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Genetics of udder health in dairy ruminants

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1. INTRODUCTION

Udder health troubles in dairy ruminants mainly include intra mammary infections, or mastitis, caused by a pathogen that enter the gland through the teat canal and develop in the gland cistern. Mastitis is a multifactorial disease; it's origin, severity and outcome are highly variable and depend on environment, a wide variety of pathogens (mostly bacteria) and host. In dairy cattle, mastitis is especially severe for environmental causing pathogens such as enterobacteriaceae (Escherichi coli), and during the critical post partum period. Dairy sheep and goat experience less frequently acute mastitis (<5% on average) than cattle (20-40%), but essentially subclinical infections due to staphylococci and mycoplasma bacteria. Evidence of individual genetic variability of resistance to mastitis in dairy ruminants has been demonstrated for a long time. Resistance to mastitis is a complex trait that involves different aspects: avoid entry of the pathogen, limit its development in the udder, control pathogenic effect such as tissue damages, eliminate and recover from infection. Genetic basis of udder health has been established mainly on phenotypes that relate to the healthy or diseased status of animals, without any knowledge about mechanisms and genes underlying resistance. Some immune genes (MHC genes) and pathways, including recruitment and functionality of neutrophils and humoral or cellular adaptative immune response, have proved to play an important role. Understanding key mechanisms in the host's response to intra mammary infections and their genetic component is, however, still a large field for investigation Because resistance to mastitis is partly genetically determined and can be characterized using simple measures, genetic improvement has been made possible as a complementary control method to treatment and prophylaxis. Regarding major economic importance of udder health for the dairy industry and its link with animal health and food safety, genetic selection has been implemented throughout the world and is expected to lead to reduced frequency of intrammamary infections and enhanced resistance to mastitis.

This paper reviews the state of art of knowledge about genetic basis of udder health, including polygenic variation, QTL detection and candidate gene approach for health status indicators and immune response traits. In a second step, current selection strategies will be addressed with focus on actual results, questioning and prospects for the future.

2. GENETIC CONTROL OF UDDER HEALTH

Accumulating literature results over the last decades give strong evidence that dairy ruminant's ability to control udder health is under genetic control. Numerous studies reported polygenic variation for phenotypic measurements of health status and, more recently for traits related to the hosts' defence mechanisms. Additionally, some candidate genes, such as MHC genes, have shown strong evidence of association of their polymorphism with udder health. QTL detection studies accumulated show that some regions of the genome explain a large part

of variability for udder health traits such as SCC and clinical mastitis. New technologies allow investigating differential gene expression and giving some clues about gene and mechanisms involved in host's ability to combat infections.

2.1. Polygenic variability for disease status related traits

On the one hand, direct measures of intrammamary infections, also mentioned as diagnostic traits, include bacteriological determination of milk and observation of clinical cases of mastitis. The bacteriological analysis of milk provides precise and exhaustive information on infected quarters and pathogen involved, but is expensive and time consuming. PCR tests on bacterial DNA from milk samples could be an attractive diagnostic alternative in the near future and have already shown their efficiency. Conversely, clinical cases of mastitis (CM) are much easier to collect on a large scale for accurate genetic studies, but refer only partially to udder health problems, especially in small ruminants where frequency of CM is generally lower than 5%. On the other hand, milk somatic cell count (SCC) is an indirect measure, or a predictor, of the presence of an intramammary infection. Increase in milk SCC is closely associated to the afflux of white blood cells from the bloodstream into the milk to eliminate infection in the udder. SCC trait therefore gives information about the udder health status of the udder, but also about the magnitude of the host's inflammatory response. However, numerous factors influence SCC of infected and non infected animals such as physiological status of the host, infection stage and pathogen. It is therefore difficult to interpret single measures and to define fixed thresholds because distributions of SCC of infected or non infected animals largely overlap. Some authors suggest that corresponding SCC relate to two different traits and developed mixture models to account for heterogeneity of distribution and variance components of SCC of healthy or diseased animals (Detilleux and Leroy, 2000; Odegard et al., 2003; Odegard et al., 2005). Anyway, repeated SCC measurements are generally preferred for interpreting the disease status of animals over a given time period.

