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Institutionen för biomedicin och veterinär
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Is it possible to improve the bovine immune response to mastitis using immunogenetics?

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SUMMARY

Mastitis is a major problem for cows all over the world. For decades selection against mastitis has been performed through direct and indirect selection. Favorable outcomes of reduced mastitis incidence include: Economic gains, reduced use of antibiotics as well as improved animal welfare. Lately, new immunogenetic methods have emerged and the era of genomic selection has arrived. The innate and adaptive immune response, the bovine MHC and epigenetics are all of great relevance in this endeavor to improve immune response (IR) in cattle. Regarding breed differences in IR, few studies have been carried out on this subject and several factors need to be examined.

The High Immune Response (HIR) technology is a patented method developed by the University of Guelph, it identifies so called high immune responders in the population. Another technique is Genome-wide association studies (GWAS), this method can be used to find the location of immune-related traits on the bovine genome. A few different GWAS will be accounted for. For example, GWAS searching for genome associations with natural antibodies (NAb) as well as for associations with antibody-mediated IR (AMIR) and cell-mediated IR (CMIR). Genomic selection (GS) is another modern technique in which estimated breeding values (EBV) are calculated based on single nucleotide polymorphisms (SNPs). This method requires a genotyped and phenotyped reference population and animals need only to be genotyped for the indicator markers to be assigned an EBV. This has reaped great success in increasing genetic gain as well as in decreasing generation intervals.

Can these emerging methods surpass the results of the previously favored ones? The advantages as well as disadvantages are discussed. The disadvantages with the previous methods of selection for breeding are due to the time required and high expenses. Advantages with the emerging ones are faster results and shorter generation intervals. However, does the long-term genetic gain of GS actually surpass the traditional methods? Also, when manipulating the immune system, balance between responses must be considered. Otherwise, adverse effects like autoimmunity might appear. Sources of bias to the studies presented are also briefly mentioned. In conclusion, the area is still far too new to draw any major conclusions. However, it is a promising subject and further studies are of interest. The purpose of this literature study is to bring clarity to the question of whether it is possible to improve the bovine immune response to mastitis using immunogenetics.

INTRODUCTION

Mastitis is a major problem for farmers as well as for cows all over the world. It is one of the dairy industries most common and costly diseases. For decades the Nordic countries have practiced direct selection for resistance to mastitis using national health recording programs. Furthermore, indirect selection for mastitis resistance using somatic cell count (SCC) is nowadays a frequently used tool in most countries. Recently, large-scale genetic and genomic selection programs with the aim to reduce mastitis and other health disorders have been developed (Weigel & Shook, 2018).

The immune system in mammals is regulated by several thousand genes which make up 8-9% of the total genome. The sheer size of it indicates how important these traits are. Breeding for an improved immune response can therefore be of great value. The cost of disease is monumental for the industry and treatment with antibiotics needs to be used with caution due to rapidly increasing antibiotic resistance. Furthermore, using genetic approaches for enhanced health may be beneficial for other traits too, such as reproduction and growth (Mallard *et al.*, 2015).

Even though the advantages of these methods seem plentiful, caution and afterthought must be practiced when attempting to manipulate the immune system. Some concerns regarding this are raised in an article from 2012 (Rauw, 2012) written from a resource allocation perspective. The immune system can be considered a life history trait, since pathogens are one of the greatest threats to an animal's survival. This means that the immune system has to trade-off against other important functions since resources are limited and need to be evenly allocated among organs. As a consequence, it is important to examine how breeding for an improved immune response affects the animal as a whole. For example, when the components of the immune system are produced as well as activated, metabolic activity increases. These processes expend energy and resources. Activation of the innate response is considered to be more costly than that of the adaptive response. Also, responses mediated by T and B cells may cause damage to the host when deployed in excess, resulting in autoimmunity or allergy. Another concern is that pathogens may be able to evolve faster to circumvent the immune system than we are able to enhance it through breeding (Rauw, 2012).

