Genetic and Epigenetic Regulation of Immune Response and Resistance to Infectious Diseases in Domestic Ruminants

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KEYWORDS

• Genetic • Epigenetic • Disease • Resistance • Resilience • Tolerance • Immunocompetence • Ruminants

KEY POINTS

- Infection with a pathogen does not always result in disease, but infectious diseases are the outcome of interactions among 3 factors: host, pathogen, and environment. Several layers of sophisticated interactions between host and pathogens have been a major limiting factor in decoding and identifying the genetic rules of disease resistance.
- Different research groups have used different strategies to dissect this complex network and to understand the genetic rules of diseases resistance. These strategies can be grouped based on the genetic information examined (eg, candidate genes, pedigree-based genetic information, and genome-wide studies) and the phenotypic information available (eg, single-disease, immunocompetence, and reductionist models).
- Recent technological advances are helping researchers to generate big data sets of the host-pathogen-environment interactions. Nonetheless, defining the relevant phenotypes seems to be the main challenge to reveal the genetic blueprint of disease resistance.
- The possibility of negative genetic correlations in resistance to 2 pathogens (eg, intracellular vs extracellular organisms), even those causing the same disease (eg, the many diverse organisms causing bovine mastitis or pneumonia), or negative associations between resistance to a pathogen with important production traits, are other challenges to the single-disease approach, as well as other approaches to selecting for health.

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- An alternative method to scrutinize these complex interactions is to simplify the main interaction network into subsystems that can be examined in detail using in vitro methods. In any subsystems, the genetic control requires the contribution of a smaller number of genes, which may simplify the genetic pathways examined. Although reductionist models can reveal potential genes and pathways important for further examination to host defense, they may not fully reflect the intricacies of in vivo disease resistance.

INTRODUCTION

During disease outbreaks, some individuals in a population are more resistant to infection than others. Resistant individuals may survive, whereas others in the population may die or have less severe signs of disease, or may completely eliminate the infection without showing clinical signs of disease. Infection with a pathogen does not always result in disease, but, when it does, infectious diseases are the outcome of interactions among 3 factors: host, pathogen, and environment.[1–3] Each of these factors has been examined in many studies that aimed to reduce the occurrence (increase resistance or resilience) or soften the impact of infection on the host (increase tolerance) (**Box 1**). Among the factors mentioned earlier, the host (or specifically the host immune system) has gained tremendous recognition because of its direct role in protecting the host against pathogens and also the possibility of genetic improvement of this system. However, several layers of sophisticated interactions between host and pathogens have been a major limiting factor in decoding and identifying the genetic rules of disease resistance.[4] The first layer is the functional complexity of the immune system. The immune system is an intricate network of cells and molecules that applies various strategies to protect the host against a broad range of pathogens.[5,6] These strategies range from nonspecific physical barriers to specific cytotoxic activity of lymphocytes. Innate defenses classically initiate immune responses; adaptive immune responses follow if innate mechanisms are not successful to eliminate the pathogen. Cells of the innate

Box 1
Resistance, resilience, and tolerance

These terms have varied definitions in the literature, but, for the purpose of this article, the following definitions are applied. Disease resistance is defined as the ability of the host to control the infection passively (eg, absence of a target receptor) or actively (ie, mounting the protective immune responses).[5] The resistant animals can clear the pathogen, entirely. Resilience is the ability of the host to recover after a disease.[144] Resistance and resilience are closely related but not interchangeable. Resilience is always an active phenomenon, whereas resistance could be passive because of the absence of the target receptor. Resilience can also be defined through the overall performance of the host in the face of general environmental challenges, not only in the face of disease. These challenges include, but are not limited to, weaning, handling, moving to a new feedlot, and disease. Livestock may cope with these challenges via physiologic, behavioral, and also immune responses. Various studies have shown that the performance of immune response at the time of challenge can be a good indicator of the overall resilience of the animal in future.[49] The current livestock management practices make it possible to measure all components of resilience to predict the overall performance in future. Tolerance is the ability of the host to cope with the presence of the pathogen. Tolerant animals can maintain their production in the face of pathogenic infection.[5]

immune system mainly recognize microbe-associated molecular patterns via pathogen recognition receptors. Following recognition, they attempt to destroy the pathogen, which is followed by sending activating signals to the adaptive immune system. Cells of the adaptive immune system generate pathogen-specific responses. Although immune responses generally follow the pathways mentioned earlier, multifunctionality of cells and molecules, numerous subpopulations of cells, redundancy in responses, and various exceptions to the general rules are a few examples that increase the complexity of immune system.[4,7] For instance, γδ T cells, a subpopulation of T cells, belong to the adaptive immune system but can also act like cells of innate defense. These cells are prominent (50%–60% of lymphocytes) in circulating blood in the early life in ruminants, but their population decreases with age (5%–25% of circulating T lymphocytes). Nonetheless, the percentage of γδ T cells among lymphocytes, even in adult ruminants, is higher than in humans and mice (1%–5%). These cells originate from lymphocyte progenitors but are not antigen (Ag) specific and do not need to recognize Ags in the context of major histocompatibility complex (MHC) molecules. Bovine γδ T cells have both inflammatory and regulatory activities. Cattle γδ T cells respond within a few hours of viral infection (similar to innate responses) by producing large amounts of interferon-γ (IFN-γ; a proinflammatory cytokine) or interleukin (IL)-10 (a regulatory cytokine) following exposure to antigen-presenting cells.[8,9] The proportion and functional capacity of these cells show individual variation among cattle genetically selected for immune responsiveness.[10]

Another layer of immunologic complexity relates to the genetic control of immune responses. Approximately, 20% of the bovine genome (ARS-UCD1.2, ENSEMBL 95) is annotated with the immune response.[11] This portion is composed of 5369 genes that are directly involved in mounting immune responses. High-throughput technologies have shown expression of up to 71.4% of the bovine genome in one cell type, the macrophage, after exposure to a pathogen. Compared with the unchallenged control, approximately 245 to 574 genes are differentially expressed.[12] Given the possible nonadditive effects (epistatic and dominance) and epigenetic mechanisms, the genetic control of immune response is astonishingly complex.

A third layer of genetic complexity is caused by the pathogen evolving during an infection. For instance, viral pathogens are well known to escape the immune response through genetic mutations (eg, antigenic shift and drift in influenza viruses) or stabilize themselves in the host via inserting their genome in the host genome, (eg, bovine leukemia virus). This phenomenon potentially results in multistrain infection in 1 host or 1 population during an outbreak.

In this article, recent advances in genetic and epigenetic regulation of immunocompetence and disease resistance in domesticated ruminants are discussed. Moreover, because of the importance of climate change, recent studies of gene-by-environment effects on the regulation of host defense are also discussed.

GENETIC REGULATION OF IMMUNOCOMPETENCE AND DISEASE RESISTANCE

Recent technological advances have helped researchers to generate big data sets of the host-pathogen-environment interactions. Nonetheless, defining the relevant phenotypes seems to be the main challenge in revealing the genetic blueprint of disease resistance.[13,14] Various research groups have used different strategies to dissect this complex network and to understand the genetic rules of diseases resistance. These strategies can be grouped based on the genetic information (candidate genes,

pedigree-based, and genome-wide studies) and the phenotypic information (single-disease, immunocompetence, and reductionist models) (Table 1). The advantages and pitfalls of each strategy are discussed here.

Source of Genetic Information: Candidate Gene, Genome-Wide, or Pedigree

The main difference between a candidate-gene versus a genome-wide approach is the goal of the study: the candidate gene approach is hypothesis driven, whereas the genome-wide approach is discovery based. The hypothesis in the candidate-gene approach is based on a previously established biological link or association between the candidate gene and the health trait. In most cases, the candidate gene has a strong biological and well-defined role in the pathway of pathogenesis or the protective immune response. However, this information, which is the basis of the new hypothesis on the gene of interest, has usually been proved in other species or the magnitude of its effect has not been measured on the specific disease (type I studies) or immune response (type II studies) (see Table 1).

Genes of the MHC are arguably the most studied health trait genes in ruminants, as well as other species. One of the first reports of an MHC polymorphism and its association with immune response was from a study in guinea pigs published in 1975.[15] The genetic polymorphism within the bovine MHC known as the bovine lymphocyte antigen (BoLA) system was first reported in 1979.[16] Since then, the association of BoLA with many infectious and metabolic diseases, as well as characteristics of immunity, has been the subject of numerous studies.[17–23] Despite the extensive studies on BoLA, the findings have rarely been commercially used in breeding programs because of the inverse association with different pathogens and between types of immune responses.[23] An exception was specific BoLA DRB-3 and DQB alleles being used to reduce bovine dermatophilosis from 0.76 to 0.02 in Brahman-Zebu cattle on the island of Martinique.[24] This approach worked well because the disease was highly prevalent in a well-contained population of cattle.

