Persistence patterns of bacterial outcomes and somatic cell counts in Dutch cow's milk related to *Staphylococcus aureus*.

M. Fokker

Bsc. Student at the Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL, Utrecht, The Netherlands

Introduction

Mastitis is one of the biggest problems in dairy farming in frequency and economy (Vasudevan *et al.*, 2003; Wellnitz *et al.*, 2012). Most cases of mastitis are caused by bacteria. The most important mastitis bacteria in cows are *Staphylococcus aureus* (Syring *et al.*, 2012). In heifers, *Staphylococcus aureus* is number two after the coagulase negative staphylococci (CNS) (Paradis *et al.*, 2010)(Schukken *et al.*, 2009). Infections with *Staphylococcus aureus* are very often highly problematic because of the different 'strategies' the bacteria can apply to avoid the immune system of the cow and to resist or avoid antibacterial treatment (Foster, 2005). The ability of producing a biofilm gives rise to the virulence properties of this bacterium (Vasudevan *et al.*, 2003). That biofilm supports the connection with the host's tissue and forms a layer around the bacteria so it cannot be detected by the host's immune cells , e.g. macrophages and granulocytes. In human found *Staphylococcus aureus* seems to have genetic clusters encoding for these and other immune evasion strategies, however, these genetic clusters are not found in animals yet (Kim *et al.*, 2012). The fact that *Staphylococcus aureus* is able to avoid an immune reaction leads to different outcomes in somatic cell count and laboratory detection for presence of the bacterium. In other words, somatic cell count can be low while *Staphylococcus aureus* is present in the udder or milk, , but also high when there is an immune reaction. However, most cases of mastitis by *Staphylococcus aureus* are characterized by a delayed or moderate increase in somatic cell count (Bannerman *et al.*, 2004). Infection with *Staphylococcus aureus* can result in a subclinical, chronic or even life-long infection (Sutra *et al.*, 1994; Wellnitz *et al.*, 2012). When a cow is infected during the first pregnancy the somatic cell count in the lactation following tends to be 1.2 times higher than in non-infected heifers (Paradis *et al.*, 2010).

It should be noted that a high somatic cell count can't always be interpreted as negative because evidence shows that cows with a slightly higher somatic cell count have a decreased risk of intramammary infection (Suriyasathaporn *et al.*, 2000). However, the majority of studies confirm the theory that somatic cell count and risk for mastitis are positively correlated (Sears *et al.*, 1990; Schukken *et al.*, 1999; Borne, B. H. P. van den *et al.*, 2011). When *Staphylococcus aureus* enters the

teat canal it is able to cause tissue damage and inflammation, but due to the biofilm, mentioned before, the bacteria can stay for a long period without clinical signs, sometimes characterized by elevated somatic cell count. Cows infected in that way are a threat for the herd because transmission to other cows is possible. Since *Staphylococcus aureus* is a cow-connected causative agent for mastitis, the milking system is playing a big role in the transmission. Given that fact, most effective to protect the herd from transmission are proper milking procedures, disinfection of teats after milking, biosecurity to prevent introduction and careful segregation or culling of chronic infected cows (Barkema *et al.*, 1998). Cure rate of infected animals is very low but negatively correlated with the duration of the infection (Barkema *et al.*, 1998).

Because *Staphylococcus aureus* is difficult to combat and an important causative bacteria for mastitis preventive strategies should be developed. Therefore, it is important to know more about the behavior of the bacteria in healthy cows. Aim of this study is to investigate the pattern of bacterial presence in milk around calving, from dry cow treatment to 7-21 days postpartum. Analyses are done to investigate the relation between such a pattern and the somatic cell count at the moment of dry cow treatment.

