Biological Basis for Selection on Udder Health Traits

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1. Introduction

Udder health has been an important component of dairy farming for the last decades. Udder health disorders were always related to a decreased profitability, and an increase in unexpected culling. More recently, udder health is becoming more important due to strict milk quality regulations. In all dairy exporting regions (such as Europe, and North America), bulk milk somatic cell count has been used a criteria for milk quality (Schukken et al., 1992). This has further increased the attention of the dairy industry towards udder health. Udder health is affected by a number of interrelating components such as presence and pathogenicity of micro organisms, environment and management, cow factors such as conformation and immunological performance, and treatment and prevention strategies. A number of these components may be steered through genetically based processes (Emanuelson et al., 1988; Shook 1989; Poso and Mantysaari, 1996). Therefore the importance of genetic selection for functional traits besides milk production has become eminent. This is especially important since a positive genetic correlation exists between milk production on one hand and somatic cell score and clinical mastitis incidence on the other hand (Poso and Mantysaari, 1996). Hence, selection solely on milk yield may have a deteroriating effect on udder health. In this presentation, the biological basis for selection on udder health traits is discussed.

2. Udder health

A healthy udder is not infected with major pathogens, is not affected by clinical mastitis, and is not the cause of unwanted culling. Healthy udders should have a low somatic cell count (SCC). In a recent paper, Schepers et al. (1997) studied SCC in uninfected cows without a history of clinical mastitis. Mean SCC in uninfected quarters was 20.000 cells/ml, with a slight increase towards the end of lactation (40.000 cells/ml), and very little difference between parities. Hardly any un-infected quarter showed SCC above 250.000 cells/ml. In infected quarters SCC increases sharply, and values above 10.000.000 are not an exception. At the herd level, bulk milk SCC is considered acceptable at level below 250.000 cells/ml. For regulatory reasons, BMSCC should be below 400.000 within the European Community (EC).

Clinical mastitis should preferably not occur in a healthy cow. However, approximately 20% of cows are experiencing one or more cases of clinical mastitis per lactation (Barkema et al., 1997; Hogan et al., 1987; Miltenburg et al., 1997). Clinical mastitis incidence is highest in early lactation, especially in heifers (Figure 1). Incidence of clinical mastitis increases with parity (Figure 2). Variability of clinical mastitis incidence between herds is large, and range from 0% to 100% of cows showing at least once a case of clinical mastitis per lactation.

Mastitis, both clinical and subclinical, is mostly caused by bacteria. These may be divided in three major groups: gram negative, where E.coli is the most important pathogen, and two groups of gram-positive bacteria: S.aureus, and non-agalactiae streptococci (S.uberis and S.dysgalactiae) (Figure 2). In a recent study, Lam (1996) studied the infection patterns of these three groups. This is summarized in Table 1. A clear distinction in infection pattern between E.coli and S.aureus is shown in these data. Infections caused by E.coli are predominantly isolated clinical cases and hardly ever chronic infections, whereas infections caused by S.aureus predominantly lead to subclinical and chronic infections. Streptococci show a patterns that is in between these two.

Table 1. Infection patterns of major mastitis pathogens in seven dairy herds, followed during 18 months (Data from Lam, 1996).

		Clinical			Subclinical chronic
Pathogen	N	Isolated	Acute-chronic	Chronic	
E.coli	105	89	6(14)[1]	4(4)[2]	6
S.aureus	171	21	18(6)	23(33)	109
S. non-agal.	159	59	20(8)	22(36)	58

[1] Between brackets is the number of clinical flare-ups after the initial case.
[2] Between brackets is the total number of clinical cases.

Clearly, udder health problems can not be treated as a single trait, but should be approached as a combination of multiple disease processes (parity, pattern and pathogen specific).

3. Defence mechanisms

The difference in disease processes is partly related to the defence mechanisms that are available to combat infection. A number of defence mechanisms are available to attack invading micro organisms.