The genome-wide association and pedigree-based studies are descriptive studies designed to discover novel associations (type V and VI studies) or to estimate breeding values for individuals in the population (type III and IV studies) (see Table 1). The genome-wide genetic information can be combined with pedigree information to increase the reliability of breeding value estimates by accounting for polygene effects. These estimates are known as genomic breeding values,[25] which are currently being used by breeding companies, such as the Semex Alliance, to improve accuracy of selection for various traits, including for Immunity+. However, the findings of genome-wide association studies should be validated (eg, using candidate gene studies), and the findings of pedigree-based studies only hold within the population

Table 1
Classification of studies on genetic control of immune response and resistance to infectious disease based on genetic and phenotypic information

		Genetic Polymorphism		
		Candidate Gene	Pedigree	Genome-wide
Phenotype	Single Disease	Type I	Type III	Type V
	Immunocompetence	Type II	Type IV	Type VI

The pedigree information can be used alone, or it can be added to the genome-wide study to account for polygenic effects. The pedigree information can also be used to select samples in candidate gene approaches to maximize the diversity of the samples.

or the breed of the studied population, depending on the structure of the sample population.[26]

In genetic association studies, single nucleotide polymorphisms (SNPs) have been the primary source of genetic information used to identify variation between individuals. Over the past decade, technological advances in sequencing and bioinformatics have provided other sources of genetic information, mainly structural polymorphisms at the genomic, epigenomic, and transcriptomic levels (**Box 2**).[27] These structural variants are novel and remain expensive to detect. Therefore, the studies on ruminants using structural polymorphism are limited. Copy number variation (CNV) and splicing variants are two types of novel polymorphisms that have been reported in cattle, water buffalo, sheep, and goat.[28–31] Studies on the association of the structural variants mentioned earlier with health traits in ruminants are rare. However, there is a limited number of reports on the association of CNVs with bovine clinical mastitis, somatic cell score, and resistance to gastrointestinal nematodes in cattle and resistance to retroviral infection in sheep.[32–35] The methods to detect structural variants and the challenges in their application in breeding have been reviewed by Bickhart and Liu.[36]

Source of Phenotypic Information: Single Disease or Immunocompetence

In the single-disease approach, the goal is to identify alleles (type I studies) or to estimate genetic variance components (type III and V studies) by comparing animals classified as resistant, resilient, or tolerant to a disease or syndrome following infection (natural or experimental challenge) with animals classified as susceptible. Any type of investigation of the genetic regulation of immune response and disease resistance needs a large sample size. The sample size required to achieve adequate statistical power can be ambiguously estimated using the predicted number of quantitative trait loci (QTLs) that control the trait, minor allele frequency of QTLs, the threshold to detect QTL effects, effective population size, and estimated environmental effects.[37,38] Although environmental effects can be controlled or removed in experimental challenges, this type of study in large ruminants (ie, to investigate the genetic control of disease resistance) is less feasible because of the space needed for the containment facility and the high cost to study a large sample population. These limitations have led some researchers to choose case-control studies following natural infection as the most feasible approach to study the genetic control of health traits in ruminants.[39] The most critical step in case-control studies is to accurately define the phenotype and assign the samples to the appropriate class. During natural infections, the time of occurrence of the infection, the dose of infection, and any prior exposure to the pathogen are often unknown factors. Therefore, animals might be classified

Box 2
Novel sources of genetic variation

Copy number variation (CNV). CNV is a type of structural variation defined as a segment of DNA (more than 1 kb) with more than 1 copy in the genome. These segments have undergone inversion, deletion, or duplication mutations, but the sequences of these segments are very similar to each other. These segments are called copy number polymorphism when their frequency in the population is more than 1%.[145]

Spicing variants. During messenger RNA (mRNA) maturation, intron segments are removed from pre-mRNA, and exons are joined together. Through this process, alternative combination of exons and residues of introns can result in different variants of a mature mRNA, called splicing variants. The association of splicing variants and mastitis has been reported in cattle.[146,147]

incorrectly. For instance, one individual might be classified as resistant but was not exposed to the pathogen, or the animal might have been previously exposed and developed immunologic memory. These types of errors can potentially introduce prominent experimental "noise," resulting in an inability to detect QTLs with small effects or rare alleles. However, when the mortality of a disease is very high (eg, highly pathogenetic avian influenza) or the genetic defect is lethal (eg, bovine leukocyte adhesion deficiency [BLAD]) the study noise does not obscure identifying cases and controls. Therefore, identification of genetic regulation at the level of causal mutation is feasible. For instance, BLAD was found to be caused by a mutation on the cluster of differentiation (CD) 18 gene that encodes an adhesion molecule (β2 integrin) on the leukocytes surface causing major defect in phagocytosis, chemotactic response, and other normal functions of neutrophils.[40] In investigating the genetic regulation of diseases with lower mortality or morbidity rate, the study noise must be carefully considered in case-control studies and modified designs are being proposed for complex diseases, such as bovine mastitis.[41]

Genetic regulation of resistance to complex diseases, such as clinical and subclinical mastitis, Johne disease, lameness, calf diarrhea, bovine leukosis, bovine tuberculosis, and helminth infestations, are the subject of numerous investigations by using the single-disease approach. The heritabilities of these traits are low compared with production and immune response traits (**Table 2**). Therefore, the genetic gain will be slow depending on selection intensity.[42]

Table 2
Heritabilities of health traits in cattle

Health Trait	Heritability	References
Disease Resistance		
Clinical mastitis	0.02–0.04	Govignon-Gion et al,[148] 2016; Koeck et al,[149] 2014
Subclinical mastitis	0.04–0.06	Narayana et al,[150] 2018
Johne disease	0.04–0.06	Brito et al,[151] 2018; Kirkpatrick & Lett,[152] 2018
Lameness	0.01–0.09	Chapinal et al,[153] 2013; Koeck et al,[149] 2014
Bovine leukosis	0.08	Abdalla et al,[154] 2013
Bovine tuberculosis	0.10	Raphaka et al,[51] 2018
Nematode infestation	0.06–0.23	Passafaro et al,[155] 2015
Immunocompetence		
Cell-mediated immune response	0.18	Mallard et al,[156] 2018
Natural antibody	0.27–0.31	de Klerk et al,[65] 2018
Specific antibody response	0.46	Emam et al,[157] 2014
Cellular Traits		
Percentage of CD4+ lymphocytes	0.46	Denholm et al,[73] 2017
Percentage of CD8+ lymphocytes	0.41	Denholm et al,[73] 2017
Percentages of monocytes	0.15	Denholm et al,[73] 2017
Percentages of monocytes	0.42	Denholm et al,[73] 2017
In vitro nitric oxide response of bovine monocyte-derived macrophages	0.78	Emam et al[75]

Additional challenges to the single-disease approach, as well as other approaches to selecting for health, are introduced by the possibility of negative genetic correlations in resistance to 2 pathogens, even those causing the same disease (eg, the many diverse pathogens that cause bovine mastitis or pneumonia), or negative associations between resistance to a pathogen and important production traits. Mahmoud and colleagues[43] investigated the genetic correlation between 9 calf and 14 cow pathogens/diseases. In addition to a strong favorable correlation between resistance to the most common causal agents of mastitis (*Escherichia coli* and *Staphylococcus aureus*), they also reported negative correlations between rotavirus and chorioptic scabies, and between *E coli* and daily weight gain in calves.[43] Rupp and colleagues[23] reported similar negative associations in relation to mastitis, somatic cell count, and immune response traits. However, other studies have not shown these negative associations.[44–46] For example, breeding for enhanced immunity in cattle has not been associated with any notable negative impact on production, growth, or reproduction.[47–50] This lack of association may be caused by overall better health minimizing any decreases in feed intake caused by illness or beneficial shared genes that control a range of fitness traits.

It is possible to define customized breeding programs to increase genetic resistance to disease by combining epidemiologic data on the common pathogens in any geographic location with the results of single-disease approaches. Bovine tuberculosis (bTB) is a significant threat to the cattle industry globally and a distressing problem in the United Kingdom, where the carrier of the pathogen, badgers, are protected by law. Nonetheless, Raphaka and colleagues[51] predicted that the risk of transmission of bTB between the herd mates can be reduced by 50% in 6 generations by selecting the top 25% of bTB-resistant sires (heritability of 0.1). Improving resistance to subclinical mastitis by reducing the somatic cell score in dairy cows has been used for decades. In addition, Scandinavian countries have been selecting for improved resistance to clinical mastitis, and more recently this has been added in Canada and the United States as other examples of the commercially available programs to improve health in dairy cattle.[26] In March 2016, Zoetis Inc introduced a Wellness Trait Index (WT$) on their Bovine Clarifide Plus SNP chip into the United States as part of their genomics program. The index includes genomic information on combined health traits, including mastitis, retained placenta, metritis, displaced abomasum, ketosis, lameness, and polled. In Canada, the Canadian Dairy Network offers a similar combined health index.[52] The limitation of these indices relates to the accuracy of disease event recording and the low heritability of these traits. In 2018, the Semex Alliance began to offer a genomics test for immunocompetence as part of their Elevate program. Rather than being based on lowly heritable clinical scoring data, the genomics test for immunity is based on direct measurements of the more highly heritable information on both antibody-mediated and cell-mediated immune responses, which control responses to a wide range of bacterial and viral pathogens.

