binary (multinomial) logistic regression  
5 (SPSS 14.0, Chicago USA; Urban, 1993). Binary dichotomous dependent variables comprised  
6 clinical mastitis (CM) during the first five days of lactation, during the first 100 days of  
7 lactation, during the entire lactation, and a lactational geometric mean SCC > 100,000  
8 cells/ml (“high SCC”; HSCC). Independent variables (covariates, factors) were the number of  
9 days before calving the teat canal of a given quarter was open, number of culture-positive  
10 findings per quarter prior to calving and the identification of bacteria (group, genus, species).  
11 Values for the latter variable could be minor pathogens (including CNS and *Corynebacterium*  
12 *bovis*), major pathogens including *S. aureus*, *S. uberis* and coliforms if the pathogens were  
13 found exclusively in a given udder quarter for one to three times. If bacteriological analysis  
14 yielded more than one type of pathogen during all precalving samples from a given quarter,  
15 this variable was rated as mixed. A backwards stepwise elimination process was used for  
16 model selection, applying a  $P$ -value < 0.05 for retention. Models were calculated on both  
17 quarter and cow level. Odds ratios (OR) with 95% confidence intervals (95% CI) were  
18 calculated. The association between culture-status precalving and SCC in the subsequent  
19 lactation was determined using standard ANOVA methods. To approximate the normal  
20 distribution, a  $\log_{10}$  transformation of SCC was used. Statistical significance was assumed at  $P$   
21  $\leq 0.05$ .

22

23 **3. Results**

24

25 An increase of open teat canals was detected in the precalving period (Table 1). No open  
26 teat canals were found 80 days before parturition. From this time, the number of open teat

1 canals increased continuously until calving; 60% of the quarters were already open at two  
2 months precalving. Opening pattern included two patterns: opening of all quarters of an udder  
3 at the same sampling event, or accumulative opening of the quarters over two or three  
4 sampling events (57 and 43% of the cows, respectively). Almost 66% of secretion samples  
5 from quarters with open teat canals were culture-positive (Table 1). A time-dependent  
6 infection pattern was observed: during the intervals of 90 to 61, 60 to 31, and 30 to 0 days  
7 before calving, skin inhabitants (CNS, *C. bovis*), cow-related, and environment-related  
8 microorganisms prevailed, respectively. Infections with CNS and with *S. aureus* persisted  
9 beyond parturition. While the number of culture-positive secretion samples increased  
10 continuously towards calving (63% of all quarters by the last pre-calving sampling), the  
11 number of positive secretion samples dropped to 18% in the first week after calving.

12 In 77% of the quarters that were culture-positive after calving the same bacterial species  
13 was found before calving. With increasing days in milk (DIM) prevalence of CNS IMI  
14 decreased, while the prevalence of *S. uberis* IMI increased. After calving, three quarters in  
15 two animals became non-functional. A total of 27 clinical mastitis cases occurred in 25  
16 animals during the entire first lactation. Of these, 15 cases (56%) had developed by 5 DIM,  
17 while 26 (96%) cases did so by 100 DIM. The pathogen responsible for the 15 clinical  
18 mastitis cases in the first 5 DIM corresponded, as far as morphological, serological and  
19 biochemical characteristics are concerned, to the one found at the last precalving sampling  
20 event. 85% of all clinical mastitis cases during the first 100 DIM and 74% of all clinical  
21 mastitis cases in the first lactation were found in quarters that had an open teat canal at 10  
22 days before parturition.

23 Precalving secretion samples with a major pathogen IMI had a 19.8 (95% CI: 5.0 – 77.2)  
24 higher odds for clinical mastitis during the first five DIM. Similar results were encountered  
25 for the development of a clinical mastitis case in the first 100 DIM, over the entire first  
26 lactation, and for HSCC (Table 2). The goodness-of-fit of the models was acceptable with

1 deviance values of 51 with 129 degrees of freedom (df) for CM in the first 5 DIM, 71 (df 128)  
2 for CM in the first 100 DIM, 74 (df 128) for CM in the first lactation, and 71 (df 128) for  
3 HSCC. In general, cows with culture-positive findings before calving were more likely to  
4 develop clinical mastitis in each of these three periods.

5 It was not possible to determine a significant association between the days a quarter was  
6 found open and the incidence of CM 0-5, 0-100 DIM, over the first lactation and HSCC.  
7 However, the duration being open was positively associated with the number of positive  
8 bacteriological findings before calving. The risk to develop an udder disease, especially  
9 subsequent to parturition, increased proportionally with the time the quarter has been infected  
10 and the degree of pathogenicity of the infecting agent (Table 2).

11 Mean geometric SCC of all lactating quarters for all four sampling events after calving  
12 was 18,000 cells/ml milk (range: 5,000 – 11,255,000 cells/ml). The analysis of variance with  
13 repeated measurements showed a significant influence of the number of culture-positive  
14 findings per quarter prior to calving on the SCC of quarter milk, considering the animal and  
15 its effects ( $F = 6.22$ ;  $P < 0.001$ ). The one-way analysis of variance yielded another significant  
16 influence of number of culture-positive findings per quarter prior to calving on the quarter  
17 SCC of the first sample after parturition ( $F = 9.78$ ;  $P < 0.001$ ). The subsequent post-hoc  
18 analysis (LSD) demonstrated that particularly a culture-positive finding more than one month  
19 before calving lead to increased SCC at first sampling after calving.