Materials and Methods

Data for this analysis was extracted from the dataset resulting from an investigation by the Animal Health Service in Deventer, Holland. Milk samples were taken from animals with a low (heifers <150.000 cells/ml and cows <250.000 cells/ml) somatic cell count based on the last results of the milk production registration. Sampling was done at three times, for each quarter: when dry cow treatment was started, within 24 hours after calving and 7-21 days after calving. Samples were also taken in cases of clinical mastitis while the project was running, i.e. from starting the dry cow treatment till day 100 of lactation. On all these samples bacterial examination was done using agar plates. Somatic cell count was also investigated. For this particular paper results of investigation were used from cows that tested positive at least once for *Staphylococcus aureus*. The seven possible outcomes will be referred to as A. B. C. D. E. F. G. as shown in table 1. Frequencies of these patterns were counted.

In the overall project 100 common-managed farms were involved. In this analysis 66 different farms are selected. In total 130 cows were included. All the animals had parity higher than two.

Pattern Category	Moment of dry cow treatment	Moment of calving	Moment of 7-21 days lactation
A	1	1	1
B	1	0	1
C	1	1	0
D	1	0	0
E	0	1	0
F	0	0	1
G	0	1	1

Table 1: Different bacterial outcomes; 0 is for negative and 1 for positive outcome of bacterial examination for Staphylococcus aureus. The patterns are the product of subsequent outcomes at dry off, calving and three weeks of lactation

Because of the low frequency, the patterns A, B and C were omitted from the analyses. Somatic cell count was investigated and initially divided into three categories. Category 1 (somatic cell count 0-250.000 cells/ml) is interpreted as good udder health and category 2 (251.000 - ∞ cells/ml) is interpreted as subclinical or clinical mastitis in multiparous cows. These borders were taken because of the fact that quarters with cell counts below 250.000 cells/ml in multiparous cows are mentioned to be healthy under Dutch circumstances (Borne, van den, 2010). Since the frequency of category 2 was very low this category is combined with category 3 which is the new category 2 (see figure 4a and 4b). Logistic regression was done to investigate the relation between somatic cell count on the moment of dry cow treatment and the somatic cell count on the moment of the 14 days lactation.

Odds ratio is calculated to investigate the predictive value of somatic cell count category at dry off for the value of the somatic cell count category in early lactation (7-21 days postpartum).

Results

The frequency of the patterns A, B, C, D, E, F and G were 4, 0, 1, 14 43, 37, 34 respectively (figure 1, table 2). The patterns A, B and C showed a very low frequency. These are the patterns with a positive outcome of bacterial investigation on the dry off moment. Since only cows with a somatic cell count below 250.000 cells/ml were selected in the project this is not an unexpected result. Pattern D had a positive outcome of bacterial investigation at dry off too, so visibly healthy cows can actually test bacterially positive. This is also visible in figure 2a and 2b where the outcomes of the bacterial investigation where showed by somatic cell count category at dry off and early lactation.

PATTERN	FREQUENCY	RELATIVE FREQUENCY
A(1-1-1)	4	3,01%
B(1-0-1)	0	0,00%
C (1-1-0)	1	0,75%
D(1-0-0)	14	10,53%
E(0-1-0)	43	32,33%
F(0-0-1)	37	27,82%
G(0-1-1)	34	25,56%
TOTAL	133	100,00%

Table 2: Absolute and relative frequency of different patterns explained in table 1.