As reported in earlier dairy cattle reviews (Mrode and Swanson, 1996; Detilleux, 2002; Rupp et Boichard, 2003) and in more recent papers (Carlen et al., 2004; Koivula et al., 2005), results consistently indicate low heritability for clinical mastitis, i.e. around 0.03 whereas heritability estimates of single SCC range from 0.05 to 0.14 for monthly test-day and increase around 0.15 for lactation measures. Similar heritability estimates for lactation mean SCS, ranging from 0.11 to 0.18, were found for various sheep breeds (El Saied et al., 1999; Othmane et al., 2002; Serranno et al., 2003; Rupp et al., 2003; Legarra and Ugarte, 2005). Genetic correlations between adjacent test-day SCS and between lactation measures of SCC or CM in successive parities (Rupp and Boichard, 2003; Carlen et al., 2004; Koivula et al., 2005) are high (>0.7 in general) so that genetic determinism of SCC is partially similar within and across lactations. Low heritability for clinical mastitis values, i.e. from 0.02 to 0.04, obtained on the observed binary scale, generally increase around 0.07, with values up to 0.10, when threshold models are applied. Those threshold models assume that a clinical case occurs when a non-observable normally distributed trait exceeds a threshold on an underlying scale. Despite low heritability values, genetic variability for clinical mastitis is large. Indeed, genetic variability is diluted in a very large phenotypic variability probably due to diversity of pathogen and environment conditions responsible for udder infections. The genetic standard deviation of CM is around 0.05, this means that for a mean frequency of 20% in a given environment, extreme genotypes present a large range of incidence, varying at least from 10 to 30%. Differences between breeds are of the same magnitude. Most estimates of genetic correlation between SCC and clinical mastitis range from 0.50 to 0.80, with an average of 0.70 (review by Rupp and Boichard, 2003; Carlen et al., 2004; Koivula et al., 2005). This

reasonably high value suggests that resistance mechanisms that lead to better resistance to either persistent intramammary infection (continuously high SCC) or acute clinical episodes (CM) are partially but largely common although those forms of mastitis might be associated to different environmental conditions, different pathogens, and different animal's physiological status.

Genetic parameters for other traits than SCC or clinical mastitis are scarce. In particular, there are very few data on intramammary infection assessed by bacteriological analyses, which would more directly and exhaustively refer to udder health status. Heritabilities for intra mammary infection varied from 0.02 to 0.04 from study of Weller et al. (1992) based on 9784 cows, and were somewhat higher (0.10 to 0.20) for Detilleux et al. (1994) and Wanner et al. (1998), based on 1237 and 756 cows, respectively. Interestingly, genetic correlation between SCC and bacterial infection was estimated to be near unity (Weller et al., 1992), indicating that SCC and subclinical infections are essentially the same trait. Additional data, however, would be useful, especially to validate this result, to explore genetic determinism of the host's resistance to different species of pathogens and to draw general conclusions on universality of mastitis resistance.

2.2 QTL detection studies

Many QTL detection studies, allowing detecting and localising chromosomal regions controlling udder health in dairy ruminants, are already available. Results have been reviewed by Rupp and Boichard (2003), Khatkar et al (2004) and Smaragdov (2006). The latter review includes 19 references for QTL of mastitis resistance, most about SCC and only 3 with clinical mastitis information. All autosomal chromosomes but 3 are found to carry one or several QTL and most regions are found by several studies. Although much more limited in number, CM QTL are found on chromosomes 8, 9, 11, 14, 18, 21, 25, and 27. According to this large number of results, it appears that the individual contribution of each QTL to the total genetic variance is likely to be much smaller than originally estimated. Following these primo-detection results, intensive fine mapping work has been conducted by several teams to characterize some of these QTL, particularly on chromosomes 6 (Opsal et al, 2006), 15, 18 (Kuhn et al., 2003), 22 (Sugimoto et al., 2006), 27. Many hopes are put in the use of high-throughput SNP genotyping techniques and use of linkage disequilibrium to achieve very fine mapping and discover causal mutations. Most likely, merging international efforts into consortia would be necessary to reach satisfactory power and mapping resolution, as already illustrated by Bennewitz et al. (2003, 2004). Recently, Sugimoto et al. (2006) showed that a polymorphism of the bovine forebrain embryonic zinc finger-like gene (FEZL), located in the region of a QTL for SCC on BTA22, was associated with high and low SCC and with its transcription activity, leading to control of cytokine expression. If this conclusion is confirmed, it is probably the first QTL fully characterized for mastitis resistance.