3.1. First line of defence

The first line of defence is the mechanical prevention of infection of the mammary gland. The teat and the teat canal allow the flow of milk from the udder to the outside, but should prevent the entrance of micro organisms. Factors that have been associated with the quality of this defence are udder depth, fore udder attachment, teat length, teat shape, and milkability (Leslie, 1995; Schutz et al., 1990). It is likely that high udders, with a good attachment and small teats are less prone to teat lesions, and therefore to a mechanical lesion to the teat canal. Milkability is associated with an increased width of the teat canal, Roets et al. (1986) showed that this was strongly correlated with the ratio of alpha-2 and beta-2 receptors in teat musculature, and the presence of these receptors on white blood cells. Teat shape is of importance, since its relationship with the amount of keratinisation during lactation. Pointed teats-ends are more prone to keratinisation than flat or hollow teat-ends.

Broadly speaking, immunity against foreign material falls into two categories: innate and adaptive immunity. Adaptive immunity is highly specific for each pathogen and alters with repeated exposure to the same pathogen. Innate immunity is non-specific, and does not lead to a 'memory'.

3.2. Innate immunity

Cellular innate immunity in the mammary gland consists of macrophages that are present in milk. These macrophages ingest and kill pathogens. They also produce endogenous mediators such as interleukins, the mediators signal to the immune system that an invasion occurs, and this leads to chemo attraction of leukocytes to the place of infection. Kehrli et al. (1994) showed that intra-mammary infections are less likely to occur in cows with a higher SCC (280.000 vs 90.000 ml). This may indicate that the number of macrophages present at the time of initial infection is critical for prediction of outcome of this infection.

Other components of innate immunity are solubles such are lactoferrin, minerals (zinc and iron) and also complement factors. Lactoferrin binds iron, and this will limit the reproductive ability of micro organisms. Complement has a series of functions, including lysis of (gram-negative) microorganisms, chemotaxis of white blood cells, and opsonisation of bacteria such that they are recognized better by macrophages.

Innate immunity is especially important in the first few days after infection. Innate immunity is effective, and often the major immune response in case of infections with gram-negative (e.g. E.coli) bacteria. The outer cell wall of these micro organisms contains LPS which is susceptible to lysis by complement (Roitt et al., 1996; Kremer et al., 1991). Relatively little is known about the genetic background of innate immunity. Bovine leukocyte adhesion deficiency (BLAD) clearly limits the influx of leukocytes to the site of infection (Kehrli and Shuster, 1994), macrophage function may also be controlled genetically (Roitt et al., 1996), and finally, steroid receptors, necessary to down-regulate the immune response, may be genetically affected (Mallard et al., 1995).

3.3. Adaptive immunity

Adaptive immunity plays an important role in udder defence. Adaptive immunity involves lymphocytes: B and T cells. Immunity involving B-cells is related to antibody formation. Once a pathogen is recognized in the mammary gland, a process of clonal selection will lead to a massive response with antibodies that are produced by B-cells. These antibodies bind to the antigens of the microorganism on one side, and to macrophages on the other side. This will lead to phagocytosis of the microorganism. Once the antigen has been recognised by the immune system, it will be recognised immediately upon reinfection. This secondary response then is faster and more efficient. In the case of S.aureus infection a rise in antibodies against (capsular) antigens has been observed (Lohuis et al., 1995).

Immunity involving T-cells is related to either an enhancement of intracellular killing (T-helper) by macrophages, or to lysis of infected cells (T-cytotoxic). In both cases, an essential component is the presentation of components of the bacterial antigens by molecules of the major histocompatibility complex (MHC or bola). When macrophages have ingested microorganisms, antigen components are bound to MHC-class II molecules, and expressed at the outer membrane of the macrophage (or other 'antigen presenting cells'). T-cells recognise this combination of MHC-molecules and antigen and will bind to it. This triggers the release of cytokines (such as interferon-gamma and tumor necrosis factor) from the T-cell, and these cytokines activate killing mechanisms within the macrophage (T-helper). Similarly, infected cells will use MHC-class I molecules to present antigen at the surface, and this will bind cytotoxic T-cells. This will lead to release of cytokines and enzymes that will lyse the infected cell. Several studies have shown the importance of certain bola alleles in the prevention of especially S.aureus intra-mammary infections (Schukken et al., 1994; Aarestrup et al., 1995; Mallard, 1995).