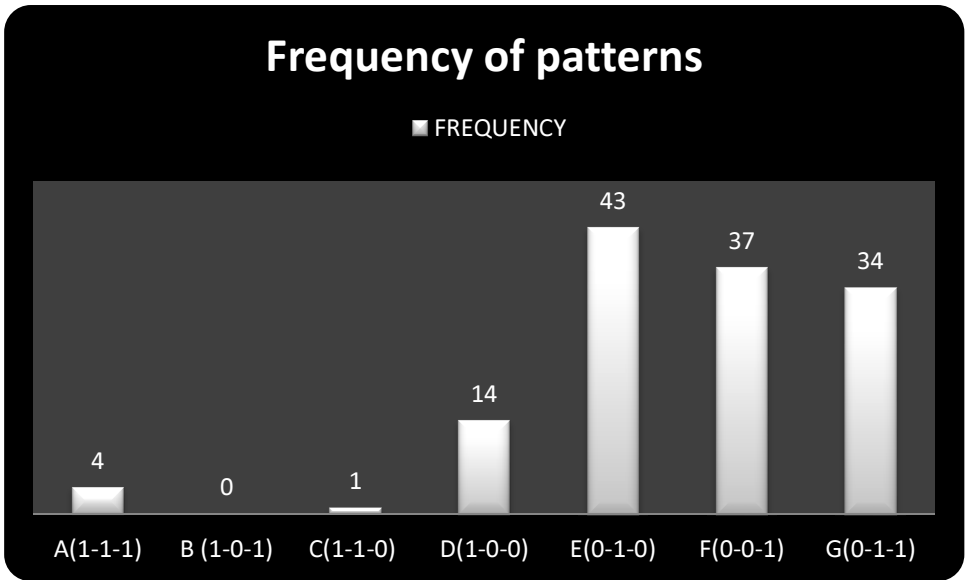

Figure 1. Table 2 converted in a graphical representation. Patterns are explained in table 1.

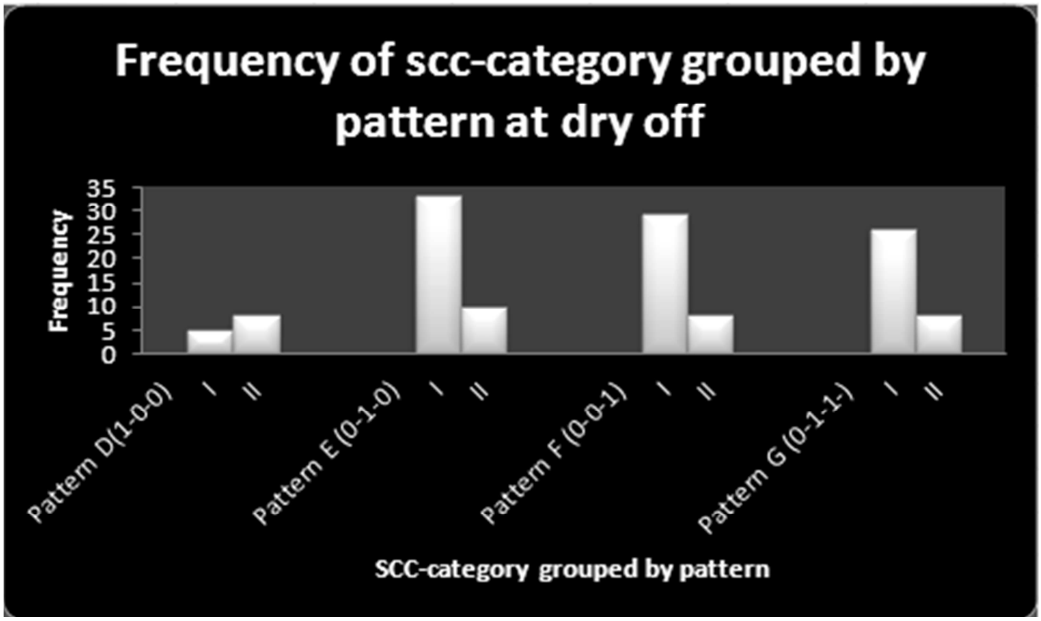

Figure 2a: Frequency of somatic cell count categories explained in table three grouped by pattern at dry off. Due to the low prevalence the patterns A, B and C are not included in the results.