Improvement of immunocompetence (also known as overall immune responsiveness) is another approach to increase resistance against infectious diseases. The rationale behind the immunocompetence approach is the beneficial direct link between the type and magnitude of an immune response with protection of the host. This approach makes sense because the immune system is the body's defense against infectious disease and cancer. The idea of selection based on immune response traits dates back to Biozzi and colleagues[53] in 1972 and the mouse model they generated. These mice are currently known as the ABH strain.[54] Biozzi selected these mice for many generations for increased antibody and decreased cellular responses to investigate the genetic regulation of susceptibility and resistance to

infectious diseases. In livestock, the idea was first introduced in the late twentieth century and continued research led to the first commercially available health index for dairy cattle in 2013, based on their immunocompetence.[6] Improving the overall immune responsiveness provides the opportunity for resistance to a broad range of pathogens when heritable, well-balanced, and broad-based aspects of immunity are contained within the selection index. Simultaneously, responses to vaccines have been reported to improve, and, in ruminants, their colostrum contains higher amounts of immunoglobulin (Ig) to protect their newborns.[48,6,55] Cows classified as having superior immunocompetence have a lower occurrence of various diseases, such as mastitis, metritis, and pneumonia.[6] This finding is true both in research and commercial application.[56] Also, as mentioned earlier, this approach has not been found to adversely affect production or reproductive traits of dairy cattle.[56,57]

The main challenge in the immunocompetence approach is to develop methods to measure immune responses that can reflect the overall performance of this complex system. The immune system is composed of an integrated network of innate and adaptive immune responses. The antibody-mediated and cell-mediated immune responses are the effector mechanisms of the adaptive immune system. Measuring serum antibody as the indicator of antibody-mediated immune response is accurate, inexpensive, and technically simple. However, defining an index to measure antibody-mediated and cell-mediated immune responses, as well as capture innate cellular responses, is much more challenging. The cell-mediated response can be evaluated by measuring T-cell cytotoxicity, interferon-γ, delayed-type hypersensitivity (DTH), or other measures of T-cell effector functions.[58,59] Although there are various methods to capture these T-cell responses, they are generally not as simple, cost-effective, or as accurate as measuring antibody. One of the advantages of measuring DTH is that it involves a variety of important leukocyte populations (neutrophils, macrophages, dendritic cells, and natural killer cells) from the innate system, as well as T and B lymphocytes from the adaptive immune system that are involved in mounting the DTH response.[60] Not surprisingly, the genetic control of this response is complex and involves many genes on various chromosome. The results of genome-wide association studies of serum antibody and DTH responses in dairy cattle show the differences in the genetic control of these two responses. Two major QTLs on chromosomes 21 and 23 are associated with antibody response to a type II antigen, whereas associations of SNPs with DTH are scattered over the entire bovine genome. Both traits are under polygenic control, but the nature of that control differs. To date, studies indicate that the heritability of antibody-mediated response is about 2-fold larger than the heritability of DTH response in dairy Holsteins[58] (see **Table 2**). This difference may simply be caused by the large number of leukocyte populations involved in DTH or the difficulty of accurately measuring this response in vivo. Nonetheless, the heritability estimates for both of these immune response traits are moderately high at 0.18 to 0.46, which is more than those for clinical scores of diseases. This difference is partly caused by the immune system directly controlling host defense and disease outcome, whereas clinical scores are an indirect indicator of the host response. For this reason, in 1999 Wilkie and Mallard[61] proposed that heritable adaptive immune responses measured after exposure to carefully selected nonpathogenic antigens can reflect general immunocompetence and be used in a selection index based on estimated breeding values to improve animal health (**Fig. 1**). From a technical point of view, this method is similar to a highly controlled experimental challenge because the background response, the dose of antigen, and the time duration from exposure to the test antigens to sample collection are fully controlled. Moreover, the added benefit of this test method compared with an experimental disease challenge is that

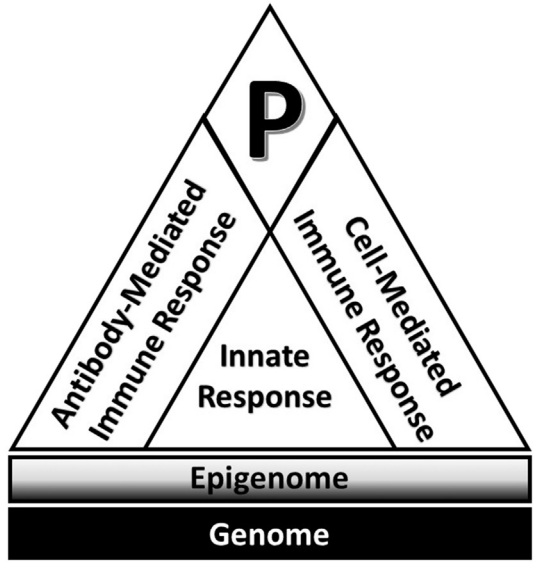

Fig. 1. In 1999, Wilkie and Mallard[61] proposed that the overall performance of the immune system (phenotype [P]) is shaped by adaptive immune responses, including cell-mediated immune responses and antibody-mediated immune responses. Because innate responses initiate adaptive immune responses, adaptive immune responses can reflect the performance of innate defenses. Furthermore, all of these responses are coded by immune response genes in the host genome that can be influenced by the epigenome. (*Adapted* from Wilkie B, Mallard B. Selection for high immune response: an alternative approach to animal health maintenance? Vet Immunol Immunopathol. 1999;72(1-2):231-235. http://www.ncbi.nlm.nih.gov/pubmed/10614513; with permission.)

there is no need for a containment facility because of the nonpathogenic characteristics of the test antigens.[62] These controlled factors result in high heritability of these health traits (see **Table 2**).

Measuring innate host defense can be more challenging than adaptive immune responses because of their varied mechanisms of action, broad specificity, and assorted tissue locations. Measuring innate cellular responses in vivo is very difficult, except for some limited measures in birds (eg, measuring macrophage phagocytosis using carbon clearance test).[63,64] In contrast, humoral innate responses, such as natural antibodies (NAbs), are simpler to measure, and their roles in disease resistance have been well studied in ruminants. NAbs, produced mainly from B1 lymphocytes, are present in the body before exposure to any foreign antigens and are classified as one of the humoral innate responses.[65] NAb provides one of the first barriers to infection. Binding to the pathogen, activating the complement system, and facilitating phagocytosis are the effector mechanisms that are mediated by NAb.[66] Studies on the genetic regulation of IgM and IgG NAbs have shown these as heritability traits in dairy cattle (see **Table 2**). The genes that control specific adaptive antibody responses versus NAb differ, indicating that these are distinct traits, each making unique contributions to defense of the host.[65,67]

Reductionist models

As mentioned previously, disease pathogenesis is the outcome of a highly complex set of interactions between host, pathogen, and environment. An alternative method

to scrutinize these complex interactions is to simplify the main interaction network into subsystems that can be examined in detail using in vitro methods. In any subsystems, genetic control requires the contribution of a smaller number of genes. These genes and their mechanisms of action can, in some cases, be studied in fully controlled in vitro environments in which infectious dose and virulence of the pathogen are set by the investigator.[68] Therefore, the chance of detecting QTLs with smaller effects or discovering causal mutations is higher than with other approaches. The other advantage of the reductionist model is the smaller sample size needed for these types of studies.[37] The downside of using reductionist models to study the complexity of disease resistance is that focusing on a single subsystem in vitro might be misleading. These in vitro models may not represent the in vivo condition, or their effect on the in vivo system might be small. Therefore, the results of the reductionist models should subsequently be evaluated on the in vivo outcome of host-pathogen-environment interactions.

The reductionist concept to study the genetic control of disease resistance was first introduced in 2009 by Ko and colleagues[69] and later was reviewed in a 2014 publication entitled "The Marriage of Quantitative Genetics and Cell Biology: A Novel Screening Approach Reveals People Have Genetically Encoded Variation in Microtubule Stability."[68] Dennis Ko and his colleagues have developed an in vitro model, called high-throughput human in vitro susceptibility testing (Hi-HOST), to screen the response of B lymphocytes followed by a genome-wide association study to investigate host-pathogen interactions. Using the Hi-HOST model, in 2017 they discovered an association between *VAC14*, a gene responsible in the metabolism of phosphoinositide, and resistance to *Salmonella enterica* serovar Typhi.[70] In 2018, an independent study on an African population found the association of the same gene with bacteremia in children.[71,72] These findings represent the potential of the reductionist approach to reveal the genetics rules in resistance to infectious disease.

In dairy cows, the genetic regulation of blood leukocyte proportions was first reported by Denholm and colleagues[73] in 2017, who found the heritability of the percentage of cells from the myeloid and lymphoid lineage in bovine blood ranged from 0.18 to 0.81. However, the researchers did not find any significant associations between these cellular proportions and the infectious diseases they examined.[73] Emam and colleagues[74] studied the effect of host genetics on the function of bovine monocyte-derived macrophages (MDMs) in response to *E coli* and *S aureus*, using a cellular immunogenomic approach in 2018. Using a highly controlled in vitro culture system, they showed a pedigree-based heritability of 0.776 for in vitro nitric oxide production by MDMs against *E coli*.[75] The genome-wide association study on this cellular trait revealed the association of 8 SNPs on chromosome 4, 5, 6, 9, and 27 describing 78% of the phenotypic variation.[76]

Results from reductionist models should be confirmed using other experimental systems. These confirmatory studies can be association studies in an independent population or investigation using genome editing or other appropriate technologies. Inducing disease resistance in ruminants using gene editing has previously been reported. Resistance to *S aureus* in a transgenic cow expressing lysostaphin in epithelial cells of the mammary gland is an example of this approach.[77] At present, new technologies with high efficiency and accuracy in inserting, deleting, or substituting single nucleotides are available. Clustered regularly interspaced short palindromic repeats–associated protein 9 (CRISPR/Cas9) is a tool to edit a specific sequence of the genome with single nucleotide accuracy. Some research groups have reported the successful application of this method in cattle. However, the publications are yet to come because of the long generation interval in cattle and the novelty of the

technology.[78] The reductionist models in conjunction with genome editing methods will likely provide the foundation of genetically resistant ruminants in future.