In most situations innate and adaptive immunity will both be involved to prevent intra-mammary infection. However, E.coli infections are often short term infections (Kremer, 1991; v Werven, 1995) and therefore the innate immune system appears to play an initial key role. Also, complement is able to lyse the cell wall of gram-negative bacteria (Roitt et al., 1996). In contrast, gram-positive (S.aureus) infections are often long term. Adaptive immunity, both soluble and cellular, appears to be the major mechanism to eradicate infection (Daley et al., 1991).

4. Udder health problems

When the immune system acts inappropriately, udder health problems may occur. When the first line of defence fails, intra-mammary infection will occur. This may or may not lead to a major inflammatory response. When the immunological response acts appropriately, a short term activation of the immune response will occur. This may be observed when daily SCC or conductivity is measured. Also a short term and very modest number of clinical signs indicating inflammation (rubor, tumor, dolor) might be observed. Hence, a short term increase in SCC with or without accompanying clinical signs are on one hand a symptom of a failing first line of defence but on the other hand indicating an appropriate immunological reaction.

Severe clinical signs indicate the presence of a massive immune response to infection. Massive production of cytokines is observed especially with gram-negative infections. Large amounts of cell wall carbohydrates (LPS) trigger macrophages to unrestricted production of cytokines. Occasionally, super antigens such as ß-toxin of S.aureus also trigger massive production of cytokines. This massive production of cytokines may lead to shock-like symptoms and eventually to death of the animal (toxic shock syndrome). Infection experiments with

E.coli microorganisms have shown that the rapidity of initial growth of the microorganisms, and the speed of influx of white blood cells into the udder are strongly related to the severity of symptoms (Hill et al., 1979; v.Werven et al., 1995). Feed back mechanism of cytokine production is partly through the Hypothalamus - Pituitary - Adrenal (HPA) - axis. Steroids, produced in the adrenal glands are strong inhibitor of leucocyte (macrophage) activity. A failure to express or activate steroid receptors may be involved in toxic shock syndrome in high producing dairy cattle.

Chronic infections occur when bacterial killing is not efficient. Both S.aureus and S.dysgalactiae have been shown to survive within macrophages (Daley et al., 1991). This may lead to a cycling process where presence of pathogens in the milk leads to an inflammatory response (Daley et al., 1991). Pathogens are ingested, and the inflammatory response is down-regulated. However, pathogens survive within the macrophage and eventually appear again in the lumen of the gland. Another inflammatory response is mounted and cycling continues. These patterns are frequently observed with gram-positive pathogens (Lam, 1996).

5. Selection traits

5.1. Conformation

The conformation traits are univocal arguments that can be used to improve udder health. Udder depth, fore udder attachment, and teat placement have shown to be correlated both genotypically as well as phenotypically with low SCC (reviewed by Leslie, 1995). Out of theoretical considerations, milkability and receptor ratio could be added as a potential trait for genetic selection with regard to better udder health. Finally, teat shape may show to be useful as a genetic trait. Pointed teats are more prone to keratinisation compared to flat teats. This may prove to be associated with udder health.

Essentially, the effectiveness of the first line of defence may be monitored by calculating new infections. As proposed by Dohoo and Leslie (1991), new infections can be measured using a rise in SCC in combination with all cases of clinical mastitis.

5.2. SCC and EC

Currently, SCC is the most frequently used trait for udder health. Further development of automated milking may result in large data bases with electrical conductivity (EC) measurements, and eventually replace SCC. Heritability of SCC appears to be around .1 to .15, and therefore selection is possible (Pose and Mantysaari, 1996; Leslie, 1995; Shutz, 1990). In chronic infections, SCC is increased, and therefore related to a failure of the immune response. However, since mean lactational SCC is used, a single high count may also affect mean lactational SCC. It may be of greater biological value to interpret SCC patterns in a lactation, and use these patterns as genetic traits. Macrophages are part of innate immunity, and therefore of great use to the defence system. An optimal udder health should be a combination of low SCC and no clinical events, it appears likely that this will not occur at the lowest possible level of SCC.

