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# Teat canal closure in non-lactating heifers and its association with udder health in the consecutive lactation

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**Abstract**

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

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

## 1. Introduction

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

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

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

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

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

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

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

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

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

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

## 2. Materials and methods

### *2.1. Herds and Animals*

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

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

*2.2. Sampling and laboratory procedures*

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

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

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

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

*2.3. Statistical analyses*

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

**3. Results**

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

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

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

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

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

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

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

## 4. Discussion

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

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

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

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

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

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

## Conflict of interest

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

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Table 1

Time-related opening of quarters and corresponding bacteriological findings in 84 German dairy heifers

| Examination Date[a] | Open quarters (n) | Neg. (%)[b] | CNS (%)[b] | *C. bovis* (%)[b] | *S. uberis* (%)[b] | *S. aureus* (%)[b] | Coliforms[c] (%)[b] | Other (%)[b] |
|---|---|---|---|---|---|---|---|---|
| -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[d] | 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 |

[a] Days in relation to calving

[b] % of the open quarters

[c] *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp. or *Citrobacter* spp.

[d] 3 quarters were non-functional

Table 2

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

| | Covariate/Factor | Quarter Coefficient | S.E. | *P* | Odds ratio | 95% CI (OR) |
|---|---|---|---|---|---|---|
| CM5 | Intercept | -5.97 | 1.07 | 0 | | |
| | Interaction of the number of positive bacteriological findings (PBF[a]) and bacteriological results (MIP[b], MIX[c], MJP[d]) | | | | | |
| | 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 |
| | PBF x N[e] | Reference | | | | |
| CM100 | 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 |
| | PBF x N | Reference | | | | |
| | ODQ[f] | -0.03 | 0.01 | 0.02 | 0.97 | 0.95, 0.99 |
| CMLAC | 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 |
| | 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 |
| HSCC | 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 | | | | |

| | Covariate/Factor | Cow Coefficient | S.E. | *P* | Odds ratio | 95% CI (OR) |
|---|---|---|---|---|---|---|
| CM5 | ODQ[g] | 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 | | |

[a] positive bacteriological findings

[b] open quarter & positive 1 to 3 times for CNS-positive or *Corynebacterium bovis*

[c] open quarter & positive 1 to 3 times for different pathogens

[d] open quarter & positive 1 to 3 times for *Staphylococcus aureus*, *Streptococcus uberis* and coliforms

[e] open quarter & bacteriologically negative

[f] open days of a given quarter

[g] open days of the first quarter of the udder