EPIGENETIC CONTROL OF THE IMMUNE SYSTEM IN DOMESTICATED RUMINANTS

Epigenetics is defined as the control of gene expression by mechanisms that do not change the underlying DNA sequence.[79,80] The epigenome can be influenced by environmental factors, including diet, stress, hormones, pathogens, toxins, and drugs, resulting in both permanent and reversible changes to gene expression. The epigenome encompasses all epigenetic modifications, including DNA methylation, histone modifications, and microRNA (miRNA) regulation, which are among the major regulatory elements that dictate chromatin accessibility and gene transcription. The immune system is dynamic and possesses the ability to respond to infection and other stressors while also regulating its own response. Epigenetic modifications regulate gene expression, which drives adaptive and innate immune cell phenotypes, establishing cell memory, cell polarization, and regulation of the immune response.[81]

DNA Methylation

The DNA methylome of the ruminant immune system is influenced by species,[82] tissue and immune cell types,[83–91] disease state,[85,92] stimulation,[93] age,[94] and physiologic event.[83,87,88,93,95–97] Studies investigating the DNA methylation levels and profiles of immune response genes have been performed on immune-associated tissues,[89] specific immune cells,[85,97–99] as well as other non–immune-related tissues[83,86,87,100] in domesticated ruminants species. Studies investigating DNA methylation and the immune system have reported on both the methylation of individual cytokine genes[93,99] and the global DNA methylome.[98] In cattle, CD4+ T-cell polarization depends on expression and secretion of cytokine genes including IFNγ and IL4. Expression and secretion of cytokines by isolated CD4+ T cells showed decreased DNA methylation at the promoter regions of IFNγ and IL4.[93,99] In addition, differential DNA methylation was observed at transcription factors GATA3 and RORC in alveolar macrophages of cattle infected with *Mycobacterium bovis*.[98] In agreement with this observation, examination of MHC-I revealed that DNA methylation of MHC-associated CpG islands are associated with downregulation of MHC-I.[101]

Differentially methylated regions (DMRs) have been identified in cattle using global methylation analysis technologies on different types of immune cells, which are summarized in **Table 3**.[82,85,94,97,98] Evaluation of global DNA methylation can reveal regions of the genome that change in response to a treatment, or that change in a tissue-specific manner. DMRs can differ depending on factors such as the tissue type, comparison of treatments, environmental impacts, and infection. As such, care must be taken when choosing the type of tissue or cell type isolated for DNA methylation analysis. Furthermore, the time of sample collection could affect the presence of DNA methylation or intermediary DNA methylation.[85] Age and diet are also well-known factors that affect global DNA methylation and therefore DMRs.[88,90,91,94] Examination of global DNA methylation in non-–immune-related tissues showed an association between decreased DNA methylation at immune-related genes, including TLR4, and increased chromatin accessibility and gene expression in cattle that were fed high-concentrate diets compared with their counterparts that were fed a low-concentrate diet.[95] Overall DNA methylation is associated with transcriptional regulation in ruminants and thus can influence immune cell phenotype and function.

Table 3
Summary of global DNA methylation studies in dairy cattle

Cell Type	Method	Comparison	Number of DMRs	References
CD4+ T lymphocytes	Reduced-representation bisulfite sequencing	*M bovis*–infected cattle vs noninfected cattle	765 DMR infected vs noninfected cattle	Doherty et al,[98] 2016
Fibroblast	Reduced-representation bisulfite sequencing	Difference between 5 mo and 16 mo of age stimulated with lipopolysaccharide	14,094 DMR (5065 gene regions, 1117 promoters, 1057 gene exons, 2891 gene introns)	Korkmaz & Kerr,[94] 2017
Peripheral blood mononuclear cell	Whole-genome MeDIP-seq	High milk yield and average milk yield	72 DMR high vs average milk yield 252 DMR herd environment	DeChow & Liu,[97] 2018
Alveolar macrophage	Whole-genome bisulfite sequencing	*M bovis*–infected cattle vs noninfected	0 DMR between infected and noninfected	O'Doherty et al,[85] 2019
Blood cells	Reduced-representation bisulfite sequencing	Creole cattle vs Iberian breeds	334 DMR	Sevane et al,[82] 2019

Abbreviation: MeDIP-seq, methylated DNA immunoprecipitation sequencing.

Histone Modifications

There are limited studies that report on histone modifications in domesticated ruminants, especially in the context of the immune response. Genome-wide assessment of the gene repressor marker, H3K27me3,[102] in bovine peripheral blood lymphocytes identified that this epigenetic mark is predominantly found 2 kb upstream of transcription start sites (TSSs) and in introns.[103] The presence of H3K27me3 at TSSs was generally associated with transcriptional repression for most genes.[103] He and colleagues,[104] investigated the impact of *S aureus* on H3K27me3 levels in blood mononuclear cells in cattle.[104] Most H3K27me3 was found to be intergenic and 20 kb upstream of TSSs, suggesting it is associated with regulatory factors outside the promoter region, such as enhancers.[104] There was a negative correlation between H3K27me3 and gene expression. In this case, the TSS was a key area of regulation for genes that function in immune-related processes in innate and adaptive immune responses when comparing cows with mastitis with healthy cows.[104] More research is needed to better understand the regulatory role of histone modifications and the histone code in bovine species and how they relate to immunity and immune function.

BOVINE COLOSTRUM AND MILK EXOSOMAL microRNAs

MicroRNAs are abundant in bovine colostrum and milk either free or enclosed within exosomes.[105–107] Notably, immune-related miRNAs are highly expressed in milk, particularly in colostrum, suggesting they are crucial for mammary gland immune regulation as well as promoting development of the calf gut mucosal immune system.[108]

Studies from the Mallard laboratory assessed the bioactivity of bovine colostrum and milk exosomes containing miRNA on gut health. Purified exosomes were noted to have typical phenotypic features similar to those in other body fluids[109] based on size (20–100 nm) and protein markers essential for their interaction with host cells.[110,111] Fluorescently labeled exosomes cocultured with human intestinal epithelial (Caco-2 cells) were taken up and visualized in the vicinity of the nucleus of cultured cells at 2 and 24 hours.[111] Furthermore, colostrum and milk exosomes cocultured with Caco-2 cells were not only noncytotoxic but enhanced cell viability using methylthiazoletetrazolium (MTT) cell proliferation/viability assay. Although both colostrum and milk seem to support Caco-2 cell viability for up to 72 hours, MTT activity was significantly ($P<.0001$) higher in cells cocultured with milk compared with those with colostrum. Furthermore, differences in Caco-2 cells' metabolic activity cocultured with exosomes from cows with different immune response phenotypes was observed. Specifically, metabolic activity after coculture with colostrum and milk exosomes from high immune response cows was significantly greater than those with low immune response exosomes ($P = .0198$). Of note, classification of those cows as high or low immune response was based on estimated breeding values using the patented High Immune Response (HIR) technology. Viability of Caco-2 cells cocultured with either colostrum or milk exosomes from high immune responder cows was significantly greater ($P<.0024$ and $P<.0048$) than that of low responders at 72 hours. A similar observation was reported in porcine milk exosomes.[112]

High-throughput next-generation sequencing of milk exosomal miRNA from average immune responder cows identified 680 mature miRNAs.[110] This study was the first to profile bovine exosomal miRNA isolated by differential ultracentrifugation and report their abundance compared with those identified in bovine milk exosomes by microarray (n = 79[105]) and in porcine milk exosomes (n = 218,[113] n = 491,[114] and n = 234[115]). Similar to the aforementioned studies, immune-related miRNAs, such as miR-148a, let-7 family, miR-21, and miR-26, were highly expressed in the study of cows classified based on their immune response phenotypes.

Important immune-related miRNAs (including miR-148a, miR-155, miR-21, miR-26a, and miR-29b) were also confirmed by quantitative reverse transcription polymerase chain reaction in colostrum and milk exosomes with significantly ($P<.05$) higher expression of miR-155 in colostrum compared with milk exosomes. Further, miR-155, miR-21, miR-26a, and miR-29b were differentially expressed among high and low immune responder cows.[110]

GENE BY ENVIRONMENT EFFECTS ON REGULATION OF IMMUNOCOMPETENCE OF DOMESTICATED RUMINANTS

The environmental component of host-pathogen-environment interactions that dictate disease profiles is discussed next. This area of research is important and topical because the changing climate is affecting livestock health and welfare directly through increased environmental temperatures and drought[116] and indirectly through ecosystem changes that alter the availability of feed resources and the distribution or epidemiology of animal diseases.[117,118] This article defines heat stress (HS) and resilience to climate change and how these factors relate to immunocompetence in domestic ruminants.

Because ruminants are endothermic homeotherms, they can maintain a physiologic body temperature within a certain ambient thermal neutral zone through passive cooling mechanisms (conduction, convection, and radiation).[119] However, when the surrounding ambient temperature is greater than an animal's thermal neutral zone, the

animal must expend energy and mobilize body reserves to maintain euthermia through active cooling (sweating and panting).[119,120] In general, cold ambient temperatures are manageable through protective shelters, increasing body size, insulation, and the heat generated through metabolism.[119,120]

Resilience can be defined as the ability of a species to survive and recover from a perturbation.[121] Animals with greater resilience to HS are able to maintain euthermia for longer through heat dissipation before becoming physiologically compromised.[118,122–124] Resilience depends on multiple factors, including region (adaptability), species, breed, sex, and productivity.[117,119] For example, among cattle, an increase in body temperature under HS was less pronounced in Brown Swiss compared with Holstein cows, suggesting that Brown Swiss are more resilient.[125,126] Among dairy animals, goats were identified as the most adapted species to HS in terms of production, reproduction, and disease resistance.[127,128] The question of whether animals more resilient to climate HS can be identified within breed and whether they are more resistant to disease is an area of emerging research.