5.3. Clinical events

In a number of countries, clinical mastitis events are recorded. These events appear to have a heritability that is lower than SCC (.05 to .10). Although there is generally a very low phenotypic correlation between SCC and clinical mastitis, genotypic correlation is much higher. Recently Poso and Mantysaari (1996) estimated the genotypic correlation between clinical mastitis and SCC at .2 to .6, depending on lactation number (Table 2). They concluded that these traits are essentially separate but correlated. A prime source of correlation would be the clear association of both with the primary defence mechanisms. From an immunological point of view, gram-negative infections and gram-positive infections appear different, this may be reflected in differences in genetic control of immune response to these infections. Since gram-negative infections are mostly associated with clinical mastitis and innate defence mechanisms, and less with chronic infections (Table 1), it appears likely that indeed clinical mastitis and SCC are different traits.

Table 2. Heritabilities (on diagonal), genotypic (above diagonal) and phenotypic (below diagonal) correlations between SCC, clinical mastitis and milk yield in first and second lactation (Data from Poso and Mantysaari, 1996).

	MAS 1	MAS 2	SCC 1	SCC 2	MY 1	MY 2
MAS 1	.023	.71	.37	.21	.46	.58
MAS 2	.09	.042	.66	.61	.25	.35
SCC 1	.05	.07	.16	.78	.10	.01
SCC 2	.03	.15	.40	.18	.10	-.11
MY 1	.00	.02	-.05	.02	.40	.87
MY 2	.03	.01	-.02	-.10	.56	.25

5.4. Markers of immune response

Markers of immune response have shown to be associated with udder health traits. A prime group of markers is the BoLA system, however other options both in the innate (such as neutrophil function, cytokine profile, steroid receptors, adhesion molecules and others) and adaptive (BoLA, humoral immune response, lymphocyte function, vaccine response and others) immune system appear to be present (Mallard et al., 1995). Further studies in this area may show to be effective in identifying genetically encoded responses to udder pathogens. However, since udder health is a combination of multiple processes, it is unlikely that single immune functions will improve general udder health.

6. Discussion and conclusions

Udder health is genetically controlled by a number of traits. Some of these, such as components of first defence are associated with improvement of udder health in general. Other (marker) traits such as BoLA alleles and the Bovine Leukocyte Adhesion Deficiency (BLAD) allele address a very specific component of the immunological defence against invading pathogens.

The overall goal of udder health is clear: low SCC and no clinical events. However the methods to obtain this goal are not straightforward. Selection on only a single trait in a single parity such as SCC bears the risk of improvement in one area and no improvement or even a potential loss in another area. On the other hand, clinical events are difficult to measure, and may be prone to diagnostic errors. When designing selection programs, the goal should be to apply the best possible traits, and solve subsequently the problems of measurement, data collection and data analysis.

A combination of udder health traits into an udder health index appears to be the best way to obtain long term improvements. Such an index should contain conformation traits, milkability, an SCC index (based on patterns) and a clinical event trait.