The aim of this literature study is therefore to shed light on recent developments in the area of bovine immunogenetics. Hopefully reaching a conclusion on whether these upcoming methods have the potential to actually improve the bovine immune response, and reduce the mastitis prevalence among cows.

MATERIALS AND METHODS

Databases: PubMed, Web of Science and Google scholar.

Search words: Bovine OR dairy OR cattle OR cow* AND immunology OR “immune response” AND mastitis OR “intermammary infection”

“Mastitis pathogens” OR “Staphylococcus aureus” OR “Escherichia coli”

Review-article sources.

LITERATURE REVIEW

Mastitis

Mastitis is usually defined as an inflammation of the mammary gland. The cause is most often bacteria but it can have other origins. It all begins when pathogenic bacteria enter the mammary gland. This is often due to the first line of defense; the teat canal, having been compromised. Mastitis infections can be classified in several different ways. First of all, it can be either subclinical or clinical. Subclinical mastitis means that no visual changes are observed in either the milk or udder, although infection is present. Clinical mastitis on the other hand involves an inflammatory response, resulting in visibly altered milk. Furthermore, the severeness of clinical mastitis can differ. In mild or moderate mastitis, pain, heat, redness or swelling in the udder can occur. In severe mastitis, the response is systemic. This can involve anorexia, fever or shock. If the infection persists for longer than 2 months it is termed chronic (Thompson-Crispi *et al.*, 2014a).

The spectrum of pathogenic bacteria causing mastitis varies, not only between different countries but also between subclinical and clinical mastitis. As for the situation in Sweden, two different studies will bring clarity to the matter. One of these is a nationwide survey on the etiology of subclinical mastitis on Swedish dairy farms. The results of 590 bacteriological diagnoses are given in Table 1. Samples with no growth or contamination constituted 22% and 18% of the diagnoses, respectively (Persson *et al.*, 2011). The second study is also a nationwide study, but focuses on the etiology of acute clinical mastitis in 829 Swedish dairy cows. In this study, samples with no growth or contamination constituted 10.6% and 4.5% of the diagnoses, respectively. Results are summarized in Table 1 (Ericsson Unnerstad *et al.*, 2009).

Table 1. Prevalence of mastitis pathogens in clinical and subclinical mastitis, respectively

Mastitis pathogens	Mastitis in percent (%)	
	Clinical mastitis	Subclinical mastitis
<i>Staphylococcus aureus</i>	21.3	19
<i>Coagulase-negative staphylococci</i>	6.2	16
<i>Streptococcus dysgalactiae</i>	15.6	9
<i>Streptococcus uberis</i>	11.1	8
<i>Escherichia coli</i>	15.9	2.9
<i>Streptococcus spp</i>	-	1.9
<i>Arcanobacterium pyogenes</i>	6.1	-
<i>Klebsiella spp</i>	4.2	-

Common mastitis pathogens

***Staphylococcus aureus* (S. aureus)**

Most cases of *S. aureus* mastitis are subclinical and the infections have a tendency to become chronic. Clinical mastitis due to *S. aureus* can also occur but are less common. *S. aureus* mastitis is difficult to treat and very contagious. Spread of the infection can occur through direct as well as indirect contact, either between infected individuals or contaminated objects. Infected cows must be culled or separated from the herd until successfully treated. Once the infection has taken hold, it is hard to treat since it does not respond well to antibiotic treatment. *S. aureus* produces toxins which damage the udder tissue, this results in scar tissue and reduces the milk yield. Leukocytes are recruited to the area to fight the infection. However, *S. aureus* is able to hide within the neutrophils as well as other host cells. This enables *S. aureus* to hide from the immune system and escape antibiotics. The bacteria also have the ability to form abscesses in order to avoid detection and treatment (Petersson-Wolfe *et al.*, 2010).