The temperature-humidity index (THI) has been widely used as an indicator of HS in livestock.[129–131] THI is calculated by combining ambient temperature and humidity (see **Box 1**).[132] Various THI HS thresholds have been estimated, depending on species, breed, and region. For example, estimated THI thresholds for HS in Holstein cows range from 60 (which could correspond with a temperature of 21°C and a relative humidity of 62%; see **Box 1** for calculation of THI) for Holsteins in Germany[133] to 78 for Holsteins in a US subtropical environment.[134] The THI threshold for HS is likely to be higher for animals with lower production rates and for nonlactating animals.[132] Curtis and colleagues[135] used rumen temperature and feed intake to estimate a THI HS threshold of 75.5 for black angus feedlot cattle. Given these diverse ranges for THI thresholds, it is important for studies in various geographic locations to determine the relevant THI thresholds for each study.

Livestock are likely to experience days during the summer months when the THI exceeds their THI HS threshold. HS intensifies when the THI exceeds the threshold for consecutive days because the animal has reduced opportunity to dissipate body heat at night.[136] As an example, analysis of climatic data from a temperate region in southern Ontario, Canada, revealed that livestock frequently experience days, often consecutively, on which the THI HS threshold was likely exceeded (**Fig. 2**).

Stress Responsiveness and Immunocompetence

The environment plays a key role in the nature and outcome of host-pathogen interactions,[137] and it is therefore important to improve understanding of how climate extremes affect animals' responses to pathogens. Furthermore, climate change could induce shifts in the spread and types of diseases to which livestock are exposed.[118] The health and welfare of animals as the climate changes will be dictated in part by their resilience to extreme temperatures as well as their natural resilience to infections agents. As such, it is desirable to select for animals with both enhanced ability to resist disease[49,55,138,139] and superior resilience to HS.

There is evidence for favorable associations between disease resistance and stress responsiveness in livestock.[124] Recently, Aleri and colleagues[49] showed a favorable and significant association between a preferred response to stress and above-average immune competence in Holstein-Friesian and Holstein-Friesian × Jersey heifers. For example, heifers with above-average immune competence had lower serum cortisol concentrations compared with their below-average counterparts, suggesting that they had enhanced ability to cope with management-induced stress.[49] Reduced cortisol production in response to stress is desirable because high

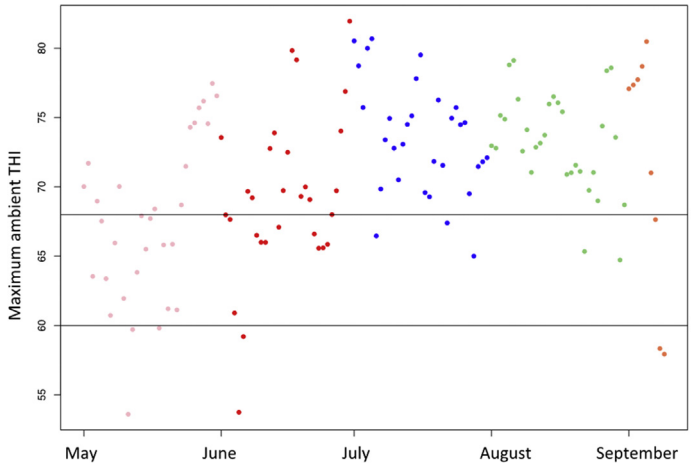

Fig. 2. The THI calculated for the summer months (May to September) of 2018 at a temperate region in southern Ontario, Canada. Suggested THI heat shock thresholds for dairy cows are indicated. [a] Estimated by Zimbelman and colleagues.[132] [b] Estimated by Brügemann and colleagues.[133] (*Data from* Zimbelman RB, Rhoads RP, Collier RJ, Duff GC. A re-evaluation of the impact of temperature humidity index (THI) and black globe humidity index (BGHI) on milk production in high producing dairy cows. Proc Southwest Nurr Man Conf. 2009;(January):158-169 and Brügemann K, Gernand E, von Borstel UU, König S. Genetic analyses of protein yield in dairy cows applying random regression models with time-dependent and temperature x humidity-dependent covariates. J Dairy Sci. 2011. https://doi.org/10.3168/jds.2010-4063.)

concentrations can suppress immune function and decrease the ability to cope with stress.[140,141] Because HS also results in increased serum cortisol concentrations,[142,143] it will be informative to investigate associations between immune competence and resilience to HS. For this reason, the Mallard laboratory is investigating the connection between cattle classified based on estimated breeding values of immune responsiveness and their response to HS. Preliminary results indicate substantial individual variation in response to both in vivo and in vitro HS in Holsteins, as well as beef cattle of mixed breeds. Knowledge of the genetic link between resilience to HS and immunocompetence would provide scope for breeding animals that are more resilient to HS and disease for a future with predictions for increased frequency and duration of HS events and changes to the distribution or epidemiology of pathogens.

SUMMARY

Disease resistance has a complex phenotype because of the dynamic interaction between host, pathogen, and environment. Discovering the mechanisms of how the genome shapes this phenotype is an exceptionally complex process with more than 5000 genes controlling host defense. Various strategies that have been used by researchers are limited. Although new technologies and bioinformatic methods are promising to collect and analyze much more complex data, the bottleneck of the investigation seems to be at the starting point: how to accurately translate a biological phenomenon (disease resistance) to a parsable data set. Recently, after decades of researches on the genetic regulation of disease resistance, some technologies became commercially available. However, these technologies are mainly based on association studies and, in some cases, the heritabilities are very low. Therefore, the

genetic gain might be limited because of the loss of association over generations or the low heritabilities. Novel strategies and sources of data are required to deepen the current study beyond association, free from study noise, to discover the causal mechanisms with near-perfect heritability to overcome these limitations. Reductionist models, structural variants, and epigenomic research are all examples of novel approaches and source data in an attempt to show the genetic blueprint of disease resistance. It should also be noted that livestock production and health will likely face new challenges with climate change. Emerging or remerging diseases, or the compromised performance of the current traits under environmental stress, such as HS, are just a few examples of the potential future challenges. All these factors warrant further investigation to identify the genetic regulation of disease resistance to improve livestock health now, as well as to be prepared for future challenges.

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REFERENCES

1. Casadevall A, Pirofski L. Host-pathogen interactions: redefining the basic concepts of virulence and pathogenicity. Infect Immun 1999;67(8):3703–13. Available at: http://iai.asm.org/content/67/8/3703.short.
2. Lazzaro BP, Little TJ. Immunity in a variable world. Philos Trans R Soc Lond B Biol Sci 2009;364(1513):15–26.
3. Casadevall A, Pirofski L-A. What is a host? Incorporating the microbiota into the damage-response framework. Infect Immun 2015;83(1):2–7.
4. Villani A-C, Sarkizova S, Hacohen N. Systems immunology: learning the rules of the immune system. Annu Rev Immunol 2018;36(1):813–42.
5. Bishop SC, Woolliams JA. Genomics and disease resistance studies in livestock. Livest Sci 2014;166:190–8.
6. Mallard BA, Emam M, Paibomesai M, et al. Genetic selection of cattle for improved immunity and health. Jpn J Vet Res 2015;63(Suppl 1):S37–44. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25872325.
7. Chaplin DD. Overview of the immune response. J Allergy Clin Immunol 2010; 125(2):S3–23.
8. Toka FN, Kenney MA, Golde WT. Rapid and transient activation of γδ T cells to IFN-γ production, NK cell-like killing, and antigen processing during acute virus infection. J Immunol 2011;186(8):4853–61.
9. Guzman E, Hope J, Taylor G, et al. Bovine γδ T cells are a major regulatory T cell subset. J Immunol 2014;193(1):208–22.
10. Hine BC, Cartwright SL, Mallard BA. Analysis of leukocyte populations in Canadian Holsteins classified as high or low immune responders for antibody- or cell-mediated immune response. Can J Vet Res 2012;76(2):149–56. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23024458.
11. Zerbino DR, Achuthan P, Akanni W, et al. Ensembl 2018. Nucleic Acids Res 2018;46(D1):D754–61.