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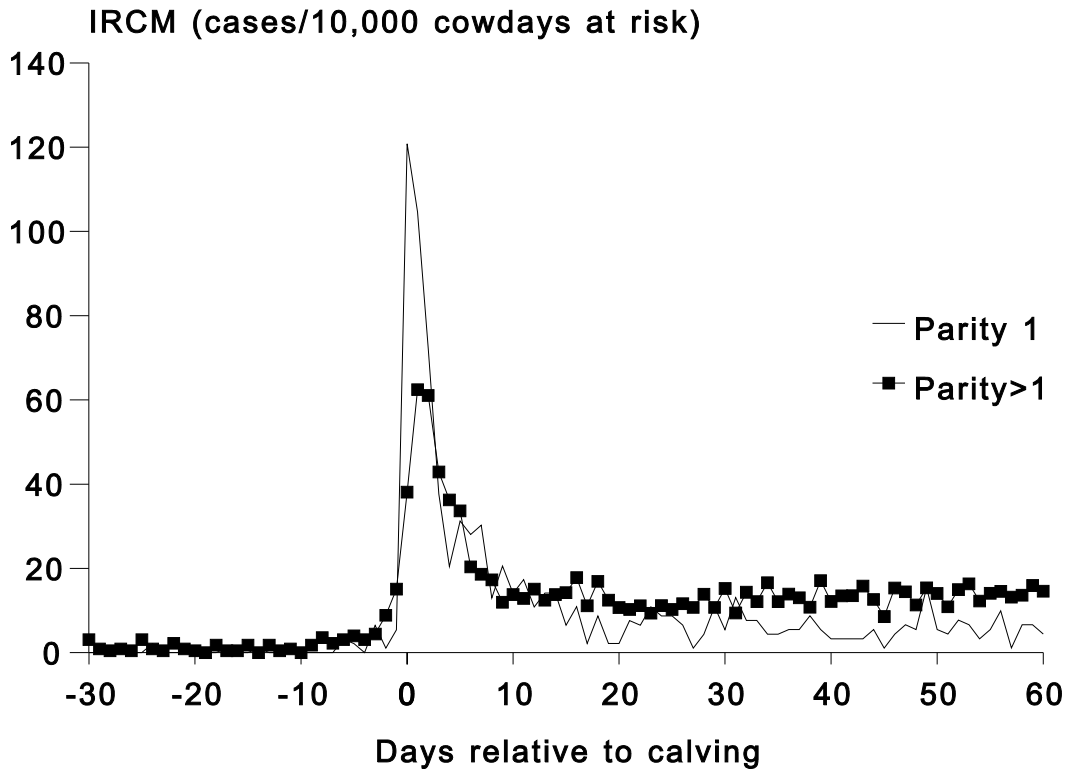

Figure 1. Incidence rate of clinical mastitis per day of lactation in first and later lactations. (Data from Barkema et al., 1997).

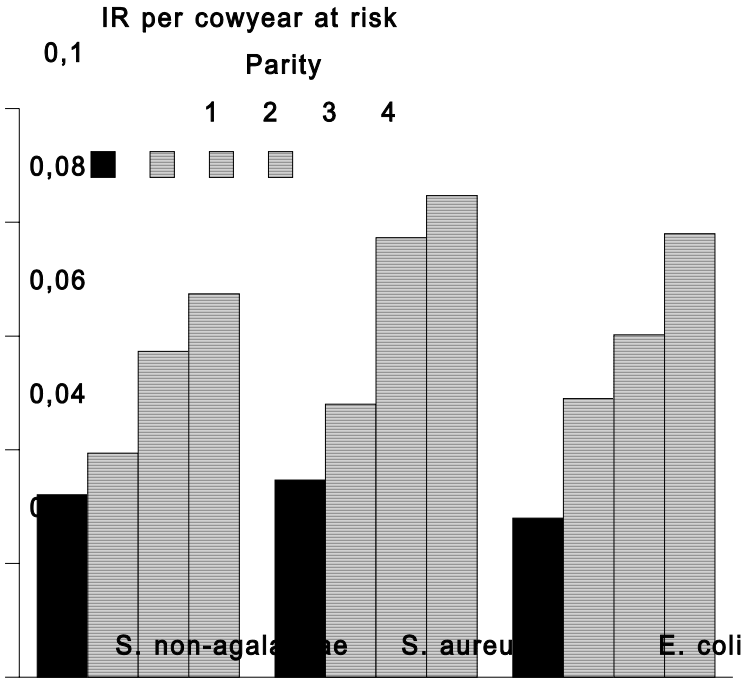

Figure 2. Incidence rate of clinical mastitis of the three major groups of pathogens per lactation. (Data from Barkema et al., 1997).