***Escherichia coli* (E.coli)**

E. coli is a common cause of mastitis in dairy cattle and it is a part of the normal intestinal flora in humans as well as animals. Despite several studies on the subject of mastitis caused by *E. coli*, many questions concerning the clinical course and pathogenesis still remain unanswered. In early lactation the cow is more susceptible to *E. coli* induced mastitis, it also tends to be more severe during that period. This is most likely due to the pregnancy taking a toll, negatively affecting the immune system in the peripartum period. However, some evidence has shown that mastitis can originate from the late dry period and become clinical first in early lactation. So the severity and outcome of *E. coli* mastitis vary even for the same individual, during different lactation stages. The host response plays a major role in *E. coli* mastitis; the progress of the disease and the final outcome is based on the reactions of the host (Lehtolainen, 2004).

Favorable outcomes of reduced mastitis incidence

- Reduced use of antibiotics

Mastitis often requires treatment with antibiotics. In Sweden, two thirds of the antibiotics prescribed to cattle is used to treat mastitis (Hårdemark, 2014). Also, the use of antibiotics contributes to the development of antibiotic resistance in bacteria. Therefore, antibiotics should be used with caution and only when necessary.

- Economical aspect

In broad terms there are two types of costs to consider, direct and indirect. The direct costs consist of discarded milk, veterinary fees and increased labor as well as reduced milk yield and quality. In Sweden only veterinarians are allowed to prescribe medication for treatment of mastitis. To consult a veterinarian for treatment of clinical mastitis (CM) the average cost is €119. Indirect costs are also a result of mastitis but can be less obvious. Some of these are: Increased risk of secondary disorders, reduced fertility, increased risk of culling and in some cases increased mortality. As a consequence, the total cost can become very steep (Nielsen, 2009).

- Animal welfare

Aside from the economic losses and the questionable use of antibiotics, it is important to also consider the suffering mastitis causes the animal in question. The ethical aspect of disease which are related to animal welfare should be accounted for. Also, in recent years, consumers have become more interested in animal welfare as well as production methods (Heringstad *et al.*, 2000). According to the theory of supply and demand, the consumers wishes have a huge impact on the industries actions

History of genetic selection in the Nordic countries

The foundation of genetic selection for improved health and mastitis resistance was pioneered in the Nordic countries. It began in the 1960s when Norwegian farmers, veterinarians and academics realized the number of veterinary treatments on cows were increasing. A health recording system was put in place to deal with the issue. This system was implemented in the other Nordic countries and later spread across continental Europe and North America (Weigel & Shook, 2018).

Somatic cell count (SCC) and clinical mastitis (CM)

Breeding for increased resistance against mastitis can be executed using direct and indirect selection. The most common indirect factor is SCC. Somatic cells include neutrophils, macrophages, lymphocytes and epithelial cells. Bacterial infection causes a shift in the cell composition and numbers. Heritability estimates have reported ranges from 0.08 to 0.19 for SCC. However, SCC and CM are not the same traits, although both are measures of udder health. SCC sheds light on the subclinical or chronic cases while CM neglects these. In this way

they complement each other. The heritability of CM was estimated in several studies based on Nordic health-recording system data. Most values are in the interval of 0.02-0.03. The Nordic countries record information on SCC and some also use CM (Heringstad *et al.*, 2000).

Progeny trial

In the Nordic countries progeny trials have formed the basis for estimated breeding values (EBV). A bulls daughters are monitored using veterinarian field records to evaluate their performance in production. The models and data used differ slightly between countries but are strikingly similar. The average daughter group in Sweden 1992 consisted of 140 individuals. Despite the low heritability of some traits, accuracy as well as genetic gain can be quite high. A trait with heritability of 0.03 can have an accuracy of 0.66, 0.78 and 0.88 for progeny groups of 100, 200 and 300 respectively (Heringstad *et al.*, 2000).

The bovine immune system

The innate and adaptive immune response

The innate immune response is the first line of defense against pathogens. It responds quickly but lacks in diversity and specificity. The innate response is not only cellular but also include the physical and physiological barriers that prevents pathogens from entering the body. Pattern recognition receptors (PPR) recognizes invaders either through pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs). If the innate immune response is not enough, the adaptive response commences after at least 5-7 days. The adaptive immune system can be divided in two parts, the antibody mediated immune response (AMIR) and the cell-mediated immune response (CMIR). AMIR is classified as a type 2 immune response and defends the body against extracellular pathogens, for example bacteria. CMIR is primarily a type 1 immune response and takes care of intracellular pathogens, such as obligate intracellular bacteria. AMIR and CMIR are genetically and epigenetically regulated (Paibomesai, 2017).