12. Casey ME, Meade KG, Nalpas NC, et al. Analysis of the bovine monocyte-derived macrophage response to mycobacterium avium subspecies paratuberculosis infection using RNA-seq. Front Immunol 2015;6:23.
13. Greives TJ, Dochtermann NA, Stewart EC. Estimating heritable genetic contributions to innate immune and endocrine phenotypic correlations: a need to explore repeatability. Horm Behav 2017;88:106–11.
14. Minozzi G, Williams JL, Stella A, et al. Meta-analysis of two genome-wide association studies of bovine paratuberculosis. PLoS One 2012;7(3):e32578.
15. Geczy AF, Geczy CL, de Weck AL. Histocompatibility antigens and genetic control of the immune response in guinea pigs. II. Specific inhibition of antigen-induced lymphocyte proliferation by anti-receptor alloantisera. Eur J Immunol 1975;5(10):711–9.
16. Caldwell J. Polymorphism of the BoLA system. Tissue Antigens 1979;13(5): 319–26. Available at: http://www.ncbi.nlm.nih.gov/pubmed/91208.
17. Pokorska J, Kulaj D, Dusza M, et al. The influence of BoLA-DRB3 alleles on incidence of clinical mastitis, cystic ovary disease and milk traits in Holstein Friesian cattle. Mol Biol Rep 2018. https://doi.org/10.1007/s11033-018-4238-0.
18. Hayashi T, Mekata H, Sekiguchi S, et al. Cattle with the BoLA class II DRB3*0902 allele have significantly lower bovine leukemia proviral loads. J Vet Med Sci 2017;79(9):1552–5.
19. Duangjinda M, Jindatajak Y, Tipvong W, et al. Association of BoLA-DRB3 alleles with tick-borne disease tolerance in dairy cattle in a tropical environment. Vet Parasitol 2013;196(3–4):314–20.
20. Leach RJ, O'Neill RG, Fitzpatrick JL, et al. Quantitative trait loci associated with the immune response to a bovine respiratory syncytial virus vaccine. PLoS One 2012;7(3):e33526.
21. Maillard J-C, Berthier D, Chantal I, et al. Selection assisted by a BoLA-DR/DQ haplotype against susceptibility to bovine dermatophilosis. Genet Sel Evol 2003;35(Suppl 1):S193–200.
22. Sharif S, Mallard BA, Wilkie BN, et al. Associations of the bovine major histocompatibility complex DRB3 (BoLA-DRB3) alleles with occurrence of disease and milk somatic cell score in Canadian dairy cattle. Anim Genet 1998;29(3):185–93.
23. Rupp R, Hernandez A, Mallard BA. Association of bovine leukocyte antigen (BoLA) DRB3.2 with immune response, mastitis, and production and type traits in Canadian Holsteins. J Dairy Sci 2007;90(2):1029–38.
24. Maillard J-C, Chantal I, Berthier D, et al. Molecular immunogenetics in susceptibility to bovine dermatophilosis: a candidate gene approach and a concrete field application. Ann N Y Acad Sci 2002;969:92–6.
25. Zhang X, Lourenco D, Aguilar I, et al. Weighting strategies for single-step genomic BLUP: an iterative approach for accurate calculation of GEBV and GWAS. Front Genet 2016;7:151.
26. Weigel KA, VanRaden PM, Norman HD, et al. A 100-year review: methods and impact of genetic selection in dairy cattle-From daughter-dam comparisons to deep learning algorithms. J Dairy Sci 2017;100(12):10234–50.
27. Cui H, Dhroso A, Johnson N, et al. The variation game: cracking complex genetic disorders with NGS and omics data. Methods 2015;79-80:18–31.
28. Liu GE, Van Tassell CP, Sonstegard TS, et al. Detection of germline and somatic copy number variations in cattle. In: Pinard M, Gay C, Pastoret P, et al, editors. Animal genomics for animal health, vol. 132. Basel (Switzerland): KARGER; 2008. p. 231–7.

29. Fontanesi L, Martelli PL, Beretti F, et al. An initial comparative map of copy number variations in the goat (Capra hircus) genome. BMC Genomics 2010; 11(1):639.
30. Jenkins GM, Goddard ME, Black MA, et al. Copy number variants in the sheep genome detected using multiple approaches. BMC Genomics 2016;17(1):441.
31. Liu S, Kang X, Catacchio CR, et al. Computational detection and experimental validation of segmental duplications and associated copy number variations in water buffalo (Bubalus bubalis). Funct Integr Genomics 2019. https://doi.org/10.1007/s10142-019-00657-4.
32. Szyda J, Mielczarek M, Frąszczak M, et al. The genetic background of clinical mastitis in Holstein-Friesian cattle. Animal 2019;1–8. https://doi.org/10.1017/S1751731119000338.
33. Xu L, Hou Y, Bickhart DM, et al. A genome-wide survey reveals a deletion polymorphism associated with resistance to gastrointestinal nematodes in Angus cattle. Funct Integr Genomics 2014;14(2):333–9.
34. Durán Aguilar M, Román Ponce SI, Ruiz López FJ, et al. Genome-wide association study for milk somatic cell score in holstein cattle using copy number variation as markers. J Anim Breed Genet 2017;134(1):49–59.
35. Viginier B, Dolmazon C, Lantier I, et al. Copy number variation and differential expression of a protective endogenous retrovirus in sheep. PLoS One 2012; 7(7):e41965.
36. Bickhart DM, Liu GE. The challenges and importance of structural variation detection in livestock. Front Genet 2014;5:37.
37. Kemper KE, Littlejohn MD, Lopdell T, et al. Leveraging genetically simple traits to identify small-effect variants for complex phenotypes. BMC Genomics 2016; 17(1):858.
38. Hayes B, Goddard ME. The distribution of the effects of genes affecting quantitative traits in livestock. Genet Sel Evol 2001;33(3):209–29.
39. Bishop SC, Doeschl-Wilson AB, Woolliams JA. Uses and implications of field disease data for livestock genomic and genetics studies. Front Genet 2012; 3:114.
40. Nagahata H. Bovine leukocyte adhesion deficiency (BLAD): a review. J Vet Med Sci 2004;66(12):1475–82.
41. Biffani S, Del Corvo M, Capoferri R, et al. An alternative experimental case–control design for genetic association studies on bovine mastitis. Animal 2017;11(04):574–9.
42. Moose SP, Mumm RH. Molecular plant breeding as the foundation for 21st century crop improvement. Plant Physiol 2008;147(3):969–77.
43. Mahmoud M, Zeng Y, Shirali M, et al. Genome-wide pleiotropy and shared biological pathways for resistance to bovine pathogens. PLoS One 2018;13(4): e0194374.
44. Stear M, Fairlie-Clarke K, Jonsson N, et al. Genetic variation in immunity and disease resistance in dairy cows and other livestock. Cambridge (United Kingdom): Burleigh Dodds; 2017. p. 509–31. https://doi.org/10.19103/AS.2016.0006.25.
45. Stefan T, Matthews L, Prada JM, et al. Divergent allele advantage provides a quantitative model for maintaining alleles with a wide range of intrinsic merits. Genetics 2019. https://doi.org/10.1534/genetics.119.302022.
46. Ali AOA, Murphy L, Stear A, et al. Association of MHC class II haplotypes with reduced faecal nematode egg count and IgA activity in British Texel sheep. Parasite Immunol 2019;e12626. https://doi.org/10.1111/pim.12626.

47. Stoop CL, Thompson-Crispi KA, Cartwright SL, et al. Short communication: variation in production parameters among Canadian Holstein cows classified as high, average, and low immune responders. J Dairy Sci 2016;99(6):4870–4.
48. Fleming K, Thompson-Crispi KA, Hodgins DC, et al. Short communication: variation of total immunoglobulin G and β-lactoglobulin concentrations in colostrum and milk from Canadian Holsteins classified as high, average, or low immune responders. J Dairy Sci 2016;99(3):2358–63.
49. Aleri JW, Hine BC, Pyman MF, et al. Associations between immune competence, stress responsiveness, and production in Holstein-Friesian and Holstein-Friesian × Jersey heifers reared in a pasture-based production system in Australia. J Dairy Sci 2019;102(4):3282–94.
50. Aleri JW, Hine BC, Pyman MF, et al. Assessing adaptive immune response phenotypes in Australian Holstein-Friesian heifers in a pasture-based production system1. J Anim Sci 2015;93(7):3713–21.
51. Raphaka K, Sánchez-Molano E, Tsairidou S, et al. Impact of genetic selection for increased cattle resistance to bovine tuberculosis on disease transmission dynamics. Front Vet Sci 2018;5:237.
52. Beavers L, Van Doormaal B. Canadian Dairy Network - Pro$ & LPI: enhancements and updates. Available at: https://www.cdn.ca/document.php?id=516.
53. Biozzi G, Stiffel C, Mouton D, et al. Cytodynamics of the immune response in two lines of mice genetically selected for "high" and "low" antibody synthesis. J Exp Med 1972;135(5):1071–94.
54. Amor S, Smith PA, Hart B, et al. Biozzi mice: of mice and human neurological diseases. J Neuroimmunol 2005;165(1–2):1–10.
55. Wagter LC, Mallard BA, Wilkie BN, et al. The relationship between milk production and antibody response to ovalbumin during the peripartum period. J Dairy Sci 2003;86(1):169–73.
56. Larmer S, Mallard B. High immune response sires reduce disease incidence in North American large commercial dairy populations. Cattle Pract 2016;25(2):74–81.
57. Thompson-Crispi KA, Sewalem A, Miglior F, et al. Genetic parameters of adaptive immune response traits in Canadian Holsteins. J Dairy Sci 2012;95(1):401–9.
58. Thompson-Crispi K a, Sargolzaei M, Ventura R, et al. A genome-wide association study of immune response traits in Canadian Holstein cattle. BMC Genomics 2014;15(1):559.
59. Thomas MG, Marwood RM, Parsons AE, et al. The effect of foetal bovine serum supplementation upon the lactate dehydrogenase cytotoxicity assay: important considerations for in vitro toxicity analysis. Toxicol In Vitro 2015;30(1):300–8.
60. Ramakrishnan L. Revisiting the role of the granuloma in tuberculosis. Nat Rev Immunol 2012;12(5):352–66.
61. Wilkie B, Mallard B. Selection for high immune response: an alternative approach to animal health maintenance? Vet Immunol Immunopathol 1999;72(1–2):231–5.
62. Thompson-Crispi KA, Mallard BA. Type 1 and type 2 immune response profiles of commercial dairy cows in 4 regions across Canada. Can J Vet Res 2012;76(2):120–8.
63. Sarker N, Tsudzuki M, Nishibori M, et al. Cell-mediated and humoral immunity and phagocytic ability in chicken Lines divergently selected for serum immunoglobulin M and G levels. Poult Sci 2000;79(12):1705–9.