2.3 Polygenic variability for udder defence mechanisms

Several authors focused on traits related to the host's defence mechanisms, as a complement or alternative to various indirect traits related to the health status of the udder mentioned above. Such traits should more directly relate to general resistance of the mammary gland, independently of the exposure history of the animal, and enable to dissect major components of the host's defence ability.

In particular, genetic aspect of functionality of neutrophils, which recruitment and activity are essential in the innate defence against udder infection (Sordillo, 1997; Rainard and Riollet, 2006), were investigated. Sire effect of *in vitro* phagocytosis of blood neutrophils

was demonstrated (LostrieTrussard et al., 1984; Kerhli et al., 1991; McDonald et al., 1994). Moreover, moderate heritabilities for migration (0.2 to 0.5) and phagocytosis (0.3-0.7) of neutrophils and for serum complement activity (0.4-0.5), using *in vitro* assays for 137 cows sampled three times around calving, was reported (Detilleux et al., 1994). Correlation with better udder health was demonstrated in that animals with low somatic cell counts, clinical mastitis frequency and intramammary infections tended to exhibit better functionality of neutrophils (Kelm et al., 1997).

Additionally, antibody mediated immune response (AMIR), known to be essential to control extra cellular pathogens such as bacteria responsible of mastitis, also showed moderate to high heritabilities in dairy ruminants (Wagter et al., 2000; Hernandez et al., 2006). Heritability of serum antibody measured several weeks after vaccination , ranged from 0.32 to 0.64 for ovalbumin and between 0.13 to 0.88 for E. *coli* antigens, for cows around calving (Wagter et al., 2000), and from 0.25 to 0.42 for HEWL antigen (hen egg-white lyzozyme) for lactating cows (Hernandez et al., 2006).

More recently, Hernandez et al. (2005) proposed to characterize cow's cell mediated immune response (CMIR), which involves T-lymphocytes as effectors and contributes to protection against intracellular pathogens. Similarly to previous development in pigs (Mallard et al., 1998), they used a simple delayed type hypersensitivity (DTH) test based on an increase in skin fold thickness following injection with several test antigens. Heritability for two DTH antigens ranged from 0.19 to 0.49 (Rupp et al., 2005; Hernandez et al., 2006) with moderate to high genetic correlations between both test antigens (0.60-0.86) (Hernandez et al., 2006). Genetic correlation between AMIR and CMIR varied from -0.29 to 0.31, which was consistent with results in pigs that indicated that both traits are genetically independent (Mallard, 1992) and with biological evidence that both traits represent type 1 and type 2 immune responses. Relation of both AMIR and CMIR traits with udder health, however, has not been clearly established. Optimum response to achieve better resistance, variation of the response according to test antigens used or depending on pathogen and environment need further investigations.