The heritability of AMIR and CMIR have been estimated in several previous studies and found to be moderate, suggesting breeding for improved immunity is a possibility. The value for CMIR ranged from 0.19 to 0.42 and for AMIR estimates were between 0.16 and 0.64. However, these two types of responses are negatively correlated, from -0.13 to -0.45. The cytokines characteristic for CMIR and AMIR tend to be antagonistic. This phenomena underscores the importance of considering both traits when breeding for enhanced immune response (IR), enabling the animal to mount a versatile defense against invaders (Thompson-Crispi *et al.*, 2012).

The bovine MHC (Bovine leukocyte antigen (BoLA))

The major histocompatibility complex (MHC) is an important part of the immune system in most vertebrates. To understand immune response it is important to define the structure, function and diversity of this system. The bovine MHC genes are located on the bovine autosome 23 and is referred to as Bovine leukocyte antigen (BoLA). The MHC consists of 3

classes of histocompatibility antigens, class I, class II and class III. Class I molecules main function is to present peptides to CD8+ T-lymphocytes and is expressed on all nucleated cells. Class II molecules present peptides from extracellular pathogens to CD4+ T-cells which in turn activate macrophages and B-cells. Class II molecules are expressed on professional antigen presenting cells (APCs). Class III molecules are also associated with the immune process, for example they regulate components of the complement system and tumor necrosis factors (TNF). Class II molecules in ruminants are divided into two subregions. One of these is the class IIa region, which contains two clusters of genes, DR and DQ. The second region is the class IIb region which includes the DMA, DMB, LMP7 and TAP genes which are involved in antigen processing and transport (Behl *et al.*, 2012).

The MHC has an important role in the induction and regulation of the adaptive immune response and has been associated with resistance to various diseases. The genes of the MHC are highly polymorphic and there are many reports regarding its connection to immune response and disease resistance. A variety of studies have reported a relationship between BoLA class I and resistance or susceptibility to mastitis, or immune response. Others have observed a connection between class II DRB and resistance or susceptibility to mastitis. Breeding programs based on MHC associations have been implemented, quite successfully (Rupp *et al.*, 2007).

Epigenetics

The term epigenetics was first established in the 1960s by Conrad Hal Waddington. It is used to describe changes in gene expression or phenotype that cannot be explained by genetic differences between individuals. This means that the epigenome affects the way genes express themselves and the change passes on from cell to cell. However, the epigenome is not set in stone, it adjusts in response to environmental stimuli. This ability allows an individual to adapt to its surroundings (Paibomesai, 2017).

Few studies have been published in the area of bovine epigenetics, especially ones regarding epigenetic profiles playing a part in the bovine immune response are lacking. However, studies have shown that the adaptive response is partly epigenetically regulated. Epigenetic mechanisms have been discovered in several of the cells involved in the immune response. For example, T-helper (Th) type 1 cells show high concentrations of Interferon-gamma (IFN- γ) and limited Interleukin-4 (IL-4) production. On the other hand Th2 cells have high IL-4 and scarcely any IFN- γ . The loci that controls IFN- γ and IL-4 are subject to epigenetic changes in response to stimuli, the processes depend on specific transcription factors in the Th1 and Th2 lineages (Paibomesai, 2017).

Immunologic breed differences

The literature on the subject is scarce. However, two studies on the breed dependent differences in innate immune responses of Holstein and Jersey cows have been performed. One focuses on the response to *E.coli* caused mastitis and the other on *S. aureus* mastitis. Several previous studies have indicated that the prevalence and risk for mastitis is greater in Holstein than in Jersey cows. These differences point towards a possible breed-dependent difference in the

innate-immune response to infection. The studies mentioned above are very similar and some of the shared factors will be mentioned briefly. All cows enrolled were in similar stages of lactation, of the same parity and subjected to the same housing and management conditions (Bannerman *et al.*, 2008b; a).