64. Cheng S, Lamont SJ. Genetic analysis of immunocompetence measures in a white leghorn chicken line. Poult Sci 1988;67(7):989–95.
65. de Klerk B, Emam M, Thompson-Crispi KA, et al. A genome-wide association study for natural antibodies measured in blood of Canadian Holstein cows. BMC Genomics 2018;19(1):694.
66. Rahyab AS, Alam A, Kapoor A, et al. Natural antibody - Biochemistry and functions. Glob J Biochem 2011;2(4):283–8.
67. Thompson-Crispi KA, Miglior F, Mallard BA. Genetic parameters for natural antibodies and associations with specific antibody and mastitis in Canadian Holsteins. J Dairy Sci 2013;96(6):3965–72.
68. Ko DC, Jaslow SL. The marriage of quantitative genetics and cell biology: a novel screening approach reveals people have genetically encoded variation in microtubule stability. Bioarchitecture 2014;4(2):58–61.
69. Ko DC, Shukla KP, Fong C, et al. A genome-wide in vitro bacterial-infection screen reveals human variation in the host response associated with inflammatory disease. Am J Hum Genet 2009;85(2):214–27.
70. Alvarez MI, Glover LC, Luo P, et al. Human genetic variation in VAC14 regulates Salmonella invasion and typhoid fever through modulation of cholesterol. Proc Natl Acad Sci U S A 2017;114(37):E7746–55.
71. Alvarez MI, Ko DC. Reply to Gilchrist et al.: possible roles for VAC14 in multiple infectious diseases. Proc Natl Acad Sci U S A 2018;115(16):E3604–5.
72. Gilchrist JJ, Mentzer AJ, Rautanen A, et al. Genetic variation in VAC14 is associated with bacteremia secondary to diverse pathogens in African children. Proc Natl Acad Sci U S A 2018;115(16):E3601–3.
73. Denholm SJ, McNeilly TN, Banos G, et al. Estimating genetic and phenotypic parameters of cellular immune-associated traits in dairy cows. J Dairy Sci 2017;100(4):2850–62.
74. Emam M, Tabatabaei S, Canovas A, et al. Cellular immuno-genomics: a novel approach to examine genetic regulation of disease resistance in cattle. Proc World Congr Genet Appl Livest Prod. 2018:738. Available at: http://www.wcgalp.org/proceedings/2018/cellular-immuno-genomics-novel-approach-examine-genetic-regulation-disease.
75. Emam M, Tabatabaei S, Sargolzaei M, et al. The effect of host genetics on in vitro performance of bovine monocyte-derived macrophages. J Dairy Sci, in press.
76. Emam M, Tabatabaei S, Sargolzaei M, et al. Identifying the genetic regions associated with bovine monocyte-derived macrophage nitric oxide production: a cellular genome-wide association study. Proceeding of the second Animal genetics and diseases conference. Wellcome Genome Campus, United Kingdom, May 8–10, 2019.
77. Donovan DM, Kerr DE, Wall RJ. Engineering disease resistant cattle. Transgenic Res 2005;14(5):563–7.
78. Wang Z. Genome engineering in cattle: recent technological advancements. Chromosome Res 2015;23(1):17–29.
79. Felsenfeld G. A brief history of epigenetics. Cold Spring Harb Perspect Biol 2014;6(1):a018200.
80. Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. Nat Rev Genet 2016;17(8):487–500. https://doi.org/10.1038/nrg.2016.59.
81. Carlberg C, Molnár F. Human Epigenomics. Singapore: Springer; 2018. https://doi.org/10.1007/978-981-10-7614-5.

82. Sevane N, Martínez R, Bruford MW. Genome-wide differential DNA methylation in tropically adapted Creole cattle and their Iberian ancestors. Anim Genet 2019;50(1):15–26.
83. Zhang X, Zhang S, Ma L, et al. Reduced representation bisulfite sequencing (RRBS) of dairy goat mammary glands reveals DNA methylation profiles of integrated genome-wide and critical milk-related genes. Oncotarget 2017;8(70): 115326–44.
84. Wang X, Chen Y, Ma S, et al. Whole-genome bisulfite sequencing of goat skins identifies signatures associated with hair cycling. BMC Genomics 2018; 19(1):1–9.
85. O'Doherty AM, Rue-Albrecht KC, Magee DA, et al. The bovine alveolar macrophage DNA methylome is resilient to infection with Mycobacterium bovis. Sci Rep 2019. https://doi.org/10.1038/s41598-018-37618-z.
86. Couldrey C, Brauning R, Bracegirdle J, et al. Genome-wide DNA methylation patterns and transcription analysis in sheep muscle. PLoS One 2014;9(7):1–7.
87. Frattini S, Capra E, Lazzari B, et al. Genome-wide analysis of DNA methylation in hypothalamus and ovary of Capra hircus. BMC Genomics 2017;18(1):1–9.
88. Wang X, Lan X, Radunz AE, et al. Maternal nutrition during pregnancy is associated with differential expression of imprinted genes and DNA methyltranfereases in muscle of beef cattle offspring. J Anim Sci 2015;93(1):35–40.
89. Zhang Y, Wang X, Jiang Q, et al. DNA methylation rather than single nucleotide polymorphisms regulates the production of an aberrant splice variant of IL6R in mastitic cows. Cell Stress Chaperones 2018;23(4):617–28.
90. Osorio JS, Jacometo CB, Zhou Z, et al. Hepatic global DNA and peroxisome proliferator-activated receptor alpha promoter methylation are altered in peripartal dairy cows fed rumen-protected methionine. J Dairy Sci 2015;99(1):234–44.
91. Lan X, Cretney EC, Kropp J, et al. Maternal diet during pregnancy induces gene expression and DNA methylation changes in fetal tissues in sheep. Front Genet 2013;4:1–12.
92. Fang L, Hou Y, An J, et al. Genome-wide transcriptional and post-transcriptional regulation of innate immune and defense responses of bovine mammary gland to Staphylococcus aureus. Front Cell Infect Microbiol 2016;6:193.
93. Paibomesai MA. Epigenetic influences on bovine T-helper 1 and T-helper 2 cytokines (interferon-gamma and Interleukin-4) in high and low immune responders around the peripartum period. [PhD thesis]. Guelph, Ontario: University of Guelph; 2017.
94. Korkmaz FT, Kerr DE. Genome-wide methylation analysis reveals differentially methylated loci that are associated with an age-dependent increase in bovine fibroblast response to LPS. BMC Genomics 2017;18(1):1–18.
95. Chang G, Zhang K, Xu T, et al. Epigenetic mechanisms contribute to the expression of immune related genes in the livers of dairy cows fed a high concentrate diet. PLoS One 2015;10(4):e0123942.
96. Magee DA, Spillane C, Berkowicz EW, et al. Imprinted loci in domestic livestock species as epigenomic targets for artificial selection of complex traits. Anim Genet 2014;45(SUPPL.1):25–39.
97. Dechow CD, Liu WS. DNA methylation patterns in peripheral blood mononuclear cells from Holstein cattle with variable milk yield. BMC Genomics 2018; 19(1):1–12.
98. Doherty R, Whiston R, Cormican P, et al. The CD4+ T cell methylome contributes to a distinct CD4+ T cell transcriptional signature in Mycobacterium bovis-infected cattle. Sci Rep 2016;6(1):1–15.

99. Paibomesai M, Hussey B, Nino-Soto M, et al. Effects of parturition and dexamethasone on DNA methylation patterns of IFN-γ and IL-4 promoters in CD4+ T-lymphocytes of Holstein dairy cows. Can J Vet Res 2013;77(1):54–62.
100. Zhou Y, Connor EE, Bickhart DM, et al. Comparative whole genome DNA methylation profiling of cattle sperm and somatic tissues reveals striking hypomethylated patterns in sperm. Gigascience 2018;7(5):1–13.
101. Shi B, Thomas AJ, Benninghoff AD, et al. Genetic and epigenetic regulation of major histocompatibility complex class I gene expression in bovine trophoblast cells. Am J Reprod Immunol 2018;79(1):e12779.
102. Wiles ET, Selker EU. H3K27 methylation: a promiscuous repressive chromatin mark. Curr Opin Genet Dev 2017;43:31–7.
103. He Y, Yu Y, Zhang Y, et al. Genome-wide bovine H3K27me3 modifications and the regulatory effects on genes expressions in peripheral blood lymphocytes. PLoS One 2012;7(6). https://doi.org/10.1371/journal.pone.0039094.
104. He Y, Song M, Zhang Y, et al. Whole-genome regulation analysis of histone H3 lysin 27 trimethylation in subclinical mastitis cows infected by Staphylococcus aureus. BMC Genomics 2016;17(1):1–12.
105. Izumi H, Tsuda M, Tsuda M, et al. Bovine milk exosomes contain microRNA and mRNA and are taken up by human macrophages. J Dairy Sci 2015;98(5):2920–33.
106. Izumi H, Kosaka N, Shimizu T, et al. Bovine milk contains microRNA and messenger RNA that are stable under degradative conditions. J Dairy Sci 2012;95(9):4831–41.
107. Chen X, Gao C, Li H, et al. Identification and characterization of microRNAs in raw milk during different periods of lactation, commercial fluid, and powdered milk products. Cell Res 2010;20(10):1128–37.
108. Liang G, Malmuthuge N, Guan LL, et al. Model systems to analyze the role of miRNAs and commensal microflora in bovine mucosal immune system development. Mol Immunol 2015;66(1):57–67.
109. Yáñez-Mó M, Siljander, Pia R-M, et al. Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles 2015;4(1):27066.
110. Atalla H, Mallard B, Karrow NA. Characterization of exosomal immune- related micrornas in colostrum and milk from average, low and high immune responder cows. J Anim Sci 2016;94(suppl_4):74–5.
111. Ross M, Atalla H, Mallard B. Bioactivity of colostrum and milk exosomes containing microrna from cows genetically selected as high, average and low immune responders based on their estimated breeding values. J Anim Sci 2016;94(suppl_4):65–7.
112. Chen T, Xie M-Y, Sun J-J, et al. Porcine milk-derived exosomes promote proliferation of intestinal epithelial cells. Sci Rep 2016;6(1):1–12.
113. van Herwijnen MJC, Driedonks TAP, Snoek BL, et al. Abundantly present miRNAs in milk-derived extracellular vesicles are conserved between mammals. Front Nutr 2018;5:1–6.
114. Chen T, Xi Q-Y, Ye R-S, et al. Exploration of microRNAs in porcine milk exosomes. BMC Genomics 2014;15(1):100.
115. Gu Y, Li M, Wang T, et al. Lactation-related microRNA expression profiles of porcine breast milk exosomes. PLoS One 2012;7(8):pe43691.
116. Intergovernmental Panel on Climate Change. Climate change 2013 – the physical science basis working group I contribution to the fifth assessment report of the intergovernmental panel on climate change. Cambridge (United Kingdom): Cambridge University Press; 2014.