2.4 Major candidate genes associated with udder health

MHC genes

The major histocompatibility Complex (MHC) plays an essential role in the induction and regulation of acquired immune response (Rothschild et al., 2002). Class I MHC molecules are expressed at the surface of all nucleated cells and interact with cytotoxic T lymphocytes (CD8+). On the other hand, class II MHC molecules, which expression is restricted to antigen presenting cells, are involved in antigen presentation to helper T lymphocytes cells (CD4+) and in the development and differentiation of T cells (Rothschild et al., 2002). Genes encoding the MHC molecules are highly polymorphic and numerous associations between allelic variants and immune responsiveness and disease resistance have been reported (Rothschild et al., 2000; Stear et al., 2001). In dairy ruminants, several studies have reported a relationship between bovine MHC (BoLA**,** bovine lymphocyte antigen) class I molecules and resistance or susceptibility to mastitis (Weigel et al., 1990; Mejdell et al., 1994; Aarestrup et al., 1995; Mallard et al., 1995) or immune response (Mallard et al., 1995). Nearly all of the most recent studies, however, focused on the exon 2 of the Class II DRB3 locus because of its high polymorphism and because it encodes the antigen binding site of MHC molecules (Rothschild et al., 2000). Several alleles were found to be associated with mastitis resistance as measured by decreased SCC and mastitis frequency (Dietz et al., 1997; Kelm et al., 1997; Starkenburg et al., 1997; Sharif et al., 1998a, Rupp et al., 2007) and with

increased or decreased AMIR and CMIR (Rupp et al., 2007). Interestingly, the association between BoLA DRB3.2 alleles (DRB3.2 *3, *24 and *22) and immune responses tended to be in opposite sign for the two AMIR and CMIR traits examined (Rupp et al., 2007), in agreement with the hypothesis that both traits are genetically independent and represent type 1 and type 2 immune responses. From those limited data (few hundreds of animals), however, there are still conflicting results regarding associations between given BoLA alleles and phenotypes across studies, making it difficult to draw conclusions about direct causal effect of BoLA alleles on udder health. DRB3.2 *16 and *3, for example, were associated with either resistance or susceptibility to mastitis according to authors. Genetic background (breed) or environment effects (nature of pathogens, ..) may explain those discrepancies, but statistical linkage disequilibrium due to selection, small data size or spurious results can not be excluded. However, consistent results across studies of unfavourable associations of alleles DRB3.2 *22, *23, and *8 with udder health (Dietz et al., 1997; Starkenburg et al., 1997; Sharif et al., 1998a; Rupp et al., 2007) were in agreement with the finding of Sharif et al. (2000) that some common amino acid motifs in the antigen binding groove of corresponding BoLA molecules are involved in susceptibility to mastitis.

Other candidate Genes

Candidate gene approaches have concerned other genes then MHC, such as those encoding CD18, lactoferrin and lyzozyme proteins. The CD18 gene (BTA1) encodes adhesion molecules expressed on the surface of leucocytes. Homozygous cattle for the deleterious allele exhibit leukocyte adhesion deficiency (Blad) (Kehrli et al., 1990) which leads to impaired diapedesis of leucocytes, extreme sensitivity in any infection and premature death. However, as reported in Rupp and Boichard (2003), attempts to show association with udder health in heterozygous animals have not been successful. On the other hand, studies on two genes encoding proteins present in milk and involved in the innate mechanisms of defence of the udder: on the one hand, lactoferrin (BTA22), an iron binding protein with bacteriostatic properties, and, on the other hand, lysozyme (BTA5) which can specifically cleave bacterial cell walls (Seyfert et al., 1996) have not been showed to be associated with udder health.

Currently other candidate genes are under investigation, such as genes coding for Toll like receptors (TLR, BTA6) (Sharma et al, 2006, Opsal et al, 2006) or acute phase protein (Haptoglobin and Serum Amyloid-A) involved in non specific innate immune response components during early stage of mastitis (Irvonen et al, 1999; Schwerin et al, 2003 ; Grönlund et al, 2005).

Recent advances in microarray technology, allow exploring expression of up to thousands of gene in the context of complex biological functions. Many other candidate genes could therefore be defined, best candidate genes being of course those co-locating with a QTL. The few recent published applications to ruminant's udder health (Schwerin et al., 2003; Pareek et al., 2005; Zheng et al., 2006; Jaffrezic et al., 2007; Sorensen et al., 2007) have mainly addressed the question of differential gene expression in mammary epithelial cells and mammary gland tissue, in the context of infection (in vitro or in vivo models). Gene expression profiles in bovine epithelial showed that CXCL5 genes were significantly over expressed after stimulation with Escherichia coli lipopolysaccharide (LPS) (Pareek et al., 2005). Zheng et al. (2006) found that after intrammammary challenged with LPS of mice, most over expressed genes in mammary gland tissue and epithelial cells were associated with the innate immune response, including chemokines (CXCL1, CXCL2), acute phase protein SAA3, which may play a role in leucocyte attraction, and LPS binding protein CD14. Comparative gene expression after intramammary experimental challenges of cows with two