The *E. coli* study showed that overall responses between breeds were similar, although temporal differences in onset, cessation and duration of a number of responses were detected. Most cytokine production and induction of acute phase protein synthesis were similar, although Jersey cows had a tendency to produce increased concentrations of IFN- γ . IFN- γ is a link between the innate and adaptive immune response and promotes a Th1 response. Along with IFN- γ initial induction of IL-1 β occurred earlier and remained elevated longer in Jersey cows. Also, the temporal induction of some responses differed between breeds. In Holsteins, levels of SAA, IL-8 and TNF- α , which are involved in neutrophil recruitment, increased earlier in the response. However, despite some small differences in infection clearance and resolution of the inflammation, the response was comparable between breeds. These data do suggest a highly conserved innate immune response in Holstein and Jersey cows in response to *E. coli* induced mastitis (Bannerman *et al.*, 2008a).

For *S. aureus* the overall duration, temporal onset as well as magnitude were strikingly similar across breeds. Unlike *E. coli*, which elicits a heightened inflammatory response often resulting in rapid elimination, *S. aureus* evokes a response of less magnitude and opens up for chronic infection. *S. aureus* infection caused a leukocytosis in both breeds, although the duration was greater in Holsteins. Holsteins also showed an increased number of circulating neutrophils in comparison to the Jersey cows. According to previous large scale surveys, Jersey cows tend to have a higher SCC than Holsteins. In this particular study however, the overall SCC responses were similar. Although, after 96 h and 240 h post infection SCC in Holsteins exceeded the Jersey cows. Another finding was that N-acetyl-beta-D-glucosaminidase (NAGase) activity was significantly higher in Jersey cows compared to Holsteins. Increased NAGase activity can act as an indicator of mammary tissue injury, which an elevated SCC is also an indicator for. Furthermore, a significant correlation between SCC and NAGase activity was found in both breeds (Bannerman *et al.*, 2008b).

Immunogenetic methods

High immune response technology (HIR)

The University of Guelph has developed a patented test-system, the High Immune Response technology (HIR), which is used to identify cows with superior immune responses (Thompson-Crispi *et al.*, 2014b). The protocol had previously been tested in several herds in Ontario as well as on dairy cattle from Florida with satisfying results. However, since AMIR and CMIR had only been tested on limited herds from the same region, the new study aimed to broaden the perspective to a national level using 680 cows from 58 dairy operations across Canada. This was part of a larger study conducted by the Canadian Bovine Mastitis Research Network (CBMRN). The objective was to evaluate immune response phenotypes in Holstein cattle outside the peripartum period, as well as to determine whether antibody isotype bias shifts

toward type 1 or type 2. The hypothesis stated that the results would vary among individual cows, herds and regions. Data on clinical mastitis was obtained from CBMRN and analyzed for the cows enrolled in the study (Thompson-Crispi & Mallard, 2012).

The cows were immunized with test antigens in order to measure their ability to establish CMIR and AMIR. Delayed-type hypersensitivity (DTH) was used to indicate CMIR, a type 1 response. For AMIR, primary (day 14) and secondary (day 21) serum antibodies of the immunoglobulin (Ig) G1 and IgG2 were used as an indicator for a type 2 response to the test antigens. The immune response towards extracellular antigens is usually a type 2 response, predominated by IgG1. In regards to intracellular pathogens a type 1 response dominated by IgG2 is usually mounted. Cytokines contribute to the bias towards either a type 1 or 2 response. IL-4 steers towards IgG1 while IFN- γ contribute to an IgG2 production. The ratio between IgG1 and IgG2 is used to determine the type of immune response towards the test antigens, vaccine or infection. Therefore, evaluation of CMIR and AMIR combined with the Ig bias can be used to determine the IR profiles (Thompson-Crispi & Mallard, 2012).