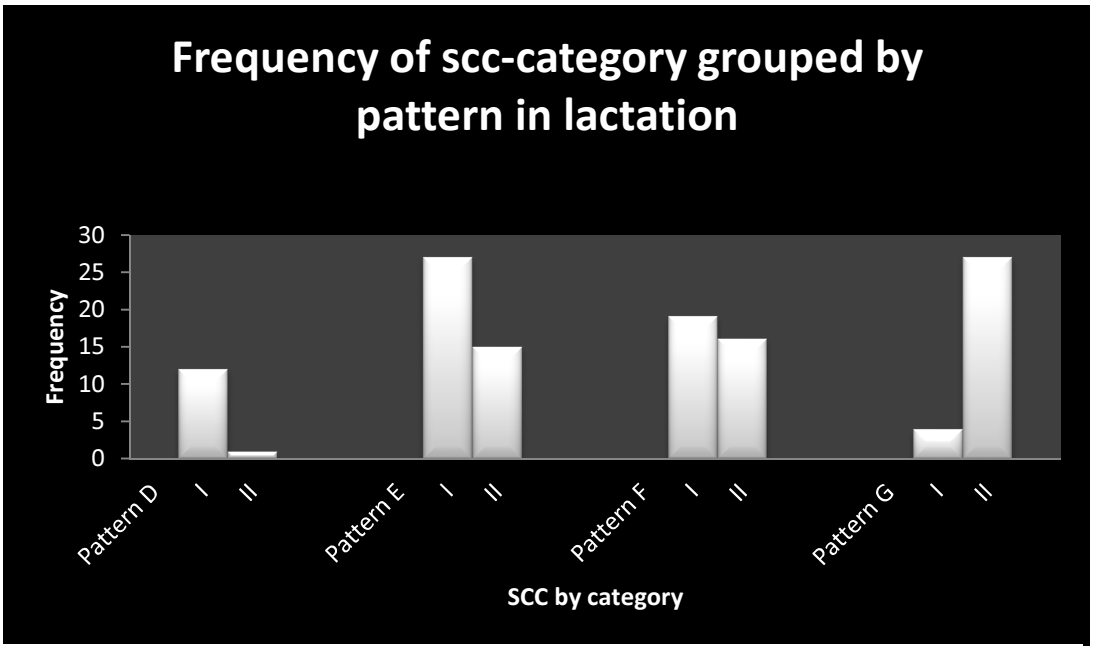

Figure 2b: Frequency of somatic cell count categories explained in table three grouped by pattern at 1-3 weeks of lactation. Due to the low prevalence the patterns A, B and C are not included in the results.

A scatterplot of the somatic cell counts at dry off and at early lactation showed no clear relation (Figure 3a). A log transformation of somatic cell counts was done to correct for the unequal variance

and a scatterplot was made again (see figure 3b). A weak negative trend is visible. The coefficient of determination, R^2, was 0.0062 which means that 0,0062% of the somatic cell count at lactation was influenced by the somatic cell count at dry off. With a p-value of 0,95 it is clear that there is no relation between the somatic cell count at dry off and in the early lactation. Odds ratio for somatic cell count category at dry off as predictive value for the somatic cell count category was 1,752 with a confidence interval between 0,810 and 3.789. Since the confidence interval contains 1 there is no indication for somatic cell count category at dry of to be predictive for the somatic cell count category in early lactation.