pathogens (E. coli and S aureus) was implemented and analyzed in the context of the EU network EADGENE (European Animal Disease Genomics Network of Excellence) (Jaffrezic et al., 2007; Sorensen et al., 2007). Results indicated that only few genes were commonly over expressed in the mammary glands infected by either E. coli or S. aureus, most of the differential expressed genes being found for E Coli infection. The most highly up-regulated genes following S aureus infection were lactotransferin and antimicrobial protein secreted in milk whereas many of the most up regulated genes following E. coli infection were associated with influx of neutrophils into the mammary gland. Combining mRNA differential display techniques for infected and non infected bovine udders, and co localisation in QTL regions, Schwerin et al. (2003) identified several genes as potential candidate involved in mastitis resistance.

Collectively, those studies support the important role of rapid influx of neutrophils into the mammary gland and the effective and early elimination of pathogens (Paape et al., 2002) as well as the importance of epithelial cells in initiating the inflammatory process (Rainard and Riollet, 2003). Microarray is a promising tool to contribute identifying genes products and pathways that are crucial in udder health. Combining this technology with other mechanistic, genomics and proteomics methods (functional studies, polymorphism investigation, fine QTL mapping) might result in a strong synergy on the way of understanding genetic basis underlying udder health.

2.5 Genetic relationships of udder health with other biological functions

Relationships with production traits

The genetic antagonism between udder health (SCC and CM) and production traits is well documented in dairy cattle, indicating that udder health has been deteriorating as a consequence of selection for production traits. Average genetic correlation between SCC and milk yield is about 0.14, with most values between 0.10 and 0.20 for cattle (reviews by Mrode and Swanson, 1996; by Rupp and Boichard, 2003; Koivula et al., 2005; Carlen et al., 2004). Genetic correlation estimates with milk yield, however, are quite inconsistent across dairy sheep studies, ranging from antagonistic (Rupp et al., 2003; Riggio et al., 2007) to favourable (El Saied et al., 1999; Othmane et al., 2002; Serranno et al., 2003; Legarra and Ugarte, 2005). The genetic antagonism between yields and clinical mastitis is more pronounced (review by Heringstad et al., 2000; Rupp and Boichard, 2003; Koivula et al., 2005; Carlen et al., 2004) with an average of about 0.35, and most values ranging from 0.20 to 0.55. Possible explanation of this antagonism between udder health and some production components, may be partially indirect, include effect of pleiotropic genes, but also involve biological competition for energy and nutrients between functions.

Relationships with udder type traits and milking ease

Udder health (SCC and CM) is favourably correlated to several anatomical characteristics of the udder (reviews by Mrode and Swanson, 1996; Rupp and Boichard, 2003). Udder depth and udder attachment generally show consistent results indicating that higher and more tightly attached udders are associated with lower SCC of the udder gland. The latter association is probably rather due to the effect of intramammary infections on udder shape (deterioration of udder tissue, sagging) than to a causative effect of the udder's morphology on risk of infection. Associations with udder balance, teat length and form have also been reported, but are less consistent across populations, breeds and studies. Anyway, such udder type traits can therefore be considered as easy to collect predictors of udder health.

As mentioned in review by Rupp and Boichard (2003), results on relationship between udder health and milking ease (or milking speed) are still conflicting. Although genetic

opposition of milking ease with SCC is consistently and well documented across numerous studies (genetic correlation about 0.40), literature data essentially indicate favourable or almost null estimates of genetic correlation with clinical mastitis. Further investigation is needed to understand biological basis of those contrasting relationships between characteristics of milk emission and different udder health related traits.