Regarding the results of this study, immune response varied significantly between cows, herds and regions. In Alberta, cows had higher DTH responses and secondary response to the type 2 test antigen compared to other regions. On the other hand, these animals had lower primary antibody responses. However, these cows also had the lowest incidence of mastitis caused by *E. coli* and *S. aureus* in comparison to the other regions. Although, it is worth noting that cows in each region have developed specific IR according to their environment and the antigens they are exposed to. Also, differences in management practices may play a part in the differences in IR found in this particular study (Thompson-Crispi & Mallard, 2012).

Another study in collaboration with CBMRN set out to compare incidence rates of CM (IRCM) between cows classified as high, average or low for AMIR and CMIR, respectively. 458 lactating Holsteins from 41 herds were immunized with a type 1 and a type 2 test antigen. A DTH for CMIR and IgG1 for AMIR were used as indicators. By using EBVs, cows were classified as high, average or low responders. In the next step IRCM was calculated as number of cases of mastitis over total time at risk during the 2-year study. Cows ranked as high-AMIR had 17.1 cases per 100 cow years. In comparison, average and low responders for AMIR had IRCMs of 27.9 and 30.7, respectively. For CMIR, no differences in IRCM were observed. These results align with previous studies which found high-AMIR cows to be prone to less mastitis in 2 out of 3 herds tested (Thompson-Crispi *et al.*, 2013).

Genomic selection (GS)

The dawn of genomic selection (GS) has revolutionized dairy cattle breeding. Genomic estimated breeding values (GEBV) provides the foundation for GS. GEBV is calculated by estimating SNP effects from prediction equations. These equations are based on a reference population that has been subjected to phenotyping as well as genotyping. This procedure ensures that all loci that contribute to a trait are accounted for, even loci with hardly any effect. Animals need only to be genotyped for the markers to find out which alleles they carry, and the estimated effect of these can be summed to get a GEBV (Hayes *et al.*, 2009).

GS has doubled the rate of genetic progress, increased selection accuracy as well as decreased generation intervals. The benefits are major for traits that are noticed late in life as well as for traits with low heritability. Also, past selection has caused an increased inbreeding in for example the US Holstein cow population born since 2000. GS can act as a means to control inbreeding in the upcoming generations by computing genomic relationships (Wiggans *et al.*, 2017).

Genome wide association studies (GWAS)

A GWAS searches the entire genome and identifies single nucleotide polymorphisms (SNPs) that could be associated to specific traits of interest. Increased availability of high-density SNP arrays and whole genome sequence data improves the chances to identify mutations. However, linkage disequilibrium (LD), the association between loci, spreads over large areas in the bovine genome. Due to this, data from multiple breeds may be required to pin traits down to a more precise location (Sahana *et al.*, 2014).

A variety of different GWAS to better understand immune system related traits in cattle have been performed. One of the studies focused on genes responsible for the variation in natural antibodies (NAb) in Canadian Holsteins. NAb are polyreactive antibodies produced by B1-cells. They belong to the innate immune system, but also links it to the adaptive immune response. There are several isotypes: IgG, IgA and IgM which is the most common one. Heritability for NAb measured in blood and milk ranges from 0.08-0.45, with IgM having slightly higher heritability estimates. Variations in NAb are due to both genetic and environmental factors. However, in this study no significant SNP associations for NAb IgM were detected despite the high heritability. This indicates that IgM is under polygenic control which makes it hard to track down its origin. For IgG, significant associations were found on bovine chromosome, 1, 20 and 21. For both IgG and IgM, peaks could be seen across the genome, although not significant. With a bigger sample population, perhaps conclusions could be drawn. Genetic association studies have found no significant correlation between NAb and specific antibodies (SpAb). The lack of correlation indicates that it could be possible to include NAb for an improved innate immune reactivity in breeding programs. However, this should be done with caution, since selecting for increased amounts of NAb could lead to autoimmunity (de Klerk *et al.*, 2018).