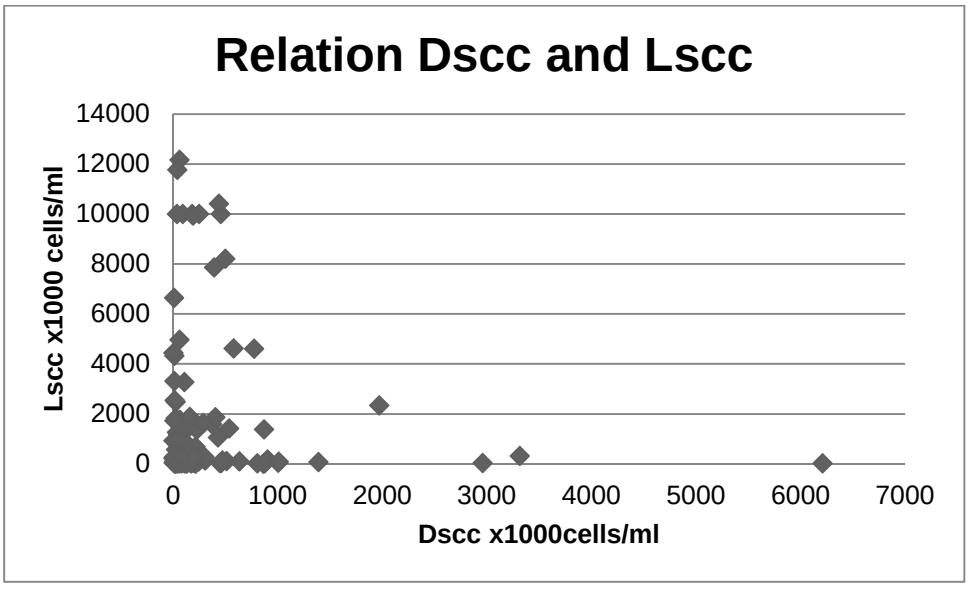

Figure 3a: Relation between the somatic cell count at dry off (Dscc) and the somatic cell count at 1-3 weeks of lactation (Lscc).

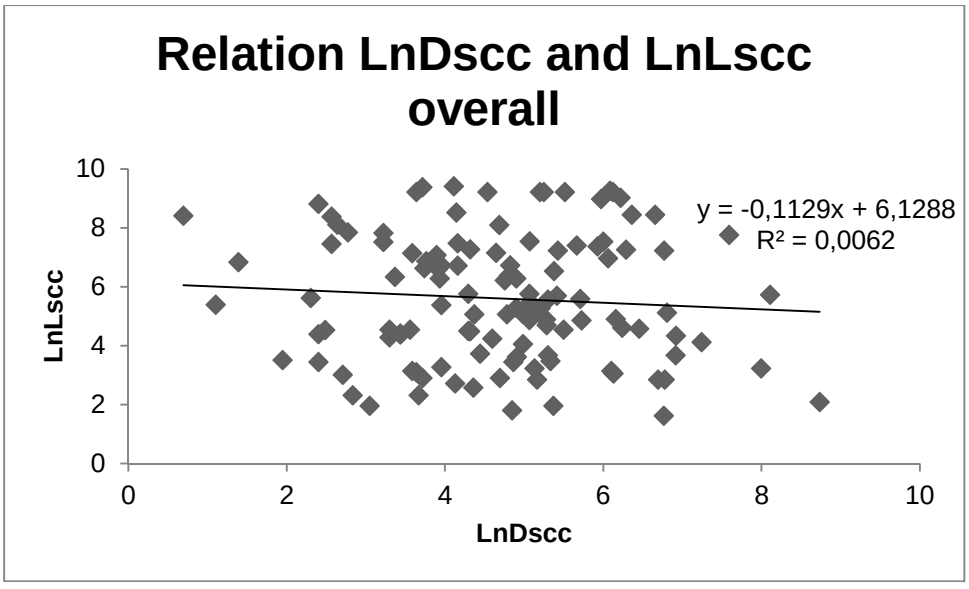

Figure 3b: Relation between the natural logarithm of the somatic cell count at dry off and the somatic cell count at 1-3 weeks of lactation. Natural logarithm is used for stabilization of variance by correction for points with huge deviations

Pattern D shows a little higher frequency of quarters with a high somatic cell count level at dry off than quarters within the low somatic cell count category. In lactation all the quarters were in the category of low somatic cell count. Pattern E shows a higher frequency of quarters within category 1 than in category 2 at dry off. In lactation was also a higher frequency of quarters in category 1 than category 2. Pattern F has the same properties as pattern E. In pattern G a high frequency of quarters within category 1 is visible at dry off but during lactation a high frequency of quarters within category 2 is seen. Significance of differences was not calculated.

Relation between somatic cell count and bacterial outcome (figure 4).