20

#### 21 **4. Discussion**

22

23 The current study demonstrated that “open quarters“, being quarters not sealed by keratin,  
24 may occur already in non-lactating heifers at 10 weeks before calving. This is in contrast to  
25 the results presented by Williamson (2002) who detected the first unsealed udder quarters in  
26 heifers as early as four weeks ante partum. These differences may possibly be related to

1 genetic effects, because the method applied for identifying open teat canals was well  
2 reproducible. In accordance with the data provided by Williamson (2002) we encountered  
3 culture-positive secretions in an approximately 66% of “open” quarters before calving. The  
4 results obtained in this survey support his thesis claiming the possibility that many early  
5 lactation mastitis cases initiated in the precalving phase. This can be assumed in 67% of the  
6 clinical mastitis cases during the first five DIM, although conclusive biomolecular  
7 comparisons of strains were not done. However, it could not be proven that those quarters  
8 with a relatively long time of being “open” suffered an increased risk of becoming mastitic  
9 after parturition. Since Dingwell et al. (2004) were able to show that forming the keratin plug  
10 at an early stage of the dry period reduced the risk of new infection significantly in cows, it  
11 may deduced that a functional keratin plug also protects heifers from new IMI.

12 The array of mastitis pathogens encountered, comprising CNS, *S. aureus* and  
13 environment-associated bacteria in descending order of relevance, during all three sampling  
14 periods (precalving, colostrum phase, early lactation) corresponds largely to bacteriological  
15 distributions provided by Oliver and Mitchell (1983), Trinidad et al. (1990), Pankey et al.  
16 (1991), Roberson et al. (1994a), Fox et al. (1995), Myllys (1995), and Aarestrup and Jensen  
17 (1997). These results suggest that a bacterial transmission via “suckling” or “flies” may be  
18 neglected, at least in the case of the animal population surveyed for the present study. It  
19 appears more likely that the infection takes place because microorganisms in the immediate  
20 vicinity of the teat canal enter this open canal spontaneously. The time-related pattern in  
21 which different microorganisms occur in the heifer secretion samples reflects the results  
22 obtained in previous papers (Fox et al., 1995; Myllys 1995; Aarestrup and Jensen, 1997;  
23 Oliver et al., 2004).

24 This study showed clearly that the moment of bacteriological colonisation and the kind of  
25 pathogen involved do have an effect on the udder health (quarter and cow levels) during the  
26 first lactation. These results are in accordance with the observations made by de Vliegher et

1 al. (2003), as well as Roberson et al. (1994b). A long-lasting colonisation of microorganisms  
2 within the juvenile gland quarter promotes the development of udder diseases after calving. In  
3 those, *S. aureus*, *S. uberis* and coliform IMIs exert a markedly stronger influence than those  
4 with CNS and coryneforms. More research (including specific pathogen isolation) will be  
5 needed if it is intended to investigate whether the colonisation by CNS really has a protective  
6 effect for the udder. Due to its extent, the present study may be referred to as a mere pilot  
7 study. The results of this study indicate that avoiding the early colonisation (i.e., precalving)  
8 of the glandular tissue by pathogenic bacteria could restrict the development of heifer  
9 mastitis. This translates fundamentally into therapeutic measures that focus on avoiding new  
10 infections in heifers' udders; however, other means, e.g. breeding for long and narrow teat  
11 canals, keeping animals in a pathogen-reduced environment, and avoiding udder and teat  
12 oedemas caused by inappropriate feeding, should not be neglected. The efficiency of the  
13 measures should depend greatly on how long a new IMI is avoided. This hypothesis is  
14 supported by two recent papers from New Zealand that tested the effectiveness of internal teat  
15 sealers in heifers. The results obtained there showed that applying a teat sealer at 39 days  
16 before calving (median value) reduced the prevalence of both postcalving IMI and the  
17 incidence of pathogen-associated clinical mastitis during the same period (Parker et al., 2007;  
18 Parker et al., 2008). The results of the present study indicate that a successful deployment of  
19 internal teat sealers is likely to depend on the moment a given quarter becomes unsealed and  
20 on the moment the product is applied.

21 The number of heifers in this study was limited. A study of a larger population is  
22 indicated before results of this study can be generalized. Nevertheless, this study strongly  
23 suggests that a high proportion of teat canals already opens several months before calving and  
24 that this is an important factor in the aetiology of heifer mastitis.

25

1 **Conflict of interest**

2

3 None of the authors (V. Kroemker, J. Friedrich) has a financial or personal relationship  
4 with other people or organizations that could inappropriately influence or bias the paper  
5 entitled “Teat canal closure in non-lactating heifers and its association with udder health in  
6 the consecutive lactation”.