Another GWAS with the objective to evaluate general immune responsiveness in cattle will be accounted for. With the ultimate goal being able to breed for immune response traits in the future. This was executed by identifying SNP markers, candidate genes and biological pathways associated with both CMIR and AMIR. The study therefore set out to find differences in genetic profiles among Holsteins classified as High or Low for AMIR and CMIR. A total of 163 cows were selectively genotyped and the results were validated using an unrelated Holstein bull population phenotyped for both AMIR and CMIR. The cows' results showed 186 SNPs significantly associated with AMIR, 93% of the markers were found on chromosome 23. 21 SNPs remained significant for CMIR. Candidate genes within 250,000 base pairs (BP) of significant SNPs were identified to determine biological pathways associated with AMIR and CMIR. The candidate genes included those within the MHC (BoLA), parts of the complement

system and various cytokines. The peak of markers found on chromosome 23 and the antigen processing pathway was significantly associated with AMIR in the cows, the results were confirmed in the unrelated bull population. The majority of SNPs associated with AMIR were located on chromosome 23, this gene region is known to mediate effective adaptive immune response. Therefore the connection to the antigen processing pathway is to be expected (Thompson-Crispi *et al.*, 2014b).

Previously it has been suspected that the selection for production in dairy cattle has decreased the MHC diversity and as a consequence increased the incidence of diseases like mastitis which is linked to BoLA. The high degree of variation on chromosome 23 associated with AMIR in this study, particularly within BoLA, suggests selection for immune responsiveness might be a way to maintain diversity in the MHC genes. The association between AMIR, CMIR and BoLA have previously been confirmed. The study also discovered significant variation in SNP profiles between cows classified as either High or Low responders for AMIR and CMIR, also confirmed by the reference bulls. This phenomena indicates that it could be possible to identify animals with superior immune responses based only on genetic profiles. The High AMIR response cows in this study have previously demonstrated a lower incidence rate of clinical mastitis compared to the Low responders. For general traits, such as immune response, which are controlled by a variety of different genes, estimated breeding values (EBV) or genomic estimated breeding values (GEBV) may be of great importance. Using these methods, complex traits can be selected for without knowing the exact genes (Thompson-Crispi *et al.*, 2014b).

Status in Sweden

Semex Sweden recently launched Immunity+. This program is based on the University of Guelphs patented HIR-technology. This is marketed on their website www.semexsweden.com. It is still far too early in time to draw any conclusions regarding whether the program can compete with other breeding strategies (Semex, 2019).

DISCUSSION

The objective of this literature study was to shed light on whether breeding cattle for improved health, in particular to reduce mastitis prevalence, through enhancing the immune response is a viable option. There are many factors to consider in this equation. Advantages as well as difficulties can be identified in regard to this particular breeding objective. First of all, one can begin to examine the previously favored methods of selection for breeding versus the emerging methods. Using either SCC, CM or progeny trials or a combination of these to estimate breeding values is an established method. The response to selection is quite high, despite the relatively low heritability. SCC and CM also complement each other (Heringstad *et al.*, 2000).

The disadvantages of the previous methods are due to them being costly as well as time consuming. Artificial insemination (AI) companies have to wait a minimum of 4.5 years before they can predict bull performances with enough accuracy for selection. During these years, hundreds of bulls are housed while waiting for the results which adds up to substantial expenses (Scheffers & Weigel, 2012). Furthermore, although SCC has been a valuable tool in mastitis

surveillance, it has been subject to some debate. Since high milk SCC is problematic from a quality point of view. Some benefits from a low SCC are longer lasting products and higher milk yield per cow. On the other hand, low SCC can cause an increase in mastitis prevalence (Alhussien & Dang, 2018). Since somatic cells are elevated in response to infection, breeding for a lower SCC may result in a lacking defense against mastitis.

GS provides a faster and cheaper way to achieve results. It was first in the mid to late 2000s that assays were developed that made it possible to genotype large numbers of SNPs at a fairly low cost. Genomic evaluations became official as late as of 2009 and the age of bulls sires has drastically decreased since GS started replacing progeny testing (Wiggans *et al.*, 2017). Worldwide, around 2 million dairy cattle have been genotyped for genomic prediction. This technique has resulted in increased genetic gain, which has been validated by genetic trend analysis in several countries. In Canada for example, the genetic gain has doubled since GS was implemented (Meuwissen *et al.*, 2016).