Relationship of udder health with resistance to other diseases

Inference on relationships between main health disorders in dairy cattle was available from large Norwegian veterinarian treatment data (Heringstad et al., 2005). Authors estimated favourable genetic correlations of clinical mastitis occurrence with frequency of milk fever, ketosis and retained placenta, with estimated values ranging from 0.11 to 0.26 in first three lactations. In agreement with the latter study, Zwald et al. (2004) reported positive genetic association between mastitis and ketosis (0.17), displaced abomasums (0.08), lameness (0.20) and cystic ovaries (0.11), but no correlation with metritis (-0.01). Positive correlations suggest that some general disease resistance factors with a genetic component exists, but may also reflect indirect statistical association due to genetic opposition of the various diseases with milk production.

Udder health mainly refers to control of intramammary infections with extra cellular pathogens such as Staphylococci and Streptococci bacteria, with crucial role of the immune response. However, immune response is known to mobilise different effectors and pathways according to species and pathogen types. For instance, whereas Type-1 responses predominantly control intracellular pathogens (bacteria and viruses), Type-2 responses predominately control extracellular pathogens such as parasites. From several divergent selection experiments in mice (Mouton et al., 1984), poultry (Pinard van der Laan et al., 2002) and pigs ((Mallard et al., 1992 and 1998;), there is accumulating evidence that different components of immune response, including type-1 and type-2 responses, and resistance to various diseases, were at least partially under independent genetic regulation. Therefore, the question is raised whether resistance to intramammary infections may be independent or have unfavourable link with resistance to other pathogens and diseases of economic and human health importance such as Toxoplasmosis (caused by the Toxoplasma gondii parasite), gastroinstinal parasitism (caused by Heamonchus parasites and others) or paratuberculosis (caused by mycobacterium bacteria).

3. APPLICATION FOR GENETIC IMPROVEMENT OF UDDER HEALTH

3.1. Breeding strategies

The Scandinavian countries were the first to consider udder health in their breeding objectives for dairy cattle, as early as in the 80's (Heringstad et al., 2000). More recently, in the last decade, many other countries similarly modified their breeding objectives for dairy cattle (Mark et al., 2002) and sheep (Rupp et al., 2002) in response to the increasing consumer's concern for better animal's health and food quality, but also to maximize profitability by reducing production costs. Current weights in combined selection indices should at least enable to decrease SCC and to stop any deterioration of CM frequency in most situations.

An accurate selection criterion must be a relevant biological trait genetically well correlated to mastitis resistance, exhibit sufficient genetic variability and have operational properties such as easy and cheap measuring procedure on a large scale. Accordingly, SCC is the most widely used criterion to achieve better udder health. Indeed, repeated SCC data are routinely recorded for individuals as part of milk recording schemes and stored in large data

bases in many countries. In different countries, accuracy of breeding values is also improved by using information of early predictors such as udder conformation, particularly udder depth, known to have moderate but favourable genetic correlations with mastitis resistance. Similar large scale recording also exists for clinical mastitis in Scandinavian for more than 20 years. Therefore, Sweden, Finland, Denmark, and Norway also include CM in the selection index. In those countries, to obtain a high accuracy of sire's breeding values, the low heritability of CM is counterbalanced by a large size of the progeny groups. Implementation of similar large scale recording is under way in several other countries such as France, and genetic evaluation for CM in cattle, in addition to SCC, will probably be generalized worldwide in the future.

3.2. Consequence of SCC (and clinical mastitis) -based selection on udder health

Currently selection strategies for improved udder health are based on a linear decrease of milk SCC and, in Scandinavian countries, on the reduction of CM occurrence as a tool to decrease both subclinical and clinical intramammary infections. However SCC (and clinical cases) is (are) used as a phenotypic black box selection tool and genes and mechanisms involved are still unknown. The long term effect and efficacy of such a selection (and especially sole-SCC selection) has therefore been questioned. In particular, the question is raised whether favourable indirect responses for clinical mastitis, for various pathogen-specific infections, and for resistance to other diseases on the long term will be observed.

Does SCC (CM) –based selection work?