Category 1 and 2 of the somatic cell counts gave both a higher frequency of a negative outcome of bacterial investigation on the moment of dry cow treatment. Lactation category 1 gave more negative outcomes of bacterial investigation but in category 2 there was a higher frequency of positive outcomes for bacteriology.

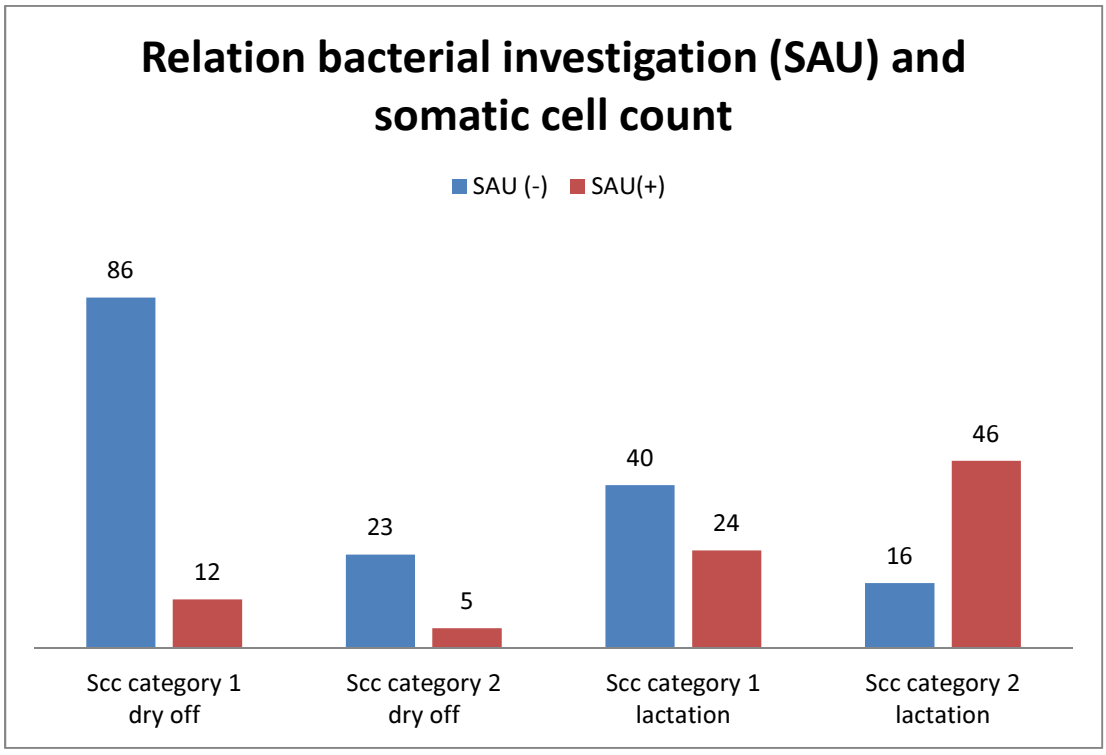

Figure 4: Outcomes of bacterial examination. This paper is only focused on Staphylococcus aureus so other bacteria are not included. At the moment of dry cow treatment the majority of cows had a low somatic cell count (category 1). Most of the cows were bacteriologically negative. In somatic cell count category 2 were relatively more SAU positive cows (18% compared with 12% in category 1)
In lactation somatic cell count category 1 had the highest amount of bacterial negative outcomes (62,5%). In somatic cell count category 2 the amount of bacterial positive outcomes was 74%. This suggests that the relation between a high somatic cell count and the presence of Staphylococcus aureus is stronger during early lactation compared to late lactation.

Odds-ratio between diagnostic results for bacterial examination from dry cow treatment and lactation samples was 0,162 with a 95% confidence interval from 0,050 till 0,518.