However, does the long term genetic gain from GS actually surpass the results of traditional phenotypic selection? Fact is, selection changes the patterns of LD between the SNP and quantitative trait loci (QTL). This means that some of the QTL variance will not be captured by genomic selection. Furthermore, phenotypic selection uses all QTL automatically, GS only uses markers which have already been discovered or previously had their effect estimated. So, it is of great importance which method is used to derive the prediction equations for GEBV. Some decay rapidly across generations while some last longer before they need to be re-estimated. One challenge in GS is therefore managing the long-term genetic gain (Hayes *et al.*, 2009). So far, not many candidate genes connected to immune function have been identified, many still remain unknown. Several immune traits seem to be connected to multiple loci, which makes them hard to identify. However, finding these traits seems to be on the agenda for several researchers. Since GS was only recently implemented the lack of information is only to be expected.

Furthermore, selecting for an improved immune response requires balance. If the inflammatory response is excessive it causes damage to the body. Also, if the immune system becomes too sensitive, it can start to react to self-antigens, causing autoimmunity. Also, as mentioned previously, an IR costs energy. However, the infection itself also uses resources. This leaves us with roughly plus minus zero in this regard. Maintaining a balanced immune response is imperial in breeding programs. Selecting against clinical mastitis can leave cattle susceptible to infections with other mastitis pathogens. Most mastitis pathogens are extracellular, which primarily requires an AMIR. Since AMIR and CMIR tend to be negatively correlated this could lead to decreased ability to fend against intracellular pathogens. Also, a variety of BoLA alleles have been associated with AMIR, CMIR and mastitis resistance. These alleles are not the same that associate with resistance to viral or parasitic pathogens. This should be considered when selecting for resistance against a specific cause (Thompson-Crispi *et al.*, 2014a).

In regards to the differences between breeds, more research is required in the area. There are many factors other than the difference in breed itself which can affect the results. In the two studies mentioned, Holstein and Jersey cows' response to induced mastitis with *E. coli* and *S.*

aureus were somewhat similar. Although some differences were detected these could be due to other factors as well. For example, one important difference between the breeds is production. Holsteins produce more milk, and the risk of CM is positively correlated with milk production. However, despite this fact, all cows in both studies did develop mastitis. Therefore, the correlation between CM and milk production may be due to a variance in susceptibility to initial infection instead of differences in the response (Bannerman *et al.*, 2008b).

The majority of the studies in this literature study show positive results regarding the emerging immunogenetic methods. However, there are some factors which are worth keeping in mind. First of all, many of the studies originate from the University of Guelph. Also, a majority of the literature is written by the same researchers. After searching for sources it became clear that while enough literature could be found on the subject, it originates from a quite limited pool. This could be a source of bias, especially for the HIR-technology. Furthermore, the HIR-technology is patented and due to this, economic interests could play a part. As for studies on GWAS and GS the information is more diverse and seems to originate from several different sources and different parts of the world. Furthermore, to determine whether the HIR-technology and GS is a good alternative to the previous methods, further testing has to be performed. Preferably on larger study populations, for longer periods of time and across several generations. However, since the HIR-method is patented, and the studies in the area began in the late 1990s, the lack of diversity in origins is to be expected (Wilkie & Mallard, 1999).

Weller asks the question “Is a selection plateau on the horizon?” (2017, s. 7). Genetic theory stipulates that sooner or later, genetic progress might plateau. Either through exhaustion of genetic variance or through development of antagonistic genetic relationships. Also, at some point, the economic gains might not be profitable enough to continue to strive for better results. In long-term selection experiments, the response usually ends after 20 to 30 generations. However, in some cases a significant response was observed for over 100 generations (Weller *et al.*, 2017).

In conclusion, being able to reduce mastitis prevalence would be a major success and a great relief for the dairy industry. Therefore, finding new improved methods to bring this problem to heel is of great importance. GS and the HIR-technology provides interesting alternatives and seem to be worth exploring further. However, caution must be exercised when attempting to manipulate the immune system, in order to avoid adverse consequences.

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