In spite of a low heritability of CM, the variety of the pathogens responsible for mastitis, and the complexity of the resistance traits, there is good evidence that SCC based selection should efficiently reduce mastitis incidence. Indeed, no strong deviation is observed in the expected covariance between relatives nor in prediction models; the genetic determinism of both SCC and CM is homogeneous across parities and lactation stages, as well as across environments or mastitis incidences; the genetic correlation between SCC and CM is high (see § 2.1) and does not show any sign of non-linearity (McDaniel et al., 1993; Philipsson et al., 1995; Cranford and Pearson 2001; Bonaiti, 2005, personal data). The practical situation, however, is more complex as the breeding objective does not aim only to decrease mastitis and somatic cell counts but also to improve milk production, milk quality and functional traits. As a consequence, limited realised selection pressure for low SCC and continuous selection for milk production (antagonistic to CM) prevent any clear and rapid decrease in CM incidence, and the expected result is a stabilisation of CM incidence in most situations. To our knowledge, only two situations clearly demonstrated the efficiency of selection against mastitis. In Norway, CM is a priority in the breeding objective and explicitly included as a selection criterion (and not SCC). The estimated annual genetic trend is about -0.25% CM incidence in the recent years (Heringstad et al, 2003). A long term experiment using AI bulls confirmed this result and showed a 10% decrease in CM incidence due to selection against CM after 5 generations (Heringstad et al, 2007). In Lacaune dairy sheep, two divergently selected lines were produced in an INRA experimental facility by using AI rams evaluated after progeny test in the Lacaune population and with extreme low or high breeding values for SCC (Rupp et al., 2006). A 2-fold SCC ratio was observed between lines, confirming the efficiency of selection on SCC. As a response to SCC selection, a clear decrease of CM and intramammary infections caused by various pathogens (measured by repeated milk bacteriological tests) was observed in the low SCC line (Rupp et al., 2006).

Can SCC get too low?

Because of biological signification of SCC, and several contrasting results on relationships between low SCC and risk of mastitis, long term effect of sole SCC selection has been questioned. It has been stated that decreasing milk SCC to very low levels by selection could impair the cow's capacity to combat intramammary infection, as some of milk resident cells such as macrophages are essential in initiating the inflammatory process in response to intrammamary invading pathogens. First evidence of the potential role of mammary epithelial cells in neutrophil recruitment (review by Rainard and Riollet, 2006) somewhat moderate the latter concern. Additionally, early studies, mainly based on experimental challenges, showed that moderate cell counts in milk play a protective role in the defense of the mammary gland (Schalm et al., 1964; Rainard et al., 1988; Schukken et al., 1994). Finally, several herd level studies, showed high clinical mastitis incidence risk in low SCC herds (Beaudeau et al., 2002; Waage et al., 1998; Elbers et al. 1998). For all that reasons, the question has been raised whether SCC is an accurate selection criterion for selection, and whether SCC can get too low.

This question has been addressed into two ways. On the one hand, linearity in the genetic relationship between SCC and CM has been investigated (see above). Although no deviation to linearity could be observed in the present populations, the genetic correlation between SCC and CM is high but not equal to 1 (see §2.1). Therefore it should be strongly recommended to explicitly account for CM in addition to SCC, as it would account for the part of the genetic determinism of CM which is not predicted by SCC and which is clearly in opposition with milk production. On the other hand, some answers can be given from several independent statistical studies analyzing the relationship between individual SCC at a given time and natural occurrence of mastitis (Coffey et al., 1986; Boettcher et al., 2002, Beaudeau et al., 1998; Rupp and Boichard 2000; Rupp et al., 2000). Results of all these studies, including contrasted herd epidemiological situations, consistently showed that cows with the lowest observed SCC were always those at the lowest risk of mastitis. In the current situation the lowest milk SCC animals do not show impaired defense mechanism against intra mammary pathogens.

Anyway, given present levels of SCC and clinical mastitis frequencies, as well as current selection pressure put on mastitis resistance (CM and SCC), milk SCC will hardly decrease dramatically in the near future. Additionally, as mentioned above, divergent selection experiments in dairy sheep and cattle and observed linearity between SCC and CM support the fact that selecting for the low SCC or for low CM frequencies should increase the frequency of healthy animals. Such a selection process probably involves selection of (still) unknown host's defence mechanisms that leads to better resistance to most frequent udder pathogens in the current epidemiological situation.