117. Hoffmann I. Adaptation to climate change–exploring the potential of locally adapted breeds. Animal 2013. https://doi.org/10.1017/S1751731113000815.
118. Rojas-Downing MM, Nejadhashemi AP, Harrigan T, et al. Climate change and livestock: impacts, adaptation, and mitigation. Clim Risk Manag 2017;16:145–63.
119. Collier RJ, Baumgard LH, Zimbelman RB, et al. Heat stress: physiology of acclimation and adaptation. Animal Front 2018;9(1):12–9.
120. Nienaber JA, Hahn GL. Livestock production system management responses to thermal challenges. Int J Biometeorol 2007. https://doi.org/10.1007/s00484-007-0103-x.
121. Williams SE, Shoo LP, Isaac JL, et al. Towards an integrated framework for assessing the vulnerability of species to climate change. PLoS Biol 2008. https://doi.org/10.1371/journal.pbio.0060325.
122. Das R, Sailo L, Verma N, et al. Impact of heat stress on health and performance of dairy animals: a review. Vet World 2016;9(3):260–8.
123. Misztal I. Breeding and genetics symposium: resilience and lessons from studies in genetics of heat stress. J Anim Sci 2017;95(4):1780–7.
124. Colditz IG, Hine BC. Resilience in farm animals: biology, management, breeding and implications for animal welfare. Anim Prod Sci 2016. https://doi.org/10.1071/an15297.
125. Johnson HD. Environmental temperature and lactation (with special reference to cattle). Int J Biometeorol 1965. https://doi.org/10.1007/BF02188466.
126. Correa-Calderon A, Armstrong D, Ray D, et al. Thermoregulatory responses of Holstein and Brown Swiss Heat-Stressed dairy cows to two different cooling systems. Int J Biometeorol 2004. https://doi.org/10.1007/s00484-003-0194-y.
127. Silanikove N, Koluman DN. Impact of climate change on the dairy industry in temperate zones: predications on the overall negative impact and on the positive role of dairy goats in adaptation to earth warming. Small Rumin Res 2015. https://doi.org/10.1016/j.smallrumres.2014.11.005.
128. Gantner V, Bobic T, Gantner R, et al. Differences in response to heat stress due to production level and breed of dairy cows. Int J Biometeorol 2017;61(9):1675–85.
129. Ingraham RH, Gillette DD, Wagner WD. Relationship of temperature and humidity to conception rate of Holstein cows in subtropical climate. J Dairy Sci 1974. https://doi.org/10.3168/jds.S0022-0302(74)84917-9.
130. Ibrahim MN, Stevens DG, Shanklin MD, et al. Model of broiler performance as affected by temperature and humidity. Trans Am Soc Agric Eng 1975;18:960–2.
131. Gaughan JB, Mader TL, Holt SM, et al. Heat tolerance of Boran and Tuli crossbred steers. J Anim Sci 1999. https://doi.org/10.2527/1999.7792398x.
132. Zimbelman RB, Rhoads RP, Collier RJ, et al. A re-evaluation of the impact of temperature humidity index (THI) and black globe humidity index (BGHI) on milk production in high producing dairy cows. Proc Western Dairy Management Conference. Reno, NV, USA. March 9–11, 2009. p. 158–69.
133. Brügemann K, Gernand E, von Borstel UU, et al. Genetic analyses of protein yield in dairy cows applying random regression models with time-dependent and temperature x humidity-dependent covariates. J Dairy Sci 2011. https://doi.org/10.3168/jds.2010-4063.
134. Dikmen S, Hansen PJ. Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? J Dairy Sci 2009. https://doi.org/10.3168/jds.2008-1370.

135. Curtis AK, Scharf B, Eichen PA, et al. Relationships between ambient conditions, thermal status, and feed intake of cattle during summer heat stress with access to shade. J Therm Biol 2017;63:104–11.
136. Garner JB, Douglas ML, Williams SRO, et al. Genomic selection improves heat tolerance in dairy cattle. Sci Rep 2016;6:1–8.
137. Shikano I, Cory JS. Impact of environmental variation on host performance differs with pathogen identity: implications for host-pathogen interactions in a changing climate. Sci Rep 2015. https://doi.org/10.1038/srep15351.
138. Burton JL, Burnside EB, Kennedy BW, et al. Antibody responses to human erythrocytes and ovalbumin as marker traits of disease resistance in dairy calves. J Dairy Sci 1989;72:1252–65.
139. Pighetti GM, Sordillo LM. Specific immune responses of dairy cattle after primary inoculation with recombinant bovine interferon-?? as an adjuvant when vaccinating against mastitis. Am J Vet Res 1996;57(6):819–24.
140. Diez-Fraile A, Meyer E, Burvenich C. Sympathoadrenal and immune system activation during the periparturient period and their association with bovine coliform mastitis. A review. Vet Q 2003. https://doi.org/10.1080/01652176.2003.9695142.
141. Eskandari F, Sternberg EM. Neural-immune interactions in health and disease. Ann N Y Acad Sci 2002. https://doi.org/10.1111/j.1749-6632.2002.tb04198.x.
142. Veissier I, Van laer E, Palme R, et al. Heat stress in cows at pasture and benefit of shade in a temperate climate region. Int J Biometeorol 2018. https://doi.org/10.1007/s00484-017-1468-0.
143. Bharati J, Dangi SS, Mishra SR, et al. Expression analysis of Toll like receptors and interleukins in Tharparkar cattle during acclimation to heat stress exposure. J Therm Biol 2017. https://doi.org/10.1016/j.jtherbio.2017.02.002.
144. Scheffer M, Bolhuis JE, Borsboom D, et al. Quantifying resilience of humans and other animals. Proc Natl Acad Sci U S A 2018;115(47):11883–90.
145. Sharp AJ, Locke DP, McGrath SD, et al. Segmental duplications and copy-number variation in the human genome. Am J Hum Genet 2005;77(1):78–88.
146. Park E, Pan Z, Zhang Z, et al. The expanding landscape of alternative splicing variation in human populations. Am J Hum Genet 2018;102(1):11–26.
147. Wang XG, Ju ZH, Hou MH, et al. Deciphering transcriptome and complex alternative splicing transcripts in mammary gland tissues from cows naturally infected with Staphylococcus aureus Mastitis. PLoS One 2016;11(7):e0159719.
148. Govignon-Gion A, Dassonneville R, Baloche G, et al. Multiple trait genetic evaluation of clinical mastitis in three dairy cattle breeds. Animal 2016;10(04):558–65.
149. Koeck A, Loker S, Miglior F, et al. Genetic relationships of clinical mastitis, cystic ovaries, and lameness with milk yield and somatic cell score in first-lactation Canadian Holsteins. J Dairy Sci 2014;97(9):5806–13.
150. Narayana SG, Miglior F, Naqvi SA, et al. Genetic analysis of subclinical mastitis in early lactation of heifers using both linear and threshold models. J Dairy Sci 2018;101(12):11120–31.
151. Brito LF, Mallikarjunappa S, Sargolzaei M, et al. The genetic architecture of milk ELISA scores as an indicator of Johne's disease (paratuberculosis) in dairy cattle. J Dairy Sci 2018;101(11):10062–75.
152. Kirkpatrick BW, Lett BM. Short communication: heritability of susceptibility to infection by Mycobacterium avium ssp. paratuberculosis in Holstein cattle. J Dairy Sci 2018;101(12):11165–9.

153. Chapinal N, Koeck A, Sewalem A, et al. Genetic parameters for hoof lesions and their relationship with feet and leg traits in Canadian Holstein cows. J Dairy Sci 2013;96(4):2596–604.
154. Abdalla EA, Rosa GJM, Weigel KA, et al. Genetic analysis of leukosis incidence in United States Holstein and Jersey populations. J Dairy Sci 2013;96(9):6022–9.
155. Passafaro TL, Carrera JPB, dos Santos LL, et al. Genetic analysis of resistance to ticks, gastrointestinal nematodes and Eimeria spp. in Nellore cattle. Vet Parasitol 2015;210(3–4):224–34.
156. Mallard B, Cartwright S, Nayeri S, et al. Genome-wide association and functional annotation of positional candidate genes for immune response in Canadian Holstein Cattle. Proc World Congr Genet Appl to livest Prod. 2018:711. http://www.wcgalp.org/proceedings/2018/genome-wide-association-and-functional-annotation-positional-candidate-genes-immune.
157. Emam M, Paibomesai MA, Thompson-Crispi KA, et al. The association between sire estimated breeding value for antibody-mediated immune response (AMIR) and offspring AMIR phenotype. Proc World Congr Genet Appl Livest Prod. 2014:522. Available at: http://www.wcgalp.org/proceedings/2014/association-between-sire-estimated-breeding-value-antibody-mediated-immune-response.