7

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- 15

1 Table 1  
 2 Time-related opening of quarters and corresponding bacteriological findings in 84 German  
 3 dairy heifers

Examination Date <sup>a</sup>	Open							
	quarters (n)	Neg. (%) <sup>b</sup>	CNS (%) <sup>b</sup>	<i>C. bovis</i> (%) <sup>b</sup>	<i>S. uberis</i> (%) <sup>b</sup>	<i>S. aureus</i> (%) <sup>b</sup>	Coliforms <sup>c</sup> (%) <sup>b</sup>	Other (%) <sup>b</sup>
-90 to -61	127	42	48	10				
-60 to -31	215	43	44	4		9		
-30 to 0	269	21	61	4	6	4	4	1
0 to 5	333 <sup>d</sup>	82	13	0	1	2	1	1
70 to +80	333	87	9	0	2	2	0	0
140 to 150	333	87	9	0	2	1	1	0
210 to 220	333	81	11	1	3	2	1	1

4 <sup>a</sup> Days in relation to calving

5 <sup>b</sup> % of the open quarters

6 <sup>c</sup> *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp. or *Citrobacter* spp.

7 <sup>d</sup> 3 quarters were non-functional

8

1 Table 2  
 2 Final logistic regression models for the probability of a quarter (n = 336; Table 2a) or cow (n  
 3 = 84; Table 2b) of developing a clinical mastitis during the first five days of lactation (CM5),  
 4 during the first 100 days of lactation (CM100), during the entire lactation (CMLAC) or  
 5 having a lactational geometric mean SCC value > 100,000 cells/ml (“high SCC”; HSCC)

	Covariate/Factor	Quarter			Odds ratio	95% CI (OR)
		Coefficient	S.E.	P		
CM5	Intercept	-5.97	1.07	0		
	Interaction of the number of positive bacteriological findings (PBF <sup>a</sup> ) and bacteriological results (MIP <sup>b</sup> , MIX <sup>c</sup> , MJP <sup>d</sup> )					
	PBF x MIP	1.19	0.41	0	3.3	1.5, 7.4
	PBF x MIX	1.51	0.41	0	4.5	2.0, 10.2
	PBF x MJP	2.98	0.69	0	19.8	5.0, 77.2
CM100	PBF x N <sup>e</sup>	Reference				
	Intercept	-3.55	0.56	0		
	PBF x MIP	1.20	0.33	0	3.3	1.7, 6.4
	PBF x MIX	1.40	0.35	0	4.0	2.0, 8.1
	PBF x MJP	2.54	0.56	0	12.7	4.2, 38.3
CMLAC	PBF x N	Reference				
	ODQ <sup>f</sup>	-0.03	0.01	0.02	0.97	0.95, 0.99
	Intercept	-2.94	0.47	0		
	PBF x MIP	1.23	0.31	0	3.4	1.9, 6.2
	PBF x MIX	1.35	0.33	0	3.8	2.0, 7.4
HSCC	PBF x MJP	2.13	0.54	0	8.4	2.9, 24.0
	PBF x N	Reference				
	ODQ	-0.03	0.01	0	0.97	0.95, 0.99
	Intercept	-3.86	0.48	0		
	PBF x MIP	0.79	0.21	0	2.2	1.5, 3.3
	PBF x MIX	1.03	0.23	0	2.8	1.8, 4.4
	PBF x MJP	2.18	0.45	0	8.8	3.7, 21.4
	PBF x N	Reference				

6

	Covariate/Factor	Cow			Odds ratio	95% CI (OR)
		Coefficient	S.E.	P		
CM5	ODQ <sup>g</sup>	0.02	0.03	0.48	1.0	0.9, 1.1
	PBF	1.26	0.59	0.03	3.5	1.1, 11.2
	Constant	-5.60	1.72	0		
CM100	ODQ	-0.02	0.02	0.20	1.0	0.95, 1.0
	PBF	1.18	0.46	0.01	3.3	1.3, 7.9
	Constant	-2.35	0.76	0		

CMLAC	ODQ	-0.02	0.02	0.15	1.0	0.95, 1.0
	PBF	1.03	0.42	0.01	2.9	1.2, 6.3
	Constant	-1.71	0.67	0.01		
HSCC	ODQ	0.01	0.02	0.96	1.0	0.97, 1.0
	PBF	0.55	0.46	0.23	1.7	0.7, 4.2
	Constant	-2.69	0.90	0		

1 <sup>a</sup> positive bacteriological findings

2 <sup>b</sup> open quarter & positive 1 to 3 times for CNS-positive or *Corynebacterium bovis*

3 <sup>c</sup> open quarter & positive 1 to 3 times for different pathogens

4 <sup>d</sup> open quarter & positive 1 to 3 times for *Staphylococcus aureus*, *Streptococcus uberis* and  
5 coliforms

6 <sup>e</sup> open quarter & bacteriologically negative

7 <sup>f</sup> open days of a given quarter

8 <sup>g</sup> open days of the first quarter of the udder