A better understanding of defence mechanisms affected or modified by such a selection would be helpful, however, to i) predict indirect responses on udder health on the long term including various pathogen-specific infections and, if necessary, to ii) modify selection modality and criteria accordingly.

3.3. Prospects

As for many functional traits with a low heritability and difficult to select with conventional approaches, many hopes are put in gene- or marker- assisted selection (MAS). However, inconsistent results on the effect of various alleles is one of the strongest impediments to selective breeding based on one of the most obvious candidate genes, MHC. In practice, MAS is still limited worldwide for mastitis resistance, because of the lack of confirmed causal genes and mutations and because of the multiplicity of QTL regions described on at least 18 chromosomes (1, 2, 5, 6, 7, 9, 10, 11, 13, 14, 15, 16, 19, 21, 22, 23, 26, 27), their individually limited part of genetic variance explained, and their poor across-

studies confirmation rate. In the French MAS programme, however, Boichard et al. (2006) showed that the efficiency was similar for SCC and milk or protein yields, although lower than for fat or protein contents. Many hopes are put on new marker technologies with high throughput SNPs and many studies are under way in different countries. Two strategies are possible, either the selection of a number of targeted regions with fine-mapped (or fully characterised) QTLs or genomic selection based on the prediction of breeding values from whole genome marker information. Although both approaches are often opposed, they are rather similar and a unified approach of genomic selection using QTL information could emerge and be extensively used in practice in the very near future.

Beside SCC and CM, other phenotypic traits are candidates as selection criteria related to udder health. The large scale development of electronic devices makes available large amount of electrical conductibility data (Norberg, 2005). Norberg (2004) showed that electrical data were highly correlated with SCC and were basically the same trait. They were as heritable as SCC (Norberg, 2004) or even more (Povinelli et al, 2005). Their genetic correlation with CM is at least equal to that of SCC (Norberg et al, 2006). Their availability potentially at each milking is a clear advantage over SCC and could make them emerge as a new udder health criterion used in selection, as far as the information systems are adapted to receive this massive amount of data.

Based on progress in understanding genetic basis of host's defence mechanisms, new phenotypes and genes may emerge to target key components of resistance of the udder gland, and potentially control resistance to various pathogens and environment-pathogen interactions. On an even more global approach, broad based resistance to disease has been targeted. Hernandez et al. (2006) developed two simple heritable tests for antibody (AMIR) and cell mediated (CMIR) immune response, to address the general immune ability of dairy cattle. They suggested using both AMIR and CMIR as an alternative, or together with other indirect traits such as SCC, to improve resistance to mastitis and other diseases. Similar combined selection for global immunocompetence had been investigated in pigs (Mallard et al., 1992, 1998; Wilkie and Mallard, 1999) and chicken (Kean et al., 1994; Pinard-van der Laan, 2002). Broad based resistance to a variety of disease in the latter experiments, however, has not always been fully demonstrated. Such approaches are promising but there's still a need for better understanding of genetic basis of defence mechanisms, relationship with udder health, and development of appropriate and practical phenotypes and measure schemes, before actual genetic improvement application.

5. CONCLUSION

Genetic control of udder health of dairy ruminants has been widely demonstrated. Accordingly, most countries have developed breeding programmes to select most resistant animals. Most evidence for genetic variability and application for genetic improvement, however, are principally based on phenotypic traits related to the healthy versus diseased status such as milk SCC and clinical mastitis occurrence. They therefore give only few clues about components of resistance involved in genetic determinism, the universality of those abilities in various epidemiological situations (environment, pathogen) and validity over time. Considerable progresses have been made in the last decade in dissecting immune mechanisms and genes that play key role in the mammary gland defences, but the function is highly complex and is still a large field for investigation. Studies combining different field approaches (genetics, QTL characterisations, immunology..), including new technology such as transcriptomics and proteomics, may be promising to better understand genetic basis of udder health, to predict long term responses to selection, and to develop new tools and strategies for genetic improvement of udder health.

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