A cross tabulation for the bacteriology outcomes and somatic cell counts in lactation showed that the ratios between somatic cell count category 1 and category 2 is 3:1 from a start situation of bacterial negativity (table 4). For the positive samples (BO =1) the ratio between category 1 and 2 for the total amount of samples is 1:3 too but there is a greater difference between the different categories of somatic cell count. A bacterial positive cow with a low somatic cell count has a chance of 64.4% to get a high somatic cell count in lactation. When a cow is bacterial positive with a high somatic cell count than de risk for a high somatic cell count is 84.6%.

For the relation between bacterial outcomes at dry off and in the lactation an Odds-ratio was found to be 0.162 with a 0.95 confidence interval between 0.050 and 0.518.

groepdscc01 * grouplscc01 * BO_lactatie Crosstabulation

BO_lactatie				grouplscc01 1,00	grouplscc01 2,00	Total
,00	groepdscc01	1,00	Count	31	11	42
			% within groepdscc01	73,8%	26,2%	100,0%
		2,00	Count	12	4	16
			% within groepdscc01	75,0%	25,0%	100,0%
	Total		Count	43	15	58
			% within groepdscc01	74,1%	25,9%	100,0%
1,00	groepdscc01	1,00	Count	17	31	48
			% within groepdscc01	35,4%	64,6%	100,0%
		2,00	Count	3	19	22
			% within groepdscc01	13,6%	86,4%	100,0%
	Total		Count	20	50	70
			% within groepdscc01	28,6%	71,4%	100,0%
Total	groepdscc01	1,00	Count	48	42	90
			% within groepdscc01	53,3%	46,7%	100,0%
		2,00	Count	15	23	38
			% within groepdscc01	39,5%	60,5%	100,0%
	Total		Count	63	65	128
			% within groepdscc01	49,2%	50,8%	100,0%

Table 4: Cross tabulation shows relation between somatic cell count and outcomes of bacterial examination. A dry off situation with a negative outcome of bacteriological examination and a low somatic cell count gives a chance of 73% for a low somatic cell count in lactation. On the same moment a high somatic cell count gives a similar probability (75%) for a low somatic cell count in the lactation. For bacterial positive cows the probability for a low somatic cell count in lactation from a low somatic cell count at dry off 35%. Bacterial positive cows with a high somatic cell count on the moment of dry cow treatment have a probability of 60,5% to come in the high somatic cell count category in lactation.

Discussion

In this study only healthy cows where selected. This healthiness was based on the somatic cell count at the last milk production registration which could be six weeks before the first sampling. This study was done at quarter level, but there is no data about the history of the quarters since a healthy cow following the criterion above can have unhealthy quarters covered in somatic cell count of the other quarters. In other words, the selected cows in this project had a low mean somatic cell count but individual quarters assessed may have had a somatic cell count higher than 250.000 cell/ml. Also, half of the udder was treated with antibiotic dry cow therapy but it is unknown which quarters in this study had received this treatment and which did not, which might have a great influence on the persistence pattern of the bacteria and the somatic cell counts. Only *Staphylococcus aureus* was involved in this study where there are many more pathogens to create mastitis. Furthermore the outcome of bacterial investigation to *Staphylococcus aureus* has a low negative prediction value because of the biofilm production, which keeps the bacteria stick in the udder. Also the fact that this bacterium can hide intracellular decreases the value of bacterial investigation of milk samples. These properties make it sometimes hard to grow *Staphylococcus aureus* on a plate.

The patterns give the result of subsequently bacteriology, but it is not known whether the infection is persistent or new. This could be investigated by genotyping and polymerase chain reactions followed by gel electrophoresis, but that was not done. More work can be done on other pathogens or in differentiation new infections or persistent infections by molecular epidemiology.

Conclusion

There is no relation between the somatic cell count at dry-off and the somatic cell count in lactation. There is evidence for a relation between the somatic cell count and the outcomes of bacterial investigation in perspective that a higher somatic cell count has more often a positive outcome of bacteriology. This relation is not the same at dry off as it is in the lactation, since this relation is better visible in lactation than at dry-off. Cows which are bacterially negative at dry off have a six times higher chance to become positive in